Latitudinal clines in floral display associated with adaptive evolution during a biological invasion

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Running head: Floral display clines in *Lythrum salicaria*

ABSTRACT

Premise: Flowering phenology strongly influences reproductive success in plants. Days to first flower is easy to quantify and widely used to characterize phenology, but reproductive fitness depends on the full schedule of flower production over time.

Methods: We examined floral display traits associated with rapid adaptive evolution and range expansion among thirteen populations of *Lythrum salicaria*, sampled along a 10-degree latitudinal gradient in eastern North America. Growing these collections in a common garden field experiment at a mid-latitude site, we quantified flowering schedule variation using Principal Coordinates Analysis (PCoA) and quantitative metrics analogous to central moments of probability distributions (i.e., mean, variance, skew, and kurtosis).

Key Results: Consistent with earlier evidence for adaptation to shorter growing seasons, we found that populations from higher latitudes had earlier start and mean flowering day, on average, when compared to populations from southern latitudes. Clines in flowering skew increased with latitude whereas kurtosis decreased, consistent with a bet-hedging strategy in biotic environments with more herbivores and greater competition for pollinators.

Conclusions: Heritable genetic clines in flowering schedules are consistent with biotic and abiotic responses to natural selection, potentially contributing to rapid evolution and range expansion of this invasive species.

INTRODUCTION

Phenology affects how organisms interact with their environment, with important consequences for their survival and reproduction. As targets of natural selection and determinants of population vital rates, the timing of growth, reproduction, and other phenological traits are crucial components of eco-evolutionary dynamics. Phenology governs interaction with mutualists (Fenster et al., 2004), antagonists (Strauss & Whittall, 2006) and abiotic factors (Jin *et al.*, 2019) that can vary over space and time. Consequently, plants experience spatial and temporal variation in natural selection on flowering phenology (Kudo, 2007), sometimes resulting in the evolution of local adaptation (Anderson et al., 2012; Colautti & Barrett, 2013; Preite et al., 2015). As such, flowering phenology is a useful composite trait for investigating evolutionary responses to past and future environmental challenges (Fitchett et al., 2015; D. W. Inouye, 2022; Panchen & Gorelick, 2017; Prather et al., 2023).

Days to first flower (i.e., flowering time) is a simple yet informative metric with which to study the ecology and evolution of phenology in plant populations. For example, latitudinal and altitudinal clines in days to first flower are commonly reported in a variety of plant taxa (Alexander et al., 2009; Colautti & Lau, 2015; Ensing & Eckert, 2019; Halbritter et al., 2018; Olsson & Ågren, 2002; Panchen, 2022), and reciprocal transplant experiments have confirmed that these clines are an important component of local adaptation (Anderson et al., 2012; Colautti & Barrett, 2013; Griffith & Watson, 2005). Flowering time clines are consistent with local adaptation models characterized by stabilizing selection within populations but a shift in optimal flowering time along environmental gradients (de Villemereuil et al., 2020; Gauzere et al., 2020; Kirkpatrick & Barton, 1997).

In contrast to the prediction of stabilizing selection, empirical measurements of selection

differentials suggest that flowering time is typically under directional selection within populations (Munguía-Rosas et al., 2011). Despite evidence for consistent directional selection, long-term phenology records show a variety of responses to climate change -- from earlier to later to no change in flowering time, depending on the species (Rafferty et al., 2020; Wolkovich et al., 2012). Thus, models and empirical observations of geographic clines and local adaptation appear to disagree with observed measurements of natural selection and variable phenotypic shifts in natural populations (Austen et al., 2017). Antagonistic selection on unmeasured traits correlated with the onset of flowering could help to resolve this apparent contradiction.

Day of first flowering is but one aspect of the "flowering schedule" of individual plants -- a time-series of flower production that characterizes reproductive opportunity (CaraDonna et al., 2014; Fox, 2003; B. D. Inouye et al., 2019; Newstrom et al., 1994). While rarely examined in studies of flowering phenology evolution, different characteristics of the flowering schedule can have important effects on reproductive fitness (Forrest & Thomson, 2010; Fox, 2003). In addition to flowering time, the shape of the flowering schedule can be quantified using ordination methods like Principal Coordinates Analysis (PCoA; Austen et al., 2014) and metrics analogous to central moments of probability distributions including the mean, variance, skew, and kurtosis (Box 1).

Not to be confused with the central moments of a statistical population or sample, the analogous characteristics of a flowering schedule are not probabilistic, but rather quantify temporal variation in relative reproductive investment through time. These schedule characteristics use the same mathematical equations as probability moments (Table 1) but are not shaped by probabilistic process and therefore may not necessarily correlate with onset or duration of flowering (Fig. 1). These metrics have rarely been used in studies of flowering

phenology, yet they have the potential to offer a novel perspective on selection and trade-offs affecting the evolution of phenology.

Central moments of individual flowering schedules describe the timing and intensity of reproductive opportunity, and thus are likely to experience strong selection from abiotic or biotic features of the environment. We are not aware of studies quantifying evolutionary divergence of entire flowering schedules among naturally occurring populations, but there are reasons to expect populations to evolve differences. For example, individuals that experience competition for pollinators may benefit from showy inflorescences that enhance pollinator attraction (O'Neil, 1997; Thomson, 1980), which would produce a flowering schedule with a more positive skew and longer duration. Herbivores and seed predators often select for delayed peak reproduction (Biere & Antonovics, 1996; Elzinga et al., 2007; Juenger & Bergelson, 1998; Wright & Meagher, 2003) but may also favour a bet-hedging strategy such that a long flowering duration, high flowering schedule variance, and positive kurtosis would be advantageous in the presence of intense herbivory.

In addition to biotic interactions, flowering schedules are likely to be shaped by abiotic factors that determine the timing and duration of environmental conditions favorable to the development of reproductive organs. Shorter growing seasons typically favour a more rapid flowering strategy (Austen & Weis, 2015; Colautti & Barrett, 2013; Griffith & Watson, 2005) characterized by an earlier onset of flowering, with a shorter duration and smaller variance of the flowering schedule. In marginal environments with less predictable growing conditions, it may be advantageous for plants to produce a few flowers over a longer timeframe as a bet-hedging strategy (Forrest & Thomson, 2010; Simons, 2011; Tufto, 2015), resulting in a long-tailed leptokurtic or skewed flowering schedule.

Environmental gradients associated with latitude can be useful for studying phenotypic evolution if natural selection covaries, for example with shorter growing seasons and weaker biotic interactions towards polar latitudes (reviewed in Zvereva & Kozlov, 2021). Adaptation along environmental gradients is commonly manifested as clines in phenotypic traits that are observable when individuals are grown in a common garden environment (Langlet, 1971; Schwinning et al., 2022). For example, adaptive flowering time clines evolved rapidly in the perennial wetland herb *Lythrum salicaria* (purple loosestrife) over a century of invasion across North America. Selection splines (Colautti & Barrett, 2010), trait correlations (Colautti & Barrett, 2011), reciprocal transplants (Colautti & Barrett, 2013), and herbarium records (Wu & Colautti, 2022) collectively support a model of selection in which shorter growing seasons toward the northern range limit favour earlier flowering. In contrast, longer growing seasons in the southern part of the introduced range relax selection on flowering time and favor plants that flower later but grow larger in stature. However, by focusing on the first day of flowering, previous studies of *L. salicaria* overlooked other characteristics of the flowering schedule, which may be important for understanding adaptation and constraint during its evolution and invasion in eastern North America.

Here, we investigate clines in flowering schedules among thirteen populations of *L. salicaria* sampled across ten degrees of latitude in eastern North America and grown in a common garden field study at the Koffler Scientific Reserve north of Toronto, Ontario, Canada. We use PCoA and central moments characteristics of individual flowering schedules to address the following questions: (1) Are common phenological metrics, like the onset and duration of flowering, associated with overlooked features of the flowering schedule such as the mean, variance, skew and kurtosis? (2) Are there latitudinal clines in flowering schedules that are

136 consistent with the adaptive hypotheses outlined above? Specifically, we predicted that the
137 longer growing season length and more intense biotic interactions at southern latitudes should
138 create stronger competition for pollinators and higher rates of herbivory in southern populations.
139 These conditions seem likely to favour genotypes with: (i) a longer flowering duration and (ii) a
140 later start and mean flowering day, but also (iii) an increase in flowering schedule variance (iv) a
141 more negative skew, with flowering focused later in the schedule and (v) a more positive
142 kurtosis, with a concentrated peak and rare flowering events far from the mean flowering day
143 (see Fig. 2). In line with these predicted adaptive changes, we expect the primary PCoA axes to
144 reflect population differentiation in flowering schedules based on latitude of origin.
145

146 **METHODS**

147 ***Experimental design --***

148 Details of the field experiment are provided in Colautti & Barrett (2010) and the seeds used were
149 collected along a 10-degree latitudinal gradient in eastern North America, as described in
150 Montague *et al.* (2008). Briefly, populations were chosen to represent a latitudinal gradient from
151 Timmins, Ontario (48.48 ºN) to Easton, Maryland (38.75 ºN) and were surveyed during the
152 summer of 2003 (see Appendix S1 for a list of locations). When 90% of plants had finished
153 flowering in the fall, 20-41 stems were randomly harvested from each population. Eight seeds
154 from each of 20 seed families from each of 13 populations were grown at the Koffler Scientific
155 Reserve at Jokers Hill, located approximately at the geographic center of the latitudinal gradient
156 (44.03 ºN). Early in the growing season (early May), plants were sprayed with insecticide to
157 reduce incidence of herbivorous insects. This was sufficient to inhibit colonization of biocontrol
158 beetles in the area, without impacting pollinator activity later in the season when plants became

159 reproductive (July through September). The primary purpose of this experiment was to
160 characterize flowering time clines and genetic trade-offs in a multi-year (2006-2008) common
161 garden study, as reported in Colautti & Barrett (2010, 2011). In 2007 we additionally recorded
162 all open flowers on every stem of a subset of 369 individuals of 216 seed families, evenly
163 represented across the 13 populations (Appendix S1). Our daily census records of this subset of
164 plants occurred over 125 days on a rotating schedule such that each individual was surveyed
165 every five days from July 1 until September 30. A 5-day cycle was chosen because stigma
166 receptivity is nearly zero after 24 h of anthesis (Waites & Ågren, 2006) and flowers are fully
167 senescent after 5 days. Additionally, unpollinated flowers begin to senesce and abscise while
168 pollinated flowers begin to close within 48 h of anthesis, limiting the risk of counting the same
169 flowers on multiple census dates. All calculations and statistical analyses were conducted in R
170 version 4.2.2 (R Core Team, 2022) in R Studio (Posit team, 2024), and are available with the
171 original data from the Dryad repository (https://doi.org/10.5061/dryad.jdfn2z3jz).
172
173 ***Characterizing flowering schedules --***
174 We characterized the flowering schedules of each individual plant using the equations shown in
175 Table 1, where the day that an open flower was first observed in the experiment is $t = 0$. The
176 number of flowers produced by plants in each population over a growing season is variable in
177 this experiment because stabilizing selection a our mid-latitude field site favours individuals that
178 balance flowering time with growth to produce more flowers relative to small, early flowering
179 phenotypes more characteristic of northern populations or large, late-flowering phenotypes
180 characteristic of more southern populations. Including flower number as a covariate in our
181 models would assume linearity between the number of flowers produced with the schedule

182 variance, skew, and kurtosis. Therefore, we instead standardized the number of observed flowers
183 for each individual on each census day as a proportion of their total number of open flowers
184 censused over the experiment. We then calculated a weighted census day by multiplying each
185 day by the proportion of flowers open on that day. The proportion of flowers and the weighted
186 census days were summed to calculate the weighted mean and other characteristics of individual
187 flowering schedules analogous to central moments (variance, skew and kurtosis) (Box 1; Table
188 1). Specific calculations are provided as custom R functions in the Dryad repository
189 (https://doi.org/10.5061/dryad.jdfn2z3jz).

190 In addition to calculating the characteristics of every individual's flowering schedule, we
191 wrote a bootstrap model in R to compute a bootstrapped mean and 95% confidence intervals
192 across 999 iterations for each population, and a similar bootstrap model to test for latitudinal
193 clines. In each iteration, individuals were hierarchically sampled with replacement from within
194 each population, and then the equations in Table 1 were used to calculate population averages.
195 The population averages of each iteration were also used to generate 999 estimates of intercept,
196 linear slope and quadratic regression coefficients to test for latitudinal clines. In this way we
197 generated bootstrap means and 95% confidence intervals for each population and for the
198 estimates of latitudinal clines. More details of the bootstrap models are available in R code from
199 the Dryad repository (https://doi.org/10.5061/dryad.jdfn2z3jz).

200 In addition to the start of flowering, duration, and central moments of flowering
201 schedules, we also used a Principle Coordinates Analysis (PCoA) to compare flowering schedule
202 shape among individuals, as described by Austen et al., (2014). Briefly, we computed a
203 dissimilarity matrix from raw flower counts using Kolmogorov-Smirnov (KS) distances (Sokal
204 & Rohlf, 1995) where each matrix cell was a KS statistic measuring the maximum distance

205 between two flowering schedules. We chose KS distance because it is robust to differences in
206 flower number and census schedules. However, the KS statistic is sensitive to differences in
207 scale, so flowering durations were standardized to run from 0 to 1 by subtracting the shortest
208 duration time from each individual and dividing by the difference of the longest and shortest
209 durations (Austen et al., 2014). We used the KS matrix as the input for the **cmdscale** function to
210 generate the PCoA axes. Since KS distances do not meet the triangle inequality condition of
211 PCoA, any variation that is unable to be converted to Euclidean distance is represented as
212 negative PCoA axes, which may lead to overestimation of variation explained by the first axes
213 (Austen et al., 2014; Legendre & Anderson, 1999; Podani & Miklós, 2002). Therefore, we
214 adjusted our estimates of proportion of variation explained to only include positive eigenvalues
215 (Austen et al., 2014). We then generated vectors to plot each flowering schedule trait in
216 multivariate space by computing the standardized covariance of the central moments with all
217 PCoA axes, filtered for positive eigenvalues (Legendre et al., 1998). It is important to note that
218 the PCoA axes characterize variation in the shape of flowering schedules among individuals but
219 not variation in onset and duration of flowering. However, projecting flowering characteristics
220 onto the first two PCoA axes provides a visualization of how different flowering schedule
221 characteristics relate to the major axes of variation in schedule shape. Additionally, we visualize
222 each individual, colour-coded by latitude, to see how schedule shape varies among and within
223 populations, and along a latitudinal gradient.
224
225 ***Correlations of flowering schedule characteristics --***
226 Our first research question addresses whether conventional metrics (i.e., onset and duration of
227 flowering) correspond to more detailed characteristics of the flowering schedule (i.e., mean,

228 variance, skew, kurtosis, and PCoA axes). To investigate these relations, we calculated pairwise
229 Pearson correlations to compare two scales of analysis, namely (A) average schedules of each
230 population (n = 13), and (B) the deviation of each individual from its population average (n =
231 369). In addition to comparing conventional metrics with other flowering schedule
232 characteristics, the correlational structure of these matrices can also be instructive for testing
233 evolutionary hypotheses. For example, the evolutionary divergence of flowering schedules along
234 biotic and abiotic gradients should produce stronger correlations among population when
235 compared to correlations among individuals within populations.
236
237 **RESULTS**
238 ***Flowering schedule characteristics --***
239 Pearson product-moment correlations revealed significant relations among commonly measured
240 traits of the flowering schedule (i.e., start and duration) and more detailed characteristics (i.e.,
241 mean, variance, skew, and kurtosis), both among and within populations (Fig. 3). There was no
242 significant correlation between days to flower and duration or flowering variance among
243 population average flowering schedules. However, there was a significant negative correlation
244 for start date with skew and a positive correlation with kurtosis, indicating that populations with
245 a later start date, on average, tended to have individuals with flowering clustered at later dates
246 (Fig. 3A).
247 For individual flowering schedules, residual within-population correlations were
248 generally weaker when compared to among-population correlations (Fig. 3B). However, there
249 were negative correlations between days to flower and duration and variance of flowing among
250 individuals within populations (Fig. 3B). These correlations were notably weaker and not

251 statistically significant among population means (Fig. 3A). Skew and kurtosis were strongly
252 negatively correlated both among (Fig. 3A) and within populations (Fig. 3B).
253
254 ***Principal Coordinates Analysis --***
255 In addition to the above characteristics, we used Principal Coordinates Analysis (PCoA) to
256 characterize similarities and differences among flowering schedules after controlling for
257 variation in start date and duration. The PCoA of all 369 individuals resulted in 369 independent
258 eigenvectors, of which 192 were positive. After adjusting the proportion of variation to include
259 only positive eigenvalues, only the first two of 192 PCoA axes had eigenvectors that explained
260 more than 10% of the variation in flowing schedule shape, and together they explained 35% of
261 the variation (Fig. 4). By comparison, the next ten eigenvectors combined explained another 35%
262 of variation, with the remaining 180 positive eigenvectors accounting for 30% of the variation.
263 Thus, we focus here on interpreting the first two eigenvectors. The first axis (PCoA1) was
264 negatively correlated with mean days to flower, after controlling for variation in start and
265 duration of flowering (Fig. 4). In contrast, higher values of PCoA2 were primarily associated
266 with higher flowering variance and lower kurtosis in flowering schedules. Despite standardizing
267 individual flowering schedules to have the same onset and duration of flowering prior to PCoA,
268 we found that the start and duration of flowering remained correlated with PCoA1 and PCoA2,
269 indicating that central moments are correlated with other, unmeasured shape characteristics.
270
271 ***Latitudinal clines --***
272 The significant correlations that we detected among population average flowering schedule traits
273 in our central moments and PCoA analyses also covaried with latitude. For example, the start,

duration, mean, and kurtosis of flowering schedules were each significantly negatively correlated with latitude (Fig. 5). This means that on average, individuals from southern populations start flowering later, have later mean flowering days, and more 'outlier' flowers opening far from the mean flowering date, when compared to northern populations. Additionally, average skew was negative for all populations, but more negative at lower latitudes, whereas kurtosis was close to zero but slightly positive in southern populations (Fig. 5). In other words, individuals from southern populations tended to produce more flowers far from peak flowering whereas flowering schedules in individuals from northern populations are more symmetric and concentrated (Fig. 5), even though there were no significant latitudinal clines in schedule variance.

DISCUSSION

There is growing evidence for rapid evolution in invasive species (reviewed in Barrett et al. 2017), but it remains uncertain how often adaptive evolution contributes to their spread. Previous studies focusing on the onset of flowering provided only a partial view of how plants allocate flowering resources (Box 1), leaving uncertainty about the extent to which flowering phenology is adaptive. To address this knowledge gap, we used a common garden study to test for latitudinal clines in complete flowering schedules in the wetland invader *Lythrum salicaria*. We found that the onset of flowering was significantly correlated with other aspects of the flowering schedule including among-population averages (Fig. 2A), and among individuals within populations (Fig. 2B). Furthermore, the significant among-population correlations were largely consistent with the evolution of correlated traits along latitudinal clines (Fig. 5). Compared to northern genotypes, plants from lower latitudes flowered later, for a longer duration, and with a more negative skew and leptokurtic shape (Fig. 5). Below we discuss the implications of these

297 results for understanding the evolutionary causes and ecological consequences of flowering
298 schedule diversity.
299
300
301 ***Onset and duration of flowering --***
302 Date of first flower and duration of flowering are relatively easy to measure and are commonly
303 reported as focal traits in phenological studies. However, the reproductive fitness of individuals
304 depends on the full schedule of flower production, and it has not been clear how well the onset or
305 duration of flowering represents other flowering schedule characteristics. In our study, the use of
306 PCoA revealed substantial variation in flowering schedules even after standardizing for the onset
307 and duration of flowering (Fig. 4). However, correlations of both PCoA1 and PCoA2 with onset
308 and duration (Fig. 4) indicated that this multi-dimensional variation in schedule appears to
309 covary with commonly measured parameters. In line with this conclusion, the onset of flowering
310 was strongly correlated with the mean flowering day, both among population averages (Fig. 3A)
311 and among individuals within populations (Fig. 3B). Therefore, the onset of flowering appears to
312 be a good predictor of peak flowering in this study system.
313 Within populations, one might predict a trade-off between the onset and duration of
314 flowering. Instead, we found that early flowering plants also flowered for a longer duration with
315 a higher flowering variance (Fig. 3B). Although counterintuitive, this result is consistent with
316 studies of other species that have reported that early flowering plants also flower for a longer
317 duration (Austen et al., 2017; Hendry & Day, 2005). Variation in microhabitat quality (e.g.,
318 nutrient flux) and/or segregation of deleterious alleles within populations (e.g., inbreeding
319 depression) could account for this correlation, with early/longer flowering plants representing

320 microenvironments or genotypes of higher quality. Yet, without evidence for trade-offs it is
321 difficult to explain how latitudinal clines in flowering time could evolve.
322 In contrast to the highly significant relations observed between onset, duration, and
323 variance of flowering within populations (Fig. 3B), the same correlations were non-significant
324 when calculated among population averages (Fig. 3A). Instead, duration and variance were not
325 significantly correlated with latitude because populations originating closer to the common
326 garden latitude site tended to have the longest duration and highest variance. This result is
327 consistent with previously proposed models of constrained adaptation in this species (Colautti,
328 Eckert, et al., 2010; Colautti & Barrett, 2011, 2013; Wu & Colautti, 2022). Specifically,
329 populations that are locally adapted to the latitude of the field experiment balance a trade-off
330 between early flowering and larger growth to maximize the flowering duration and variance. In
331 contrast, populations from more distant latitudes have lower flowering duration and variance.
332 Early flowering northern plants are smaller and run out of resources for flower production earlier
333 in the season while larger plants from the south have their flowering schedules truncated by the
334 end of the growing season. Future experiments replicating common gardens at higher and lower
335 latitudes would be necessary to test for the predicted shift from a positive cline in duration and
336 variance at southern sites to a negative cline at higher latitudes with shorter growing seasons. In
337 addition to season length, the presence of biological control beetles are likely to alter flowering
338 schedules and may have different effects on northern vs southern population, which could be
339 evaluated with sprayed and unsprayed treatments in a replicated common garden.
340

341 ***Latitudinal clines --***

342 Latitudinal clines in the onset of flowering have evolved independently in several unrelated
343 angiosperm species across North America (McGoey et al., 2020; Montague et al., 2008; Samis et
344 al., 2012; Wu & Colautti, 2022). In our study of *L. salicaria*, we additionally found latitudinal
345 clines in average mean, duration, skew and kurtosis of flowering schedules (Fig. 5). This
346 latitudinal differentiation is also reflected in Figure 4, where northern populations cluster at
347 intermediate values of PCoA1 and PCoA2 compared to mid-latitude (higher PCoA1 but lower
348 PCoA2) and southern populations (lower PCoA1 and lower PCoA2). These types of clines
349 indicate that individuals from southern environments have floral displays that peak later in the
350 season but produce a few open flowers far from the mean flowering date. These findings
351 correspond to predictions based on a bet-hedging strategy with a higher intensity of biotic
352 interactions predicted in southern environments. Previous meta-analyses have found increasing
353 biotic interactions at lower latitudes (Zvereva & Kozlov, 2021) and towards warmer range limits
354 (Paquette & Hargreaves, 2021), but we are not aware of prior studies showing evolved clines in
355 flowering schedules that correspond to clines in biotic interactions.

356 *Lythrum salicaria* is a self-incompatible outcrosser (Colautti et al., 2010; Darwin, 1877;
357 O'Neil, 1997) and individuals likely experience inter-specific competition for pollinators (King
358 & Sargent, 2012; Groulx & Sargent, 2018), which may be more intense in southern populations.
359 Additionally, several effective biological control insects have been released into eastern North
360 America, including *Gallerucella* beetles that feed on meristem tissue as larvae and can
361 drastically reduce flower production (Blossey & Notzold, 1995; Grevstad, 2006; Russell-Mercier
362 & Sargent, 2015). One of the factors along with climatic adaptation that may contribute to the

later flowering time and more negative skew in plants from southern populations is the possibility of selection from insect herbivores that intensifies at mid-growing season.

Higher values of kurtosis are consistent with the hypothesis that a strategy of producing a few flowers over a long period of time while also optimizing for an intense flowering peak to compete for pollinators occurs in southern populations. We cannot easily parse the specific effects of biotic and abiotic factors based on flowering clines alone, but we can make the prediction that clines in herbivore and pollinator activity are likely to be more patchy and less predictable by latitude compared to season length. It is therefore interesting that we detected stronger clines in average start and mean flowering dates (Fig. 5), which we predicted would be more sensitive to season length, in contrast to kurtosis, which we predicted as a bet-hedging strategy associated with biotic interactions. Skew showed an intermediate pattern (Fig. 5) and could be an adaptation to biotic selection. However, it is also possible that the pattern of skew may partly be an artifact of our mid-latitude common garden site. Specifically, a lack of pollinator activity and cooler growing conditions towards the end of the growing season could act as cues for plants to stop flowering. This would effectively truncate the flowering schedules of late-flowering plants, creating a more negative skew. Indeed, this could explain the negative correlation between days to flower and skew among population means (Fig. 3A).

Conclusions --

Our study demonstrated that populations of *L. salicaria* in eastern North America have evolved differences in flowering schedules within a century of invasion. This phenological differentiation among populations includes predictable changes in the timing of resource allocation to flower development and floral display and adds to the growing list of phenotypic traits that are targets of

selection as invasive species spread along environmental gradients (MacDougall et al., 2006; McGoey et al., 2020; Molina-Montenegro et al., 2018; Urbanski et al., 2012). The ecological and evolutionary insights drawn from observations of flowering start dates should be interpreted cautiously. Instead, more detailed features of individual flowering schedules can be characterised by metrics drawn from central moments theory (Table 1). As we have shown for *L. salicaria* from eastern North America, these more refined characteristics of individual flowering schedules help to evaluate adaptive hypotheses for flowering phenology to develop a deeper understanding of the diverse ways that species respond to changing environments.

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AUTHOR CONTRIBUTIONS

M.N.A. led the data analysis with assistance from D.R.M., S.C.H.B., and R.I.C. The conceptualization and experimental design were developed by S.C.H.B. and R.I.C., and all authors contributed to the manuscript, based on an original draft written by M.N.A.

DATA AVAILABILITY STATEMENT

Raw data and fully reproducible R code for all figures, tables, and statistical analyses are available in the Dryad database (https://doi.org/10.5061/dryad.jdfn2z3jz)

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410

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Box 1: Summary characteristics of flowering schedules

Central moments (i.e., mean, variance, skew, and kurtosis) arise from probability theory and characterize distributions that can be visualized as histograms with observed values on the x-axis and probability density, frequency, or number of observations on the y-axis. Here, we use the same equations to describe analogous characteristics of flowering schedules that arise from developmental rather than probabilistic processes. In contrast to probability histograms, flowering schedules can be visualized as a time series with number or proportion of flowers on the y-axis. Although the underlying processes are distinct, the equations are the same (Table 1). To understand the biological significance of mean, variance, skew and kurtosis, we contrast flowering schedules of different shape (Fig. 1). Standardizing to proportion of open flowers (P_t) over time (t), rather than total flower number (N_t) accounts for variation in total flower number per plant. Moreover, we can use proportions to calculate a weighted mean day of flowering. By analogy to the mean of a probability distribution, the weighted mean of a flowering schedule represents the 'balance point', which better captures the start of anthesis of a typical flower.

As described in Table 1 and visualized in Figure 1, central moments capture biologically meaningful variation in flowering schedules that may not be correlated with onset or duration of flowering. For example, the variance parameter (σ_i^2) describes how much flowering is spread out, even if the start and end dates are the same. An individual's coefficient of skew (CS_i) accounts for expected differences in symmetry and thus describes whether flowering is concentrated earlier (i.e., right skew, $CS_i > 0$ as shown by the blue dashed line) or later in the schedule (i.e., left skew, $CS_i < 0$ as shown by the purple dotted line), relative to the mean flowering date. Finally, the coefficient of kurtosis (CK_i) describes deviations from the Gaussian expectation, contrasting individuals with a concentrated cluster of open flowers and a few flowers over a longer timeframe (i.e., leptokurtic distribution, $CK_i > 0$ as shown in the gold dashed line) to individuals that spread out flowering more evenly over the entire schedule (i.e., platykurtic, $CK_i < 0$ as shown by the dashed green line).

Table 1. Equations of average flowering schedule characteristics for a population, with subscripts *i* for individual and *t* for census time point.

Summary Statistic	Calculation
Proportion (P) of flowers (F) produced at time t	$P_{i,t} = \frac{F_{i,t}}{\sum_t F_{i,t}}$
Day of first flower	$\alpha_i = \min\{t \mid F_{i,t} > 0\}$
Day of last flower	$\Omega_i = \max\{t \mid F_{i,t} > 0\}$
Duration of flowering	$\Omega_i - \alpha_i$
Day of mean flowering	$\mu_i = \sum_t t\, P_{i,t}$
Flowering schedule variance	$\sigma_i^2 = \sum_t (t-\mu)^2\, P_t$
Flowering schedule coefficient of skew	$CS_i = \frac{\sum_t (t-\mu_i)^3\, P_{i,t}}{\sigma_i^3}$
Flowering schedule coefficient of kurtosis	$CK_i = \frac{\sum_t (t-\mu_i)^4\, P_{i,t}}{\sigma_i^4} - 3$

FIGURE LEGENDS

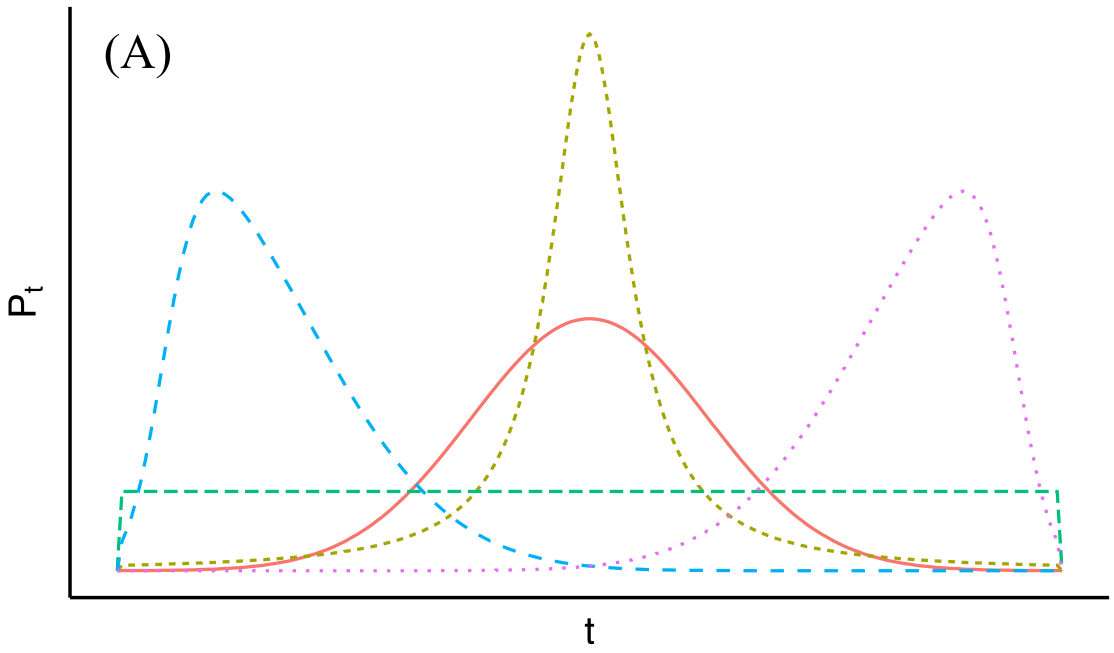
Figure 1. Hypothetical flowering schedules of five individuals with different characteristics, as described in Box 1. Flowering schedules are scaled to the proportion of total flower production per plant (P_t) over time (t).

Figure 2. Three hypothetical flowering schedules demonstrating predicted clines in central moments from northern (blue dotted line) to southern (red dashed line) genotypes, relative to a standard normal schedule at mid-latitude (solid pink line). Each schedule is scaled as the proportion of total flowers (P_t) over time (t). The northern schedule is defined by an earlier mean, lower variance and positive skew. The southern schedule is defined by a later mean, higher variance, negative skew, and higher kurtosis.

Figure 3. Matrices showing pairwise Pearson correlation coefficients among the start, duration, central moments (i.e., variance, skew and kurtosis), using either: (A) flowering schedule characteristics averaged across individuals within each population (n = 13), or (B) the residual correlations calculated on deviations of individuals from their population means (n = 369). Circles are shown for statistically significant correlations ($p < 0.05$), colour-coded by correlation coefficient (i.e., -1 to 1 as shown on the x-axis).

Figure 4. Principal Coordinates Analysis (PCoA) of Kolmogorov-Smirnov distances between flowering schedules of 369 plants from 13 populations sampled along a latitudinal gradient and grown in a common garden at the Koffler Scientific Reserve in Newmarket, ON. Point colours correspond to latitude of origin ranging from dark (48° N) to light (38° N). Bar plots show representative flowering schedules of four individual plants. Vector directions correlate to loadings on PCoA1 and PCoA2.

Figure 5. Bivariate plots showing bootstrap means and statistical relations between latitude and average flowering schedule characteristics of 13 populations of *Lythrum salicaria*, grown in a common garden field experiment at mid-latitude (44.03ºN). The bootstrapped average population means (black dots) and quadratic regression lines (red curves) are based on 999 bootstrap iterations. To visualize variation among bootstrap iterations, individual iteration averages (grey dots) and regression lines (grey curves) are also shown for a random sample of 99 bootstrap iterations.



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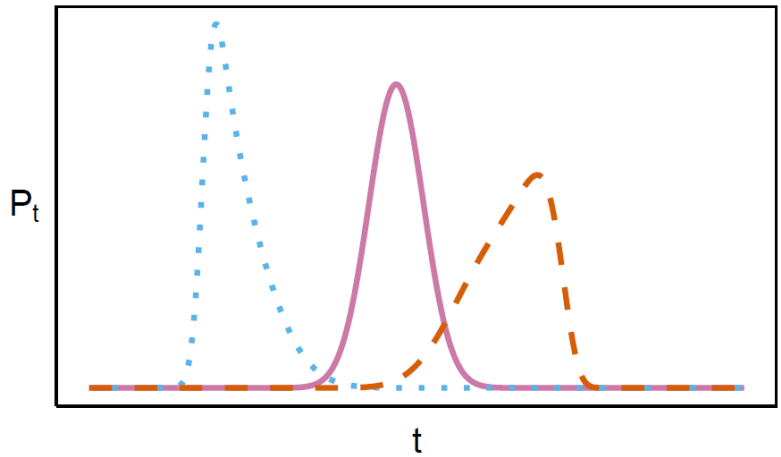

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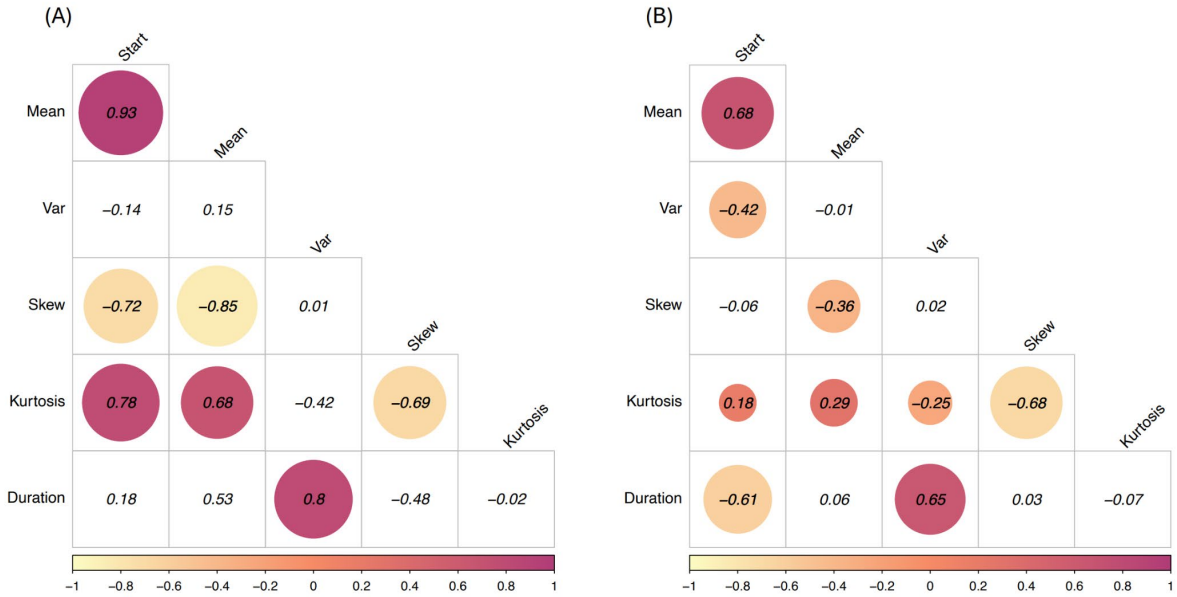

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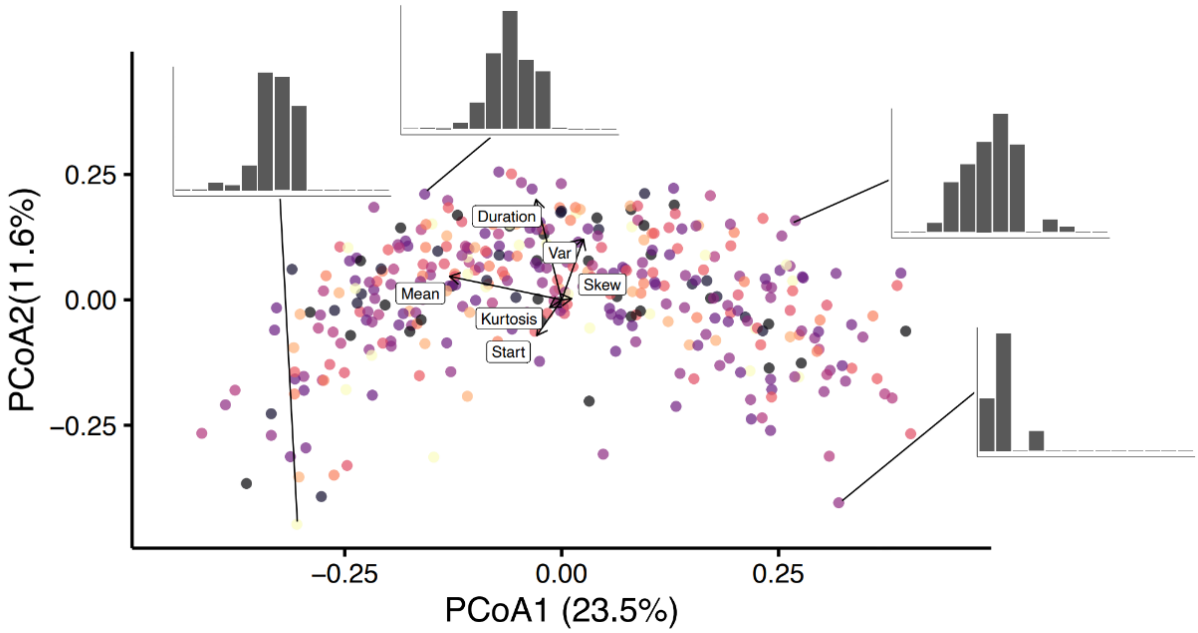

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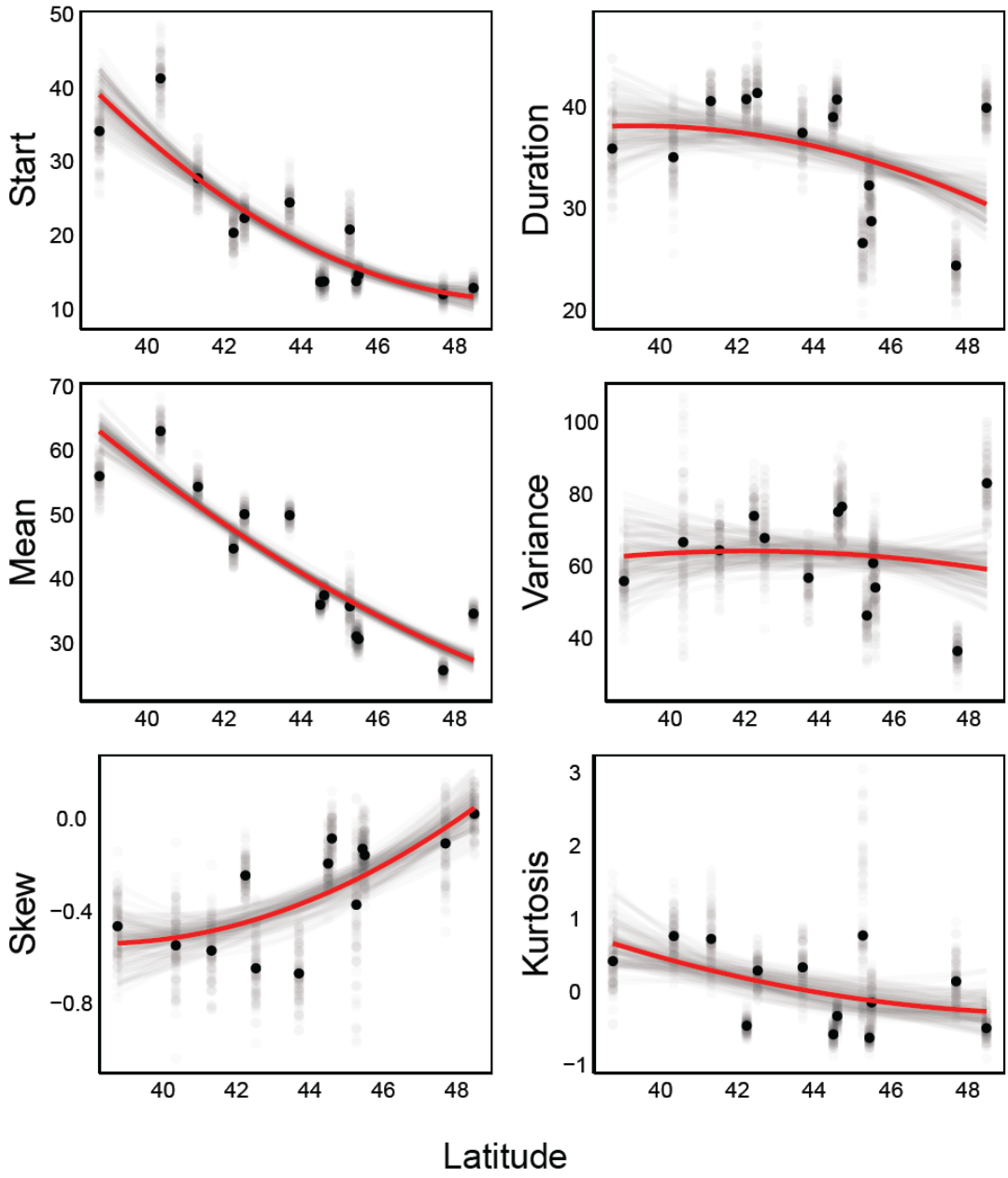

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Appendix S1. The distribution of 369 individuals from 216 seed families representing 13 populations of *Lythrum salicaria* sampled along a latitudinal gradient and grown in a common garden at the Koffler Scientific Reserve in Newmarket, ON.

Population Latitude (°)	***N* Seed families**	***N* Individuals**
38.75261	15	23
40.34025	15	33
41.31283	18	24
42.23822	20	34
42.52306	12	34
43.69719	19	21
44.49339	19	38
44.59489	19	22
45.26278	16	26
45.43194	17	36
45.49000	16	23
47.69081	14	32
48.47892	16	23