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Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae)

J. L. MONTAGUE,* S. C. H. BARRETT† & C. G. ECKERT*

*Department of Biology, Queen's University, Kingston, ON, Canada
†Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

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Abstract

Range expansion during biological invasion requires that invaders adapt to geographical variation in climate, which should yield latitudinal clines in reproductive phenology. We investigated geographic variation in life history among 25 introduced populations of *Lythrum salicaria*, a widespread European invader of North American wetlands. We detected a strong latitudinal cline in initiation of flowering and size at flowering, which paralleled that reported among native populations. Plants from higher latitudes flowered earlier and at a smaller size than those from lower latitudes, even when raised in a uniform glasshouse. Early flowering was associated with greatly reduced reproductive output, but this was not associated with latitudinal variation in abundance, and probably did not result from a genetic correlation between time to and size at flowering. As introduction to North America *c.* 200 years ago, *L. salicaria* has re-established latitudinal clines in life history, probably as an evolutionary response to climatic selection.

Introduction

The globalization of human activities has resulted in large-scale range expansion by plants and animals, especially during the last two centuries. This often involves human-mediated transport of species between continents followed by geographic spread. Expansion of the introduced range will depend on how well species cope with geographic heterogeneity in abiotic and biotic factors, especially climate. Over relatively small-scale climatic gradients, population spread may be enabled by phenotypic plasticity (e.g. Annapurna & Singh, 2003; Parker *et al.*, 2003). However, large-scale spread over broad climatic gradients is expected to involve adaptive differentiation of populations (Cox, 2004). Populations may diverge via natural selection acting incrementally during range expansion with the rate of divergence enhanced by admixture of genetic variation from several introductions from the native range (Bossdorf *et al.*, 2005). Similar patterns of geographic differentiation may also arise when preadapted genotypes are transported directly between climatically similar regions in the native and introduced ranges ('climate matching', Maron *et al.*, 2004). However, these processes are not mutually exclusive and the immigration history of invaders is often complex and may commonly involve both.

Adaptation to climate often produces clines, because many climatic factors vary geographically in a continuous manner (Endler, 1977). For example, mean annual temperature and growing season length generally decline towards higher latitudes. Geographic variation in life-history traits associated with climate has been documented in plants within their native ranges, and clinal variation is common in wide-ranging species (e.g. Winn & Gross, 1993; Van Dijk *et al.*, 1997; Jonas & Geber, 1999; Li *et al.*, 1998; Clevering *et al.*, 2001; Olsson & Ågren, 2002). Adaptation during range expansion is generally thought to be an essential component of biological invasion (Reznick & Ghalambor, 2001; Sakai *et al.*, 2001; Cox, 2004). However, few studies have investigated the extent to which invading species have re-established clinal variation in life-history traits as an adaptive response to climatic variation (Maron *et al.*, 2004; Leger & Rice, 2007).

Correspondence: Christopher G. Eckert, Department of Biology, Queen's University, Kingston, ON, Canada K7L 3N6. Tel.: +1 613 533 6158; fax: +1 613 533 6617;
e-mail: chris.eckert@queensu.ca

Among the most important life-history traits likely to influence invasion success of plants is the timing of flowering (Griffith & Watson, 2006). Because growing season length decreases with increasing latitude, individuals should initiate flowering earlier in the growing season at higher latitudes so that seeds can be matured before the onset of climatic conditions that inhibit growth. However, life-history theory predicts that early reproduction requires maturing at a smaller size, as allocation to reproduction early in development leads to physiological trade-offs because of limited resources (Reznick *et al.*, 2000). This should reduce growth, survival and future reproductive output leading to a demographic cost of early reproduction. This cost may be exacerbated by progressively less favourable environmental conditions in higher latitudes (Gaston, 2003). A demographic trade-off between the time to and size at reproduction may ultimately be manifested as a positive genetic correlation between these traits (Roff, 2000). Early reproduction is thus costly yet crucial under conditions where seasonality requires an individual to rapidly complete reproduction.

Latitudinal clines in flowering time have been demonstrated across the native ranges of many plant species (e.g. Jonas & Geber, 1999; Clevering *et al.*, 2001; Olsson & Ågren, 2002; Stinchcombe *et al.*, 2004), yet there are few demonstrations of similar clines among introduced populations (Weber & Schmid, 1998; Kollmann & Bañuelos, 2004). There is also evidence for demographic trade-offs because of reduced size at flowering within native populations (e.g. Dieringer, 1991; Ollerton & Lack, 1998), and for the expected positive genetic correlation between time to flowering and size (e.g. Dorn & Mitchell-Olds, 1991). However, whether trade-offs between time to and size at flowering are manifested among populations across latitudinal gradients has rarely been investigated (but see Griffith & Watson, 2005), and the extent to which such trade-offs might limit the geographic spread of invasive plants remains unexplored.

Here, we investigate whether latitudinal clines in life-history traits, particularly reproductive phenology, are associated with range expansion in introduced populations of *Lythrum salicaria* L. (purple loosestrife, Lythraceae), a widespread European invader of North American wetlands first reported on the eastern seaboard of northern USA in 1814 (Thompson *et al.*, 1987). Latitudinal clines in flowering time and associated life-history traits have been demonstrated in two separate regions of the species' native range (Olsson & Ågren, 2002; Bastlová *et al.*, 2004, 2006; Olsson, 2004), but nothing is known of geographic variation in life history among introduced populations. Although early immigration of *L. salicaria* probably involved multiple introductions, almost all herbarium specimens (up to 1880) were collected in the north-eastern USA. Distributional records then indicate that the species gradually spread from this region southwards reaching the south-eastern coastal plain by the 1940s and northwards into eastern and central Canada by the 1980s (Stuckey, 1980; Thompson *et al.*, 1987). The available historical data do not suggest that recent 'climatically matched' introductions have played a significant role in the contemporary spread to northern and southern range limits in eastern North America, although, of course, this possibility cannot be completely rejected.

The main goal of our study was to evaluate the hypothesis that *L. salicaria* has experienced broad-scale differentiation in life history during its spread in eastern North America. We specifically addressed the following questions: (1) is there latitudinal variation in the timing of flowering among introduced populations? We used field surveys to test the prediction that populations from higher latitudes flower earlier in the growing season than those from more southerly latitudes. (2) If clinal variation in flowering time is evident, is there corresponding latitudinal variation in size at flowering? Plants in populations at higher latitudes should initiate flowering at a smaller size than those in populations at lower latitudes. (3) Does latitudinal variation in time to flowering and size at flowering have a genetic basis, as would be the case if geographic variation in life-history results from divergent selection across a climatic gradient? We used a glasshouse experiment involving populations surveyed in the field to determine the extent to which latitudinal variation among populations is maintained in a common environment. Correspondence between the performance of populations in the field and glasshouse would be expected if geographic variation in life-history traits had a genetic component. (4) Is there a demographic trade-off between time to flowering (from the onset of the growing season) and plant size at flowering and is this manifested by variation in reproductive output among natural populations? If genetic correlations between life-history traits contribute to a demographic trade-off, we should observe a positive among-population correlation between time to flowering and size at flowering in a common glasshouse environment.

Materials and methods

Study species

Lythrum salicaria is an herbaceous, tristylous perennial found in diverse wetland habitats. Plants consist of clusters of erect primary stems that develop in late spring from winter buds formed the previous year on a persistent rootstock that does not spread or multiply clonally to any great extent. Primary stems often give rise to secondary stems from axillary meristems. In this study, the unit of observation was a 1° stem plus all associated 2° stems, which we refer to simply as a 'stem'. We define a 'plant' as a cluster of adjacent 1° stems, not more than 10 cm apart and usually spatially distinct from nearby individuals. Plants correspond to genets as we observed

that stems within a plant always produced flowers of the same style morph.

Flowering of *L. salicaria* begins in June and can last until early October. Sessile axillary inflorescences include two to seven flowers and develop acropetally along the flowering spike. Flowers are self-incompatible and pollinated mainly by bees, especially *Bombus* spp. and lepidopterans (Levin, 1970). Fruits mature *c.* 1 month after floral anthesis, and each stem produces ~100 000 very small (0.05 mg) seeds with no obvious dispersal structures (Shamsi & Whitehead, 1974; Thompson *et al.*, 1987; McCaughey & Stephenson, 2000). Seeds germinate readily over a wide range of temperatures and day lengths (Shamsi & Whitehead, 1974; Young & Clements, 2001).

We focused on the seasonal initiation of flowering by individual stems and whether this is associated with variation in the size of stems at flowering (i.e. their height, girth, node number, branching pattern and dry mass) and their fecundity. Flowering phenology in long-lived perennials can be complex, and may also involve variation in year of first flowering, as well as variation in the frequency of flowering episodes among years. However, our approach is appropriate for *L. salicaria*. Plants from all populations that we have worked with initiate flowering in their first year given favourable growing conditions. Moreover, the 1° stem is the fundamental above-ground module of an individual plant and there appears to be no morphological heterogeneity or apparent division of labour among stems within a plant. Hence, our units of observation were appropriate for detecting potential trade-offs.

Population sampling

We sampled 25 populations across most of the latitudinal range of *L. salicaria* in eastern North America: from Maryland and New Jersey through Pennsylvania and New York to Ohio and Ontario (Fig. 1). Sampled populations were ≥ 25 km apart and we avoided very small populations and those significantly damaged by insect biological control agents (Malecki *et al.*, 1993). We extensively searched for *L. salicaria* beyond the most southern and northern populations but did not find additional populations. In autumn 2003, at each sampling site, we estimated population area [the area (m^2) covered contiguously by *L. salicaria*] and stem density (the number of 1° stems per m^2) from which we calculated number of 1° stems per population. We also quantified the number of 1° stems per plant for 40 reproductive plants per population.

Climatic variation among populations

We quantified climate across the latitudinal range of *L. salicaria* in North America using data from the weather stations closest to sampled populations. We calculated annual mean temperature from 1971 to 2000 and growing season length as the number of days with mean temperature > 8 °C using 30-year daily means from 1971 to 2000; ecophysiological experiments indicate that growth of *L. salicaria* is prevented below 8 °C (Shamsi & Whitehead, 1977). We did not analyse precipitation because all the populations we studied were in standing water throughout the growing season. Daily means from NOAA (USA) had been calculated by interpolating from monthly means using a cubic spline (Greville, 1964). Data from Environment Canada were provided as daily means for each year, thus 30-year daily means were calculated from a spline function (λ = 10 000 = a relatively smooth but stiff fit to the data). Although we calculated daily means for populations in the USA and Canada somewhat differently, strong, linear regression of climate variables on latitude (see *Results*) indicated that the two components of this data set fit together seamlessly. We calculated growing degree days, a cumulative measure of heat available for growth, by subtracting the empirically determined minimum growth temperature of 8 °C from the daily mean temperature and summing across days in a growing season. We calculated day length for each day of the year according to Forsythe *et al.* (1995), using 1986 for the year variable because it was midway between 1971 and 2000 and cumulative number of daylight hours throughout the growing season was estimated using these values.

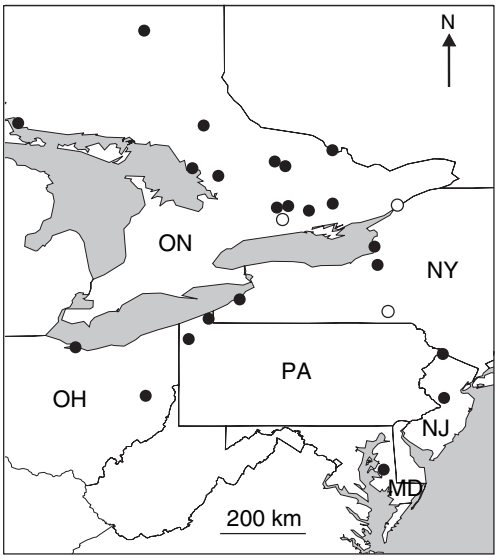

Fig. 1 Locations of introduced populations of *Lythrum salicaria* sampled across the latitudinal range of the species in eastern North America. Filled circles represent populations that provided data for both the field survey and the glasshouse experiment. Open circles represent populations that only yielded data for the glasshouse experiment (MD, Maryland, NJ, New Jersey, NY, New York, OH, Ohio, ON, Ontario, PA, Pennsylvania).

Flowering phenology, growth and reproductive output in natural populations

During summer and autumn 2004, we repeatedly visited the 25 populations to quantify flowering phenology and growth. Three populations were severely disturbed or destroyed and thus field data were collected for only 22 populations.

We visited each population every 13 days (range 8–23) and sampled an average of 260 stems (range 67–465) during each visit and classified stems as: vegetative (no buds developing), prereproductive (buds developing), reproductive (flowering), fruiting (still flowering and fruit developing) or post-reproductive (only fruits developing or mature). We visited each population until 90% of the stems were post-reproductive, which required six to seven visits per population. We calculated the weighted date when individuals began flowering for each population as:

$$\left(\frac{1}{p_t}\right)\sum_{i=1}^{k} d_i p_i,$$

where each population was visited k times, d_i is the Julian date of the ith visit to the population, p_i is the proportion of stems that began flowering since the last visit, p_t is the total proportion of stems that flowered over the course of the season (i.e. the sum of p_i across all k visits). We refer to this as 'weighted flowering date'. We then calculated the number of days from the beginning of the growing season (i.e. date when 30-year mean daily temperature > 8 °C) to the weighted flowering date, and refer to this as 'weighted days to flower'.

We randomly sampled 30 stems on their first day of flowering from throughout each population (except for NYWM that experienced frequent herbivore damage). For each population, sampling was stratified across visits roughly in proportion to the fraction of plants initiating flowering on each visit. For each plant, we measured height of 1° stem, length of vegetative portion of 1° stem, length of reproductive portion of 1° stem, number of 2° stems, diameter of the 1° stem at its midpoint and the number of internodes on the vegetative portion of the 1° stem.

During the last visit to each population, we harvested 30 randomly selected post-reproductive stems (except ONTI = 23 stems) and divided each into vegetative (vegetative portion of 1° stem and nonreproductive 2° stems) and reproductive components (reproductive portion of 1° stem and reproductive 2° stems). Each component was dried at 60 °C until constant mass and weighed to 0.1 g. Total reproductive output (number of seeds per stem) was calculated for 10 stems per population, as the product of infructescences per stem, mean fruits per infructescence (estimated from six infructescences per stem) and mean seeds per fruit (estimated from two fruits per stem).

Flowering and growth under uniform glasshouse conditions

In autumn 2003, we collected mature fruits from each of 20–41 (mean 36) randomly chosen reproductive stems from each population and dried them at 20 °C for 2 weeks. In January 2004, we sowed samples of 10 seeds from each of 30 randomly chosen maternal families (stems) for each of the 25 populations (except MDDB = 26 families; OHBV = 29 families; ONMB = 20 families; ONTI = 19 families) into 5.7-cm pots containing growth medium (Sunshine Mix™ #3, Sun Gro Horticulture, Vancouver, BC, Canada) kept saturated with water. Pots were randomized across trays (36 pots/tray), which were randomly positioned on a glasshouse bench under 16 h light/day (ambient sunlight supplemented with sodium lamps) at 18–25 °C, and repositioned every day to reduce the effects of environmental variation.

When seedlings were 10 mm tall, we randomly selected two from each family (n = 1448 total), planted each in a 10.2-cm pot of growth medium, and randomized all pots among 125 trays (12 plants/tray) on six benches in one glasshouse with environmental conditions as above. Seven weeks after sowing, we started fertilizing plants once per week with 0.5 g/tray of 20–20–20 Plant-Prod® All Purpose Fertilizer (Plant Products Co. Ltd., Brampton, ON, Canada).

We recorded the date that each plant began flowering, and on that day, made the same six size measurements as in the field survey. One plant from each family was harvested on its first day of flowering, dried at 60 °C to constant mass and weighed to 0.1 g. Each of these plants was removed from its pot and we estimated the percentage of the bottom of the pot filled by roots (root index).

Statistical analyses

For data from both the population survey and glasshouse experiment, we tested for differentiation among populations using a one-way analysis of variance (ANOVA). We used family means for traits that were measured for both replicate sibs in the common environment experiment. For all traits measured for replicate progeny per family, a mixed-model ANOVA with family as a random effect nested within population produced very similar results (not shown). For most traits, assumptions of homoscedascity could not be met, even after transformation. Accordingly, we used Welch's (1951) ANOVA allowing for unequal variances.

To assess covariation between latitudinal variation in climate and measures of time and size at flowering and reproductive output, we used Pearson correlations with latitude as a proxy for climate (see *Results*). We also tested for covariation of time to flowering, size at flowering and reproductive output using Pearson correlation. For correlations among life-history traits, we used the total height of the 1° stem as the measure of size because it

could be measured nondestructively in both field and glasshouse, and because it correlated strongly with total above-ground dry mass at flowering in the glasshouse ($r = +0.80$, $P < 0.0001$). However, we also present analyses of other size variables to more fully describe variation in plant size and growth form. We used JMP 6.0 (SAS Institute Inc., 2006) for all analyses.

Because we used seeds collected from plants grown in the field, nongenetic maternal effects could potentially contribute to variation among progeny in the glasshouse (Rossiter, 1996). Eliminating maternal effects by raising the experimental material for one or more generations in a common environment was not practical in this experiment, as the production of > 700 genetically independent families from 25 populations, while maintaining disassortative mating among style morphs and avoiding domestication effects, would have required hand-crossing thousands of plants for at least 2 years. We consider the extent to which nongenetic maternal effects may have contributed to the pattern of differentiation among populations in the discussion.

Results

Climatic variation across the latitudinal range

All climatic variables and growing season length covaried linearly with latitude (Table 1). Between the most northerly and southerly populations, mean annual temperature differed by 13 °C and growing degree days varied almost threefold. The length of the growing season, based on an empirically determined 8 °C threshold, varied from 138 to 256 days. Strong correlations between all variables and latitude indicate that latitude is a good overall predictor of climate over the sampled range. Hence, we used latitude as a reliable proxy for climate below.

Variation in population size and density across the latitudinal range

Populations varied widely in area, from 120 to 50 000 m^2, with one population (OHSF) 24-fold larger than the next largest population (median 463 m^2), but log-transformed area did not correlate with latitude ($r = -0.35$, $n = 22$, $P = 0.10$). The density of 1° stems within populations was also variable (mean ± SE 15 ± 2 stems m^{-2}, range 1–40 stems m^{-2}, CV 74%) and correlated positively with latitude ($r = +0.52$, $n = 22$, $P = 0.012$). The product of population area and 1° stem density varied from 300 to 1×10^6 stems/population (median 5475), but log-transformed stems/population did not correlate with latitude ($r = +0.08$, $n = 22$, $P = 0.72$). Number of 1° stems per plant varied among populations (mean ± SE 7 ± 1 stems/plant, range 2–21 stems/plant, CV 69%) but did not correlate with latitude ($r = +0.15$, $n = 22$, $P = 0.50$).

Table 1 Variation in climate and growing season length across the latitudinal range of *Lythrum salicaria* in North America.

Variable	Range	CV (%)	Correlation with latitude
Annual mean temperature (°C)	1.4–14.5	42	−0.95
Growing season length (days)	138–256	14	−0.93
Growing degree days (°C)	860–2775	30	−0.90
Cumulative growing season daylight (h)	2048–3343	11	−0.92

Variation is presented as the range among populations and coefficient of variation (CV). All Pearson correlations with latitude are significant at $P < 0.0001$.

Variation in flowering time across a climatic gradient

As predicted, flowering time varied clinally (Table 2, Fig. 2). Weighted date of flowering in the field did not correlate with latitude ($r = +0.34$, $n = 22$, $P = 0.12$); however, the weighted days to flowering from the start of the growing season correlated negatively with latitude among populations in the field. Significantly, we also observed the same pattern in the glasshouse. All surviving progeny flowered during the course of the experiment, and the number of days from sowing to first flower varied among populations and correlated negatively with latitude. Weighted days to flower in the field correlated positively with days from sowing to flowering in the glasshouse ($r = +0.46$, $n = 22$, $P = 0.030$).

Growth and size at flowering

All measures of size at first flower varied among populations in both the field and glasshouse (Fig. 3, Table 2). The relative amount of variation (measured by the among-population CV) was only slightly higher in the field than in the glasshouse. As expected, all measures of size at flowering correlated negatively with latitude in both the field and glasshouse, including total 1° stem height, number of 2° stems, 1° stem width and number of internodes on the 1° stem. Total above-ground dry mass at first flower in the glasshouse also correlated negatively with latitude. Plants in the glasshouse were often smaller than those in the field (Fig. 3), but population means correlated strongly between glasshouse and field environments for all measures of size, including for 1° stem height ($r = +0.82$, $n = 22$, $P < 0.0001$), number of 2° stems ($r = +0.76$, $n = 22$, $P < 0.0001$), number of internodes on the 1° stem ($r = +0.86$, $n = 22$, $P < 0.0001$) and 1° stem width ($r = +0.62$, $n = 22$, $P = 0.0027$).

Under glasshouse conditions, latitudinal variation in measures of above-ground size at first flower was also reflected by our crude measure of below-ground growth, root index, which correlated positively with 1° stem height ($r = +0.89$, $n = 25$, $P < 0.0001$), number of 2° stems ($r = +0.85$, $n = 25$, $P < 0.0001$), 1° stem width ($r = +0.87$, $n = 25$, $P < 0.0001$), number of internodes on

Table 2 Analysis of latitudinal variation in time of flowering and components of size at flowering among introduced populations of *Lythrum salicaria* observed in either natural populations (F) or a common glasshouse environment (G).

				Variation among populations			Correlation with latitude	
	Source	*n*	Mean	Range	CV (%)	r^2	*r*	*P*
Time to flowering								
Weighted days to flower	F	22	97	81–120	13	NA	−0.62	0.0020
Days to first flower	G	25	88	78–115	9	0.36	−0.72	< 0.0001
Size at flowering								
Total 1° stem height (cm)	F	21	125	68–210	26	0.62	−0.56	0.0084
Total 1° stem height (cm)	G	25	88	62–123	18	0.63	−0.73	< 0.0001
Number of 2° stems	F	21	24	9–45	38	0.33	−0.73	0.0002
Number of 2° stems	G	25	22	10–35	34	0.49	−0.73	< 0.0001
1° stem width (mm)	F	21	4.9	3.4–6.9	18	0.43	−0.42	0.057
1° stem width (mm)	G	25	3.4	2.5–4.3	15	0.58	−0.68	0.0002
Internodes on 1° stem	F	21	21	16–35	27	0.56	−0.76	< 0.0001
Internodes on 1° stem	G	25	18	14–24	16	0.54	−0.80	< 0.0001
Total biomass (g)	G	25	7.3	2.7–14.8	49	0.58	−0.74	< 0.0001
Root index (%)	G	25	72	38–92	21	0.50	−0.65	0.0004

Days to first flower was calculated from sowing in the glasshouse and weighted days to flower was calculated from the season start in the field. We used Welch's one-way ANOVA (r^2, all $P < 0.0001$) to test for variation among populations, except for weighted days to flowering, which was calculated at the population level. *n* is the number of populations sampled. Means, ranges, CVs and correlations with latitude are based on population means. See also Figs 2 and 3.

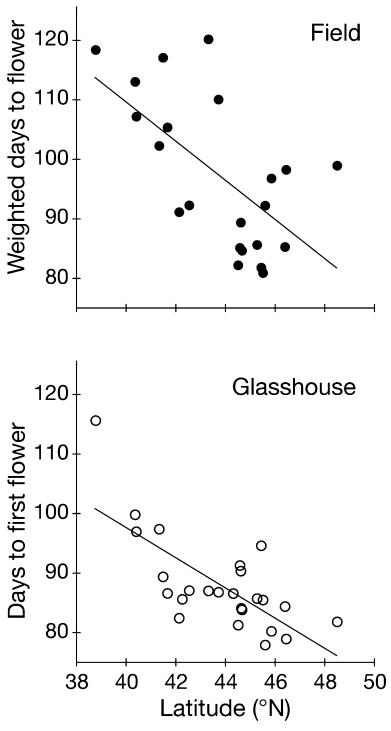

Fig. 2 Latitudinal variation in time to flowering among introduced populations of *Lythrum salicaria* spanning the species' latitudinal range in eastern North America. Weighted days to flower was calculated from the growing season start in the field (filled symbols). Days to first flower was calculated from sowing in the glasshouse (open symbols). Analysis of these data is in Table 2.

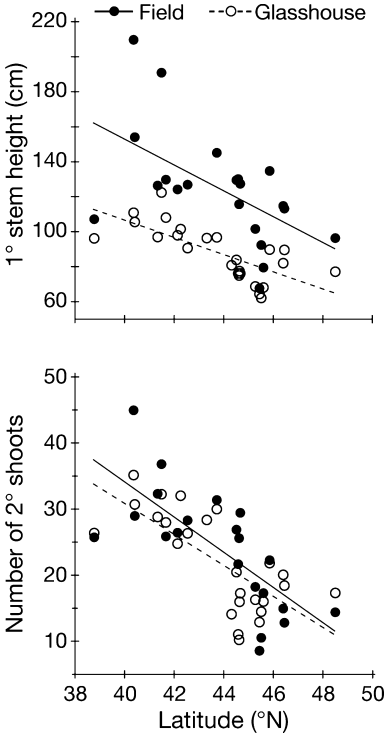

Fig. 3 Latitudinal variation in two main components of size at first flowering among introduced populations of *Lythrum salicaria* spanning the species' latitudinal range in eastern North America. Filled symbols are population means from the field survey (solid regression line). Open symbols are population means from the common glasshouse environment experiment (dashed regression line). Analysis of these data and other measures of size is in Table 2.

1° stem ($r = +0.73$, $n = 25$, $P < 0.0001$), and negatively with latitude (Table 2).

Demographic consequences of variation in flowering time

Total reproductive output of plants in the field, estimated as the number of seeds per stem, varied widely among populations, and as predicted, correlated negatively with latitude (Fig. 4, Table 3). All components of reproductive output (infructescences per stem, fruits per infructescence and seeds per fruit) also varied among populations, but only infructescences per stem correlated negatively with latitude (Table 3).

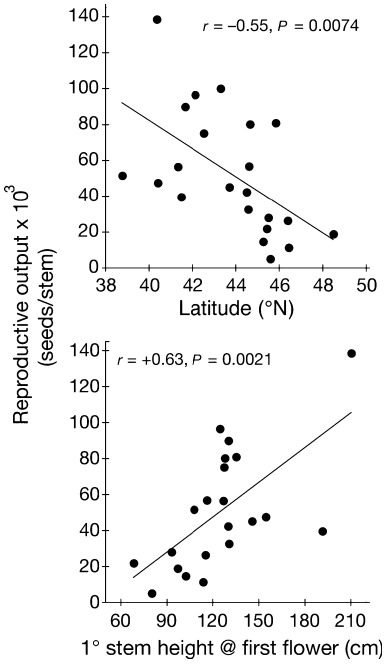

Fig. 4 Latitudinal variation in reproductive output (top panel) and covariation between size at first flower and reproductive output (bottom panel) among introduced populations of *Lythrum salicaria* spanning the species' latitudinal range in eastern North America. Pearson correlation coefficients (r) and associated P values are indicated for each relation. Analysis of these data is in Table 3.

As expected, reproductive output correlated positively with size at first flower among populations in the field (Fig. 4). Larger size at first flower also correlated positively among populations with total dry mass at the end of the reproductive season ($r = +0.81$, $n = 22$, $P < 0.0001$), which in turn correlated strongly with reproductive output ($r = +0.85$, $n = 22$, $P < 0.0001$) and negatively with latitude (Table 3). The proportional allocation of dry mass to reproductive structures, while varying among populations, did not correlate with weighted days to flower, size at first flower, final dry mass, reproductive output (all $|r| < 0.36$, $n = 22$, all $P > 0.10$) or latitude (Table 3).

The negative correlation between reproductive output and latitude is expected to arise as a demographic consequence of earlier flowering in more northerly populations mediated by size at flowering. Plant height at first flower correlated positively with weighted days to flowering among populations in the field (Fig. 5a; $r = +0.60$, $n = 22$, $P = 0.0038$). Weighted days to flower also correlated positively with reproductive output, although not quite significantly ($r = +0.41$, $n = 22$, $P = 0.061$). These correlations could arise, at least in part, from a positive genetic correlation between time to and size at flowering. However, the correlation between time to and size at flowering among populations raised under glasshouse conditions was, although positive, weak and nonsignificant (Fig. 5b; $r = +0.31$, $n = 25$, $P = 0.14$). There was considerable variation in size at flowering among populations with the same mean time to flowering. The same result was obtained using dry mass at first flower as a measure of size ($r = +0.34$, $n = 25$, $P = 0.10$). However, neither of these correlations was significantly weaker than the correlation between weighted days to flower and size at flowering among populations in the field (z-test comparing correlation coefficients: both $P > 0.2$).

Table 3 Analysis of latitudinal variation in reproductive output and its components, above-ground plant size, and reproductive allocation among 22 introduced populations of *Lythrum salicaria* measured in the field.

		Variation among populations			Correlation with latitude	
	Mean	Range	CV (%)	r^2	r	P
Seeds per stem	52 897	5285–138 700	65	0.30	–0.55	0.0074
Infructescences per stem	715	134–2299	70	0.33	–0.59	0.0037
Fruits per infructescence	3.4	2.3–4.5	20	0.38	+0.38	0.077
Seeds per fruit	68	47–84	15	0.20	–0.31	0.16
Total dry mass (g)	24.3	3.3–93.2	81	0.49	–0.55	0.0074
% dry mass in reproductive structures	24	12–40	25	0.36	+0.12	0.60

Welch's one-way ANOVA (r^2, all $P < 0.001$) tested for variation among populations. n is the number of populations sampled. Means, ranges, CVs and correlations with latitude are based on population means. See also Fig. 5.

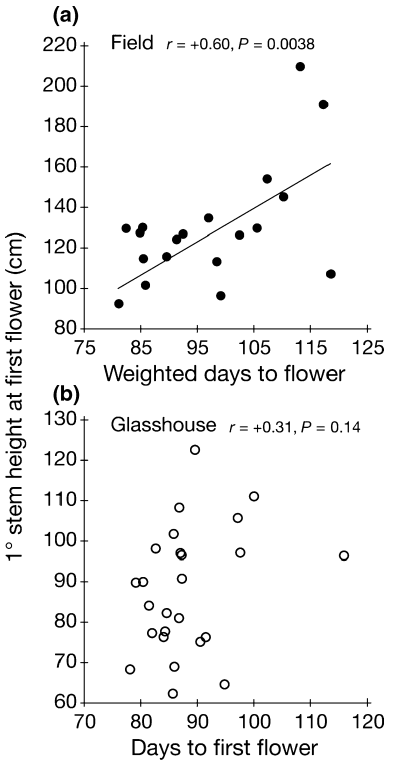

Fig. 5 Correlations between time to first flower and size at first flower among introduced populations of *Lythrum salicaria* under field conditions (a) and in a common glasshouse environment (b). Weighted days to flower was calculated from the season start in the field (filled symbols, $n = 22$ populations). Days to first flower was calculated from sowing in the glasshouse (open symbols, $n = 25$). Pearson correlation coefficients (r) and associated P values are indicated for each relation.

Discussion

Our results demonstrate a strong latitudinal cline in the seasonal initiation of flowering and size at flowering among populations of *L. salicaria* across most of its introduced range in eastern North America. Plants from higher latitudes flowered earlier in the growing season and at a smaller size than those at lower latitudes (Figs 2 and 3). We observed the same clinal patterns when populations were grown in a uniform glasshouse environment, which is consistent with a substantial genetic component to the pattern of geographic variation in the field. As predicted, we found evidence for a trade-off between time to flowering and reproductive output among populations under field conditions that appears to be largely mediated by size at flowering (Figs 5 and 6a). However, evidence that a genetic correlation between time and size at flowering contributed to this trade-off was weak (Fig. 5b). These geographic patterns are consistent with the hypothesis that introduced populations have evolved adaptive differences in phenology and life history in response to climatic selective pressures during contemporary times. We evaluate the supporting evidence and possible challenges to this hypothesis below.

Genetic versus environmental factors contributing to clinal variation

Previous studies of geographic variation in invasive species have found that clinal patterns detected in a glasshouse or common garden are sometimes not reliably expressed under field conditions (e.g. Weber & Schmid, 1998; Kollmann & Bañuelos, 2004). This was not the case in our study of *L. salicaria*. There was a remarkably close correspondence between the performance of field and glasshouse populations, despite the diversity of ecological conditions in which natural populations were growing. This consistency in performance strongly suggests that the variation in life-history traits we measured in the field has a substantial genetic basis. The pattern of geographical variation in the field could be further strengthened by phenotypically plastic responses to ecological gradients across latitude that run in the same direction as the genetic trend (co-gradient variation, Eckhart *et al.*, 2004). Little is known about the plasticity of life-history traits in *L. salicaria* (but see Mal & Lovett Doust, 2005). However, our own observations of year-to-year variation in flowering phenology indicate that latitudinal variation in climate would counteract adaptive genetic differentiation (i.e. counter-gradient variation). During relatively cool growing seasons, *L. salicaria* flowers later rather than earlier (Dech & Nosko, 2004). Hence, the putative genetic trend for earlier flowering in northern populations that we observed in the glasshouse would be dampened by plastic responses to latitudinal variation in climate.

The purpose of our common glasshouse experiment was to detect genetic differentiation among populations by removing environmental differences among them that would occur in the field. Because we used seeds collected from plants grown in the field, nongenetic maternal effects could potentially contribute to variation among progeny in the glasshouse. Such effects could result from variation in maternal growth environment, mediated through seed provisioning, or they could represent adaptive transgenerational plasticity that improves progeny fitness in maternal environments and may not involve differential provisioning (Galloway, 2005). Given the potential complexity and manifold expression of maternal effects (Rossiter, 1996), we cannot discount their influence. However, for reasons outlined below we suggest that maternal effects are unlikely to account for the strong clines we observed, and probably contributed little to the among-population differentiation observed in the glasshouse.

First, the differences in life-history traits among populations of *L. salicaria* we observed were considerable.

Time to flowering increased by 48% from north to south, a trait difference of four standard deviations (SD). Moreover, plant height and number of secondary branches increased by 100% and 240%, respectively, or > 4 SD. It is unlikely that maternal effects mediated through seed provisioning would have contributed to such large trait differences because seeds of *L. salicaria* are very small and plants were assayed under noncompetitive conditions in the glasshouse. We quantified variation in the seeds mass of families from the populations of *L. salicaria* used here. Although seed mass (mean ± SE 0.047 ± 0.0021 mg) varied among populations (range 0.030–0.063 mg, CV 21%, $r^2 = 0.29$, $F_{21,219} = 3.9$, $P < 0.0001$), it did not correlate with latitude ($r = +0.15$, $n = 22$, $P = 0.50$), or any measure of flowering time, plant size or reproductive output in the field or glasshouse (all $|r| < 0.34$, $n = 22$, all $P > 0.10$).

Second, nongenetic maternal effects are usually most pronounced in early life-history stages and are less likely to account for large trait differences that persist during later stages (Rossiter, 1996). In further experiments with *L. salicaria* (Barrett *et al.*, 2008), we grew progeny from seeds for 20 of the populations used here in a different glasshouse environment and found the same negative correlation between time to first flower and latitude ($r = -0.75$, $P < 0.0001$). We then cut back and overwintered plants from 13 of these populations and planted them the next year in a field site in southern Ontario (42.03°N latitude). Again, the latitudinal correlation in time to first flower was strong and negative ($r = -0.87$, $P < 0.0001$).

Finally, there is no theoretical expectation that adaptive maternal effects should contribute to large-scale geographic clines in plant life histories. Adaptive maternal effects are not expected when the scale of environmental variation greatly exceeds that over which gene flow would impede local adaptation (Galloway, 2005). Hence, maternal effects are unlikely to contribute to the large-scale latitudinal differentiation among the populations of *L. salicaria* we studied. Although quantitative genetic studies are required to determine the genetic basis of latitudinal variation in flowering time, our results are consistent with a genetically based cline in life history among introduced populations of *L. salicaria*. This conclusion is further supported by quantitative genetic studies demonstrating significant additive genetic variation in many life-history traits, especially flowering time, within populations of a wide variety of plant species (Mazer & Lebuhn, 1999; Geber & Griffen, 2003), including both native and introduced populations of *L. salicaria* (O'Neil, 1997; Olsson, 2004).

Evolutionary mechanisms

Clines are usually interpreted as the product of local adaptation across an ecological gradient, but they can also arise from the joint action of genetic drift and gene flow (Endler, 1977). However, latitudinal variation in life-history traits among introduced populations of *L. salicaria* closely matches the specific theoretical expectations for selection on reproductive timing across a gradient of seasonality (Griffith & Watson, 2006). Moreover, the cline among introduced populations matches the pattern of latitudinal differentiation in time to and size at flowering previously demonstrated for native populations of *L. salicaria* in two different regions of Europe (Olsson & Ågren, 2002; Bastlová *et al.*, 2004, 2006; Olsson, 2004). Parallel patterns of life-history variation in geographically disjunct clines are generally viewed as compelling evidence for adaptive evolution rather than drift (Gilchrist *et al.*, 2001). Together with the European data, our results provide some of the first evidence for the rapid re-establishment of clinal variation during biological invasion (Weber & Schmid, 1998; Huey *et al.*, 2005; Leger & Rice, 2007). Definitive proof that the latitudinal cline in flowering time is the product of natural selection will require reciprocal transplant experiments to demonstrate local adaptation combined with phenotypic selection analysis to demonstrate the latitudinal variation in sign and magnitude of selection on flowering time (e.g. Griffith & Watson, 2005).

Colonization history may also influence geographical variation in life history among introduced populations of invasive species. For example, in the most extreme case, what appears to be an adaptive latitudinal cline produced by selection during range expansion might be the result of multiple introductions of preadapted genotypes transported directly from climatically similar regions of the native range (climate matching). As discussed in the *Introduction* section, historical information on the temporal change in distribution of *L. salicaria* from herbarium specimens does not suggest marked geographic stratification of early introductions (Stuckey, 1980; Thompson *et al.*, 1987). Rather, it is likely that the species was introduced many times to the eastern seaboard of the USA through multiple vectors (e.g. ballast waste, sheep's wool and intentional planting as a medicinal herb and nectar source) and has spread from this region of introduction via ongoing transport by humans (Thompson *et al.*, 1987). Hence, evolutionary responses during range expansion were probably fuelled by admixture of genetic variation from diverse native source populations rather than new mutations occurring *in situ*. Because historical data from herbarium specimens cannot unequivocally demonstrate or refute climate matching, phylogeographic analysis would be required to evaluate the role of immigration history in the geographical differentiation of life-history traits (e.g. Kliber & Eckert, 2005). For example, genetic analysis indicated that, although the colonization of North America by *Hypericum perforatum* probably involved multiple introductions from Europe, latitudinal clines in size and fecundity among introduced populations were likely to have arisen via

local adaptation during range expansion, not climate matching (Maron *et al.*, 2004).

Demographic and genetic trade-offs associated with variation in flowering time

We detected significant latitudinal variation in reproductive output, with stems from the most northern populations producing about one-tenth of the seed per season as those in the most southern populations. This was a result of lower fruit production in northern populations rather than fewer seeds per fruit, indicating that lower reproductive output at higher latitudes is probably not a consequence of infrequent pollinator service, which is known to cause variation in seed set among native populations of *L. salicaria* at the northern limit of its range (Waites & Ågren, 2004).

Our results also suggest that geographical variation in reproductive output was caused by corresponding variation in size at first flower, which in turn correlated positively with time to flowering. Based on life-history theory (Reznick *et al.*, 2000; Roff, 2000), we expected that much of the geographic variation in reproductive output among natural populations of *L. salicaria* would result from a genetic trade-off between time to flowering and size at flowering. We found that among populations of *L. salicaria* under field conditions, days to flowering correlated positively with size at flowering and that both traits correlated positively with reproductive output. Partial correlation analysis (not shown) further indicated that, as expected, seed production correlated positively with size after controlling for time to flowering time ($r = +0.58$), whereas seed production did not correlate with time to flowering after controlling for size ($r = -0.09$). However, our results do not support the hypothesis that these associations are the result of a strong positive genetic correlation between time to flowering and size. Although the correlation between these traits in a uniform glasshouse was positive, as expected, it was weak and not significant.

Environmental variation across latitude seems likely to have played a major role in generating the strong correlation between time to and size at flowering observed in the field compared with the glasshouse. This is plausible because the environment during the period leading up to flowering is probably less conducive to growth (e.g. colder) in more northerly populations. Even if plants required the same amount of time to flower from the onset of the growing season, they would probably flower at a larger size in southern populations compared with those in the north. This explanation is supported by preliminary data from a reciprocal transplant of *L. salicaria* populations across latitude in eastern North America (R. I. Colautti, C. G. Eckert, S. C. H. Barrett, unpublished data).

Formal quantitative genetic analysis is required to estimate the genetic correlation between time to and size at flowering in *L. salicaria*. However, it is worth noting that O'Neil (1997) failed to detect a phenotypic or genetic correlation between time to flowering and plant height among 60 full-sib families within an introduced North American population of *L. salicaria*. Similarly, an analysis of paternal half-sib families failed to reveal the expected genetic correlation between time to flowering and plant size within two native populations of *L. salicaria*, even though additive genetic variation was detected for both traits (Olsson, 2004). Our preliminary analysis of genetic correlations among populations, along with these studies examining correlations within populations, raise the possibility that the evolution of flowering phenology in populations of *L. salicaria* might not be greatly constrained by a genetic trade-off with size at flowering and, ultimately, fecundity.

The strong correlations between latitude, time to flowering and reproductive output among populations in the field should be reflected in geographic variation in population growth and regional spread. However, evidence that the demographic cost of early flowering influences the invasion potential of *L. salicaria* was mixed. In populations of long-lived perennial plants that have reached demographic equilibrium, variation in seed production may have little effect on population growth rate (Ehrlén & Eriksson, 1995). However, when populations are rapidly expanding, as during biological invasion, seed production might more strongly influence population dynamics (Eckert *et al.*, 1996). However, we found no evidence of this. Per-stem seed production, which varied more than 10-fold among populations across latitude, did not correlate with log-transformed population area ($r = -0.04$, $n = 22$, $P = 0.85$), stem density ($r = -0.31$, $n = 22$, $P = 0.16$) or stem number ($r = -0.14$, $n = 22$, $P = 0.52$). Accordingly, these measures of population size did not covary with latitude, except for stem density, which correlated positively rather than negatively with latitude. Demographic studies have also suggested that the growth rate of introduced populations of *L. salicaria* is more sensitive to variation in adult survival than seed production (C. A. Lacroix and B. C. Husband, unpublished data).

Marked latitudinal variation in seed output may also influence the rate of spread at a regional scale. This is because in many plant species seed is particularly important in the establishment of new populations. Such may be the case in *L. salicaria*, as the regional distribution of *L. salicaria* in North America appears to be largely limited by seed dispersal (Yakimowski *et al.*, 2005). Based on our results, we predict that the southern limit of the range of *L. salicaria* will expand more quickly than the northern limit because of much higher seed production of southern populations. Our preliminary analysis of herbarium records combined with field observations and reports from regional biologists support this prediction. Efforts to limit the geographical spread of *L. salicaria* in eastern North America might usefully focus on reducing

the production and dispersal of seed from populations along the current southern range limit.

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