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: The timing and pattern of flowering -- 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 evolution associated with rapid adaptive evolution and range expansion among thirteen populations of *Lythrum salicaria* populations, 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 used Principal Coordinates Analysis (PCoA) and quantitative metrics analogous to central moments of probability distributions (i.e., mean, variance, skew, and kurtosis) to quantify flowering schedules..

Key Results: Consistent with adaptation to shorter growing seasons at higher latitudes, we found that populations from higher latitudes had earlier start and mean flowering day for both population average (i.e., among individuals) and population aggregate (i.e., pooled flowers). Clines in population average and aggregate flowering skew increased with latitude while kurtosis decreased, consistent with a bet-hedging strategy arising in biotic environments with more herbivores and greater competition for pollinators. Although clines were generally similar at the average vs aggregate level, we found that individual flowering schedules from more southern populations tended to flower later than their population aggregate.

Conclusions: Heritable genetic changes in flowering schedule 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, generating 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 to understanding eco-evolutionary dynamics. For example, in sexual outcrossing plants, flowering phenology governs interaction with mutualists (Fenster et al., 2004), antagonists (Strauss & Whittall, 2006) and abiotic factors (Jin *et al.*, 2019). 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; Fitchett et al., 2015; D. W. Inouye, 2022; Panchen, 2022; Panchen & Gorelick, 2017; Prather et al., 2023). As such, flowering phenology is a useful composite trait for investigating evolutionary responses to past and future environmental challenges.

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 common to a variety of plant taxa (Alexander et al., 2009; Colautti & Lau, 2015; Halbritter et al., 2018), and reciprocal transplant experiments have confirmed that these clines are an important component of local adaptation (Ågren & Schemske, 2012; Anderson & Gezon, 2015; Colautti & Barrett, 2013; Ensing & Eckert, 2019; 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 suggest that flowering time is often under directional selection within populations

71 (Munguía-Rosas et al., 2011). Moreover, long-term phenology records show a variety of
72 responses to climate change -- from earlier to later to no change in flowering time, depending on
73 the species (Rafferty et al., 2020; Wolkovich et al., 2012). Thus, there appears to be
74 disagreement between theoretical models and empirical observations of geographic clines and
75 local adaptation on the one hand and observed measurements of natural selection and phenotypic
76 changes in natural populations on the other (Austen et al., 2017). Antagonistic selection on traits
77 correlated with the onset of flowering offers one potential resolution to this apparent
78 contradiction.

79 Day of first flowering may trade off with other aspects of flowering phenology such as
80 the shape of the flowering schedule. These other aspects of flowering phenology may not be well
81 represented by flowering time (Newstrom et al. 1994; CaraDonna et al., 2014; B. D. Inouye et
82 al., 2019) but have important effects on reproductive fitness (Fox, 2003). The shape of the
83 flowering schedule can be quantified by metrics analogous to central moments of probability
84 distributions, which include the mean, variance, skew, and kurtosis (Box 1). Not to be confused
85 with the central moments of a statistical population or sample, the analogous characteristics of a
86 flowering schedule quantify temporal variation in relative reproductive investment through time.
87 These characteristics use the same mathematical equations (Table 1) but are not shaped by
88 probabilistic process and therefore may not correlate with onset or duration of flowering (Fig.
89 1A). These metrics have rarely been used in studies of flowering phenology, yet they have the
90 potential to offer a novel perspective on selection and trade-offs affecting the evolution of
91 phenology.

92 Central moments of individual flowering schedules describe the timing and intensity of
93 reproductive investment, 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 later in the growing season may benefit from showy inflorescences that enhance pollinator attraction (O'Neil, 1997; Thomson, 1980) and be characterized by a flowering schedule with a more positive skew and longer duration. Herbivores and seed predators can be unpredictable, which may select for a bet-hedging strategy and possibly delay in the onset of flowering (Augspurger, 1981; Elzinga et al., 2007; Wright & Meagher, 2003). These conditions might further result in a longer flowering duration with a higher variance and negative skew in the flowering schedule if late-season ramp-up in flowering is limited by harsh growing conditions at the end of a season.

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 an earlier onset of flowering (Austen & Weis, 2015; Colautti & Barrett, 2013; Griffith & Watson, 2005), and may favour reproduction only within a shorter growing season; this would result in flowering schedules with lower duration and variance. In marginal environments, plants may combine strategies, concentrating flower production into a narrow window but also producing a few flowers over a long timeframe as a bet-hedging strategy (Forrest & Thomson, 2010; Simons, 2011; Tufto, 2015), which would produce a long-tailed leptokurtic or skewed flowering schedule. The above examples illustrate the complex ways that biotic interactions and the abiotic environment could shape selection on individual flowering schedules, and how these differences can be quantified using equations developed for probability distributions.

Natural selection acts on phenological schedules of individuals, but variation among individuals within a population affect eco-evolutionary interactions with pollinators, herbivores, and potential mates. Just as individuals have flowering schedules, the aggregated flower production of all individuals within a population can be characterized (Box 1), which could yield emergent properties that are relevant to understanding eco-evolutionary feedbacks. We define emergent properties here as deviations in the aggregate population from the average of individual schedules comprising it (Table 1). For example, a population can have a high aggregate variance but low average individual variance in the flowering schedule if individuals vary in onset and duration of flowering. In cases like this, the central moments of the aggregate flowering schedule show characteristics that are emergent and distinct from the typical individual comprising it (Fig. 1). Although emergent properties can arise from adaptive processes acting on individuals, they can also have important ecological consequences for species communities. For example, population aggregate flowering schedules are sometimes positively skewed with an abrupt start and a long tail of flowering (Blionis et al., 2001; Rabinowitz et al., 1981), which may be an adaptation to enhance pollinator visitation to the population (Stemkovski et al., 2023; Thomson, 1980). Additionally, strong frequency-dependent selection may concentrate flowering within a narrow but synchronous timeframe to attract more pollinators (Rathcke & Lacey, 1985), increase mating opportunities (Ison & Weis, 2017), or prevent interspecific cross-pollination (Fantinato et al., 2018). Therefore, in pollinator or mate-limited environments, population aggregate flowering schedules may be very similar to the average flowering schedule. Thus, emergent properties (or a lack thereof) have the potential to provide insight into eco-evolutionary processes and ecological consequences of aggregate flowering schedules.

Environmental gradients associated with latitude can be useful for studying phenotypic evolution because selection often varies predictably due to shorter growing seasons and weaker biotic interactions towards polar latitudes (reviewed in Zvereva & Kozlov, 2021). Adaptation along gradual environments is commonly manifested as clines in phenotypic traits that are observable when genetically related individuals are grown in a common garden environment (Olsson & Ågren, 2002; Panchen, 2022; Sobral et al., 2013). For example, there is good evidence for the rapid evolution of adaptive flowering time clines in the perennial wetland herb *Lythrum salicaria* (purple loosestrife) during its invasion of 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 constrained 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 favors plants that flower later but grow larger in stature. However, by focusing on the first day of flowering, previous studies have ignored other aspects of the flowering schedule that may be important for understanding adaptation and constraint during evolution and invasion of *L. salicaria*.

Here, we investigate clines in flowering schedules among thirteen populations 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 quantify both individual and population aggregate flowering schedules to address the following questions: (1) How well do common phenological metrics, like the onset and duration of flowering, predict often overlooked features of the flowering schedule such as the variance, skew and kurtosis? (2) Are there latitudinal clines in flowering schedules that are consistent with the adaptive

162 hypotheses outlined above? Specifically, we predicted that longer growing season lengths,
163 stronger competition for pollinators, and higher rates of herbivory in southern populations will
164 have favoured genotypes with: (i) a longer flowering duration and (ii) a more negative kurtosis
165 with a concentrated peak and rare flowering events far from the average flowering day, but also
166 (iii) an increase in the flowering schedule variance and (iv) a more negative skew with flowering
167 focused later in the schedule. (3) Are there emergent properties of population aggregate
168 flowering schedules that differ from the population average of individual flowering schedules?
169 Because natural selection acts on individual flowering schedules, emergent properties represent
170 (i) potential biases in evolutionary inference when aggregate characteristics are used in place of
171 individual or population average characteristics of flowering schedules, and (ii) ecological
172 phenomena that are shaped by selection and potentially affect herbivore and pollinator
173 communities.
174
175 **METHODS**
176 ***Experimental design --***
177 Details of the field experiment are provided in Colautti & Barrett (2010) and the seeds used were
178 collected along a 10-degree latitudinal gradient in eastern North America, as described in
179 Montague *et al.* (2008). Briefly, 13 populations were chosen to represent a latitudinal gradient
180 from Timmins, Ontario (48.48 ºN) to Easton, Maryland (38.75 ºN) and were grown at the
181 Koffler Scientific Reserve at Jokers Hill, located approximately at the geographic center of the
182 latitudinal gradient (44.03 ºN). Prior to inflorescence development, plants were sprayed with
183 insecticide to reduce incidence of herbivorous insects. The primary purpose of this experiment
184 was to characterize flowering time clines and genetic trade-offs in a multi-year (2006-2008)

common garden study, as reported in Colautti & Barrett (2010, 2011). In 2008 we additionally recorded all open flowers on every stem of a subset of 369 individuals of 216 unique seed families representing the 13 populations (Appendix S1). Our daily census records of this subset of plants occurred over 125 days on a rotating schedule such that each individual was surveyed every five days from the onset of flowering in July until September. A 5-day cycle was chosen because stigma receptivity is nearly zero after 24 h of anthesis (Waites & Ågren, 2006), after which unpollinated flowers begin to senesce and abscise while pollinated flowers begin to close and fuse at the calyx, limiting the risk of counting the same flowers on multiple census dates. All calculations and statistical analyses were conducted in R version 4.2.2 (R Core Team, 2022) in R Studio v4.2.2 (RStudio Team, 2022), and are available with the original data from the Dryad database (Dryad DOI: TBD(ROB)).

Characterizing flowering schedules --

We characterized the flowering schedules of each individual plant using the equations shown in Table 1, standardized to the number of calendar days after the first flower was observed in the experiment. The number of fruits produced over a growing season is variable in this experiment because individuals that are locally adapted to mid-latitude balance flowering time with growth to produce more flowers compared to small, early flowering phenotypes more characteristic of northern populations or large, late-flowering phenotypes characteristic of more southern populations. To account for variation in total flower production, we standardized the number of open flowers every five days for each individual as a proportion of the total number of open flowers censused over 125 days of observation. We then calculated a weighted census day by multiplying each day by the proportion of flowers open on that day. The proportion of flowers

208 and the weighted census days were summed to calculate the weighted mean and other
209 characteristics of individual flowering schedules analogous to central moments (variance, skew
210 and kurtosis) (Box 1; Table 1). In addition to calculating the characteristics of every individual's
211 flowering schedule, we calculated the average of each characteristic for each population.

212 In addition, the start of flowering, duration, and central moments, we used a Principle
213 Coordinates Analysis to characterize flowering schedules of individuals as described by Austen
214 et al., (2014). Briefly, we computed a dissimilarity matrix from raw flower counts using
215 Kolmogorov-Smirnov (KS) distances (Sokal & Rohlf, 1995) where each matrix cell was a KS
216 statistic measuring the maximum distance between two flowering schedules. We chose KS
217 distance because it is robust to differences in flower number and census schedules. However, the
218 KS statistic is sensitive to differences in scale, so flowering durations were standardized to run
219 from 0 to 1 by subtracting the shortest duration time from each individual and dividing by the
220 difference of the longest and shortest durations (Austen et al., 2014). We used the KS matrix as
221 the input for the **cmdscale** function to generate the PCoA axes. Since KS distances do not meet
222 the triangle inequality condition of PCoA, we consider only eigenvectors with positive
223 eigenvalues (Austen et al., 2014; Legendre & Legendre, 1983). It is important to note that the
224 PCoA axes characterize variation in the shape of flowering schedules among individuals but not
225 variation in onset and duration of flowering.

226 In addition to individual flowering schedules, and the average of all individuals within
227 each population, we calculated characteristics of the aggregate population flowering schedules.
228 These calculations use the same equations after first pooling the flower counts for each day
229 across all individuals within a population (Table 1). For example, the aggregate start of flowering
230 of a population would be the first day that any individual in the population flowered, and

aggregate duration of flowering would be the number of days until the last open flower was observed in the population.

We used bootstrap models with 999 iterations to compute a bootstrapped mean and 95% confidence intervals for both population average and population aggregate flowering schedules. In each iteration, individuals were hierarchically sampled with replacement from within each population, and then each metric in Table 1 was calculated either from the pooled set of individuals in each population to calculate the population aggregate characteristics, or for each individual within the population to calculate the population average characteristics. Original data with reproducible R code for the bootstrap models are available from the Dryad database (Dryad DOI: TBD(ROB))

Characteristic correlations, clines and emergent properties --

Our first research question addresses whether conventional metrics (i.e., onset and duration of flowering) correspond to more detailed characteristics of the flowering schedule (i.e., mean, variance, skew, kurtosis, and PCoA axes). To investigate these relationships, we calculated pairwise Pearson correlations to compare three scales of analysis, namely (A) average schedules of each population (n = 13), (B) the deviation of each individual from its population mean (n = 369), and (C) aggregate schedules calculated on pools of individuals for each population (n = 13). In addition to comparing conventional metrics with other flowering schedule characteristics, the correlational structure of these matrices can also be instructive for testing evolutionary hypotheses because evolutionary divergence of flowering schedules would produce correlations among average (A) or aggregate (C) populations that are not evident among individuals within populations (B).

We used simple linear regression models of bootstrap means to address our second question concerning whether there were latitudinal clines characteristic of both average and aggregate flowering schedules. Each univariate linear regression included the bootstrap mean of a flowering schedule characteristic as the dependent variable, with latitude as a linear and quadratic predictor, and significance was tested using the **anova** function in R to find the minimal adequate model.

To quantify emergent properties of population aggregate flowering schedules that differ from the population average, we subtracted the average value of each population from the aggregate value for the same population. Therefore, a positive value for an emergent property means that a population had an aggregate value that was higher than the average individual comprising it. For example, all populations will have a negative emergent property for onset of flowering because the average onset of flowering will never be earlier than the day of first flower in the aggregate population. As with average and aggregate characteristics, we tested for clines in emergent properties using linear models as described in the previous paragraph.

Finally, we also considered the false discovery rate between 6 and 24 separate statistical models. We consider a range of degrees of freedom because there are 24 separate statistical models to test for clines (i.e., six characteristics across three types of comparisons) but the aggregate, average, and emergent characteristics are each calculated from the same population data, resulting in no more than 6 independent characteristics.

RESULTS

Flowering schedule characteristics --

Pearson product-moment correlations revealed significant relationships among commonly measured traits of the flowering schedule (i.e., start and duration) and more detailed characteristics (i.e., mean, variance, skew, and kurtosis), across three types of comparison (Fig. 2). At the aggregate level, populations that started flowering later also had a later mean flowering date. Additionally, populations with longer flowering duration had a higher coefficient of kurtosis indicating more flowering far from the mean flowering date (Fig. 2C). In contrast, there was no significant correlation between start date and duration among population average flowering schedules, but there was a significant negative correlation with skew indicating that populations with a later start date, on average, tend to have individuals with imbalanced flowering clustered more at later dates (Fig. 2A).

Flowering schedule variance was significantly positively correlated with flowering duration on average but not among aggregate flowering schedules. Residual, within-population correlations were generally weaker when compared to correlations among population average and aggregate flowering schedules (Fig. 2B). In addition, the correlation between onset and duration of flowing among individuals within populations was in the opposite direction to population average and aggregate. In other words, plants that started flowering earlier relative to other individuals within the same population also flowered for a shorter duration (Fig. 2B), whereas populations with earlier flowering individuals, on average, also tended to have individuals with longer flowering durations (Fig. 2A).

296 ***Principal Coordinates Analysis --***

297 In addition to the above characteristics, we use Principal Coordinates Analysis (PCoA) to fully
298 characterize similarities and differences among flowering schedules after controlling for
299 variation in start date and duration. The PCoA of all 369 individuals resulted in 369 independent
300 eigenvectors, of which 192 had positive eigenvalues. However, of these 192, only the first two
301 PCoA axes had eigenvectors that explained more than 10% of the variation in flowing schedule
302 shape, and together they explained 35% of the variation (Table 2). By comparison, the next ten
303 eigenvectors combined explained another 35% of variation, with the remaining 180 accounting
304 for 30% of the variation. Thus, we next focus on the first two eigenvectors.

305 For both the population aggregate and average, the first axis (PCoA1) was positively
306 correlated with skew and negatively correlated with the mean day of flowering (Fig. 2). PCoA1
307 was also negatively correlated with duration of flowering when comparing population average
308 but not population aggregate flowering schedules. In contrast, within-population residual
309 correlations involving PCoA1 were generally weaker than among population average or
310 aggregate, with significant correlations for mean, skew and kurtosis (Fig. 2B). Correlations
311 involving PCoA2 were quite different than PCoA1 within and among all three comparisons. For
312 example, PCoA2 was not significantly correlated with any other metrics in aggregate
313 distributions (Fig. 2C) but was negatively correlated with start and kurtosis and positively
314 correlated with skew and duration among individuals within the same populations (Fig. 2B) and
315 was positively correlated with variance and duration of average distributions (Fig. 2A). By
316 mathematical definition of the PCoA, there was no correlation between PCoA1 and PCoA2
317 among all individuals, nor was there a significant correlation among residuals of individuals
318 within populations (Fig. 2B). In other words, PCoA1 and PCoA2 change in a correlated way.

This can be seen clearly in Fig. 3, where northern populations cluster at intermediate values of PCoA1 and PCoA2 compared to mid-latitude (higher PCoA1 but lower PCoA2) and southern populations (lower PCoA1 and lower PCoA2).

Latitudinal clines and emergent properties --

The correlated changes in population aggregate and average flowering schedules are reflected in significant changes with latitude. For example, the start, mean, and kurtosis of flowering schedules were each significantly negatively related to latitude for both aggregate and average schedules (Table 3, Fig. 4). This means that southern populations start flowering later, with a later average flowering date, and more 'outlier' flowers opening far from the average flowering date, both in aggregate and on average, when compared to northern populations. However, aggregate and average schedules vary in two key characteristics – aggregate but not average schedule duration declined with latitude, whereas average but not aggregate skew was negative at lower latitudes (Fig. 4). In other words, individuals have similar flowering durations on average but are more spread out in southern populations, resulting in a longer aggregate duration towards the south. Similarly, individual schedules are more heavily weighted toward later flowering dates towards the south, but variation in start dates average out such that aggregate schedules are more evenly distributed and not significantly related to latitude.

To quantify emergent properties, we compared aggregate to average schedules and this procedure revealed considerable variation among populations and two latitudinal clines (Table 4; Fig. 4). Although the aggregate start of flowering must always be earlier than the average, resulting in a negative emergent property score, we found a significant positive correlation with latitude. Likewise, the duration of flowering must always be longer in aggregate than on average,

342 but we found a marginal negative relationship with latitude (Fig. 4, right column). Together,
343 these clines demonstrate more synchronous flowering schedules in northern populations
344 compared to those occurring in the south. We also found a nonlinear cline in variance
345 representing a lower aggregated flowering schedule variance than the average individual
346 variance at intermediate latitudes.

347 Overall, we expect about one false positive cline at $\alpha < 0.05$ with 24 independent
348 statistical tests. But of the 10 significant clines, only five remained significant after a Bonferroni
349 adjusted $\alpha = 0.0083$ for six independent tests or $\alpha = 0.0021$ for 24 tests. Despite uncertainty in
350 the detection of clines, bootstrap estimates show a relatively high level of precision for most
351 population means, as shown by the small error bars in Fig. 4.

352

353 **DISCUSSION**

354 There is growing evidence for rapid evolution in invasive species (van Kleunen et al., 2018), but
355 it remains uncertain how often adaptive evolution contributes to their spread (Colautti & Lau,
356 2015). Rapid evolution of a latitudinal cline in flowering time in response to local growing
357 conditions likely increased the rate and extent of invasion by *Lythrum salicaria* across North
358 America (Colautti & Barrett, 2013; Wu & Colautti, 2022). However, previous studies focusing
359 on the onset and duration of flowering provided only a partial view of how plants allocate
360 flowering resources (Box 1), raising uncertainty about the extent to which phenological
361 evolution has been adaptive. We used a common garden study with detailed metrics to
362 characterize entire flowering schedules and tested hypotheses about adaptative latitudinal clines.
363 We found that the onset and duration of flowering were not good predictors of other
364 characteristics of individual flowering schedules (Fig. 2B). In contrast, the average flowering

365 schedule characteristics were highly correlated among population averages (Fig. 2A) and also
366 among population aggregate flowering schedules (Fig. 2C). Furthermore, that these among-
367 population correlations were largely due to correlated evolution along latitudinal clines (Fig. 4).
368 Emergent properties were highly variable among populations and formed clines indicative of
369 more synchronized flowering schedules in northern populations. Below we discuss the
370 implications of these results for understanding the evolutionary causes and ecological
371 consequences of flowering schedule diversity.
372
373 ***Evidence for correlated evolution --***
374 Date of first flower and duration of flowering are relatively easy to measure and are commonly
375 reported as focal traits in phenological studies. However, reproductive fitness depends on the full
376 schedule of flower production, and it has not been clear how well the onset or duration of
377 flowering correlates with other characteristics of the flowering schedule. In *L. salicaria*, plants
378 that flowered later also flowered for a longer duration with a higher flowering variance and
379 slightly lower skew, relative to other individuals sampled from the same population (Fig. 2B).
380 This result is consistent with a life history trade-off between flowering earlier at a small size or
381 delaying reproduction to accumulate more resources for later floral investment – a mechanism
382 that was proposed previously for this species (Colautti & Barrett, 2013; Wu & Colautti, 2022)
383 but was not validated with floral display data until now.
384 In contrast to individual flowering schedules, measurements of the flowering schedules of
385 whole populations provide more limited insights into the evolutionary mechanisms shaping
386 flowering schedules. The different correlational structure for average (Fig. 2B) versus aggregate
387 schedules (Fig. 2C) produced significant variation in emergent property characteristics among

388 populations (Fig. 4C), suggesting that aggregate characteristics are not generally indicative of
389 individual flowering schedules within a population. Studies that have not considered the full
390 schedule shape of individual plants may therefore neglect key aspects of phenology that are
391 crucial for investigating ecological and evolutionary hypotheses. For example, there is
392 considerable among-population and among-species variability in phenological shifts due to
393 climate change (Anderson et al., 2012; Fitchett et al., 2015; D. W. Inouye, 2022; Panchen, 2022;
394 Panchen & Gorelick, 2017; Prather et al., 2023). This variation may be explained by selection on
395 characteristics of flowering schedules that are not correlated with the onset of flowering.
396 There were differences in both the strength and direction of correlations among
397 individuals within *L. salicaria* populations when compared with population average and
398 aggregate characteristics (Fig. 2). Among-population correlations were caused by either
399 statistical artifacts or genetic constraints, then we would expect to see similar correlations among
400 individuals within populations. Instead, the differing correlation matrices are more consistent
401 with correlated evolution among populations. We cannot rule out the possibility that
402 environmental effects mask genetic correlations within populations; however, this would require
403 a much larger experiment with sufficient replication to estimate correlations among relatives
404 within populations (Price et al., 1988; Rausher, 1992). Nor can we rule out the potential role of
405 stochastic processes without replicated sampling from independent geographical gradients
406 (Colautti & Lau, 2015). Nevertheless, our finding that flowering schedules change continuously
407 with latitude lends additional evidence for the correlated evolution hypothesis. Moreover, the
408 direction of the observed clines are consistent with adaptive responses to biotic and abiotic
409 selection, as discussed below.
410

411 ***Latitudinal clines and emergent properties --***

412 Latitudinal clines in the onset of flowering have evolved independently in several species across
413 North America (McGoey et al., 2020; Montague et al., 2008; Samis et al., 2012; Wu & Colautti,
414 2022). In our study of *L. salicaria*, we additionally found latitudinal clines in average mean,
415 skew and kurtosis of flowering schedules (Fig. 4, left column). These types of clines indicate that
416 individuals from southern environments have floral displays that peak later in the season but also
417 a few open flowers far from the mean flowering date. These findings correspond to higher
418 intensity of biotic interactions in southern environments reported in a meta-analysis of terrestrial
419 ecosystems (Zvereva & Kozlov, 2021).

420 *Lythrum salicaria* is a self-incompatible outcrosser (Colautti et al., 2010; Darwin, 1877;
421 O'Neil, 1997) and therefore individuals in southern populations likely experience strong inter-
422 specific competition for pollinators. Additionally, several effective biological control insects
423 have been released into eastern North America, including *Gallerucella* beetles that feed on
424 meristem tissue as larvae and can drastically reduce flower production (Blossey & Notzold,
425 1995; Grevstad, 2006). The later average flowering and more negative skew in plants from
426 southern populations is therefore consistent with selection from higher herbivory and more
427 competition for pollinators under a longer growing season. We cannot easily parse the specific
428 effects of biotic and abiotic factors based on clines alone, but we can make the prediction that
429 clines in herbivore and pollinator activity would be more patchy and less predictable by latitude
430 compared to season length. It is therefore interesting that we detected strong clines in average
431 start (β = -0.856) and mean (β = -0.881) flowering dates, which we predict to be more sensitive
432 to season length, in contrast to kurtosis (β = -0.590), which we predicted as a bet-hedging
433 strategy for biotic interactions. Skew shows an intermediate pattern (β = 0.745) and could be an

434 adaptation to biotic selection but could also be partly an artifact of our mid-latitude common
435 garden site, as discussed below.
436 Characteristics of aggregate flowering schedules were generally very different from their
437 population averages, resulting in high variability in emergent properties among populations (Fig.
438 4, right column), but these differences did not generally correlate with latitude. Two important
439 exceptions are the significant and marginally significant clines observed in the onset and
440 duration of flowering respectively, which characterized more synchronized flowering schedules
441 in the north. However, the mid-latitude location of our common garden may also be responsible
442 for these trends. Cooler temperatures that are expected with increasing latitude not only delay the
443 start of favorable growing conditions, but they also restrict insect pollinator activity. Therefore,
444 lack of pollinator activity towards the end of the growing season at our mid-latitude field site
445 may represent an artificial truncation of flowering by southern populations leading to a perceived
446 difference in flowering schedule synchrony. Overall, variation in emergent properties among *L.*
447 *salicaria* populations has important implications for field research on components of floral
448 display. It has previously been established that individuals with flowering schedules
449 uncharacteristic of their populations can potentially bias ecological and evolutionary inferences
450 drawn from aggregate population schedules (Newstrom et al. 1994; B. D. Inouye et al., 2019).
451 The emergent properties in our study suggest that such cases are likely more common than is
452 generally appreciated and that they may also be geographically structured. As such, aggregate
453 schedules should be interpreted cautiously.
454

455 ***Conclusions --***

456 Our study demonstrates that populations of *L. salicaria* in eastern North America have evolved
457 differences in flowering schedules within less than a century of invasion. This phenological
458 differentiation among populations includes predictable changes in the timing of resource
459 allocation to flower development and floral display and adds to the growing list of phenotypic
460 traits that are targets of selection as invasive species spread along environmental gradients
461 (MacDougall et al., 2006; McGoey et al., 2020; Molina-Montenegro et al., 2018; Urbanski et al.,
462 2012). Based on the observed clines in our study, we predict that longer growing seasons
463 accompanying anthropogenic climate change will alter flowering schedules. These changes are
464 likely to involve a later start date to flowering, a later mean day of flowering, and a larger
465 kurtosis, which in turn should increase seed production and promote population growth and
466 range expansion of *L. salicaria*. The ecological and evolutionary insights drawn from
467 observations of flowering start dates and population aggregate characteristics should, however,
468 be interpreted cautiously. In future, more detailed features of individual flowering schedules can
469 be characterised by multivariate methods and metrics drawn from central moments theory. As we
470 have shown for *L. salicaria* from eastern North America, these more refined characteristics of
471 individual flowering schedules might help in developing a deeper understanding of the diverse
472 ways that species respond to changing environments.

473

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AUTHOR CONTRIBUTIONS

M.N.A. led the data analysis with assistance from D.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 (DOI:)

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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 process. 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. 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 anthesis day of a typical flower.

As demonstrated in Figure 1A, 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 variance 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). 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 long-time frame (i.e., leptokurtic distribution, $CK_i > 0$ as shown in the gold dashed line) or individuals that spread out flowering more evenly over the entire schedule (i.e., platykurtic, $CK_i < 0$ as shown by the dashed green line). In addition to calculating these characteristics for individual plants, all flowers (or proportion of flowers) on each day can be pooled to yield a population aggregate flowering schedule (Fig. 1B). The aggregate schedule of a population may not be representative of the individual schedules comprising it, resulting in emergent properties that we define as deviations from the average individual flowering schedule.

Table 1. Equations of flowering schedule characteristics for an average individual within each population and for the aggregate population of pooled individuals.

Summary Statistic	Individual (i)	Population aggregate (j)
Proportion (P) of flowers (F) produced at time t	$P_{i,t} = \frac{F_{i,t}}{\sum_t F_{i,t}}$	$P_{t,j}^{\Sigma} = \frac{F_{t,j}^{\Sigma}}{\sum_t F_{t,j}^{\Sigma}} = \frac{\sum_i F_{i,t,j}}{\sum_t \sum_i F_{i,t,j}}$
Day of first flower	$\alpha_i = \min\{t \mid F_{i,t} > 0\}$	$\alpha_j^{\Sigma} = \min\{t \mid \sum_i F_{i,t,j} > 0\}$
Day of last flower	$\Omega_i = \max\{t \mid F_{i,t} > 0\}$	$\Omega_j^{\Sigma} = \max\{t \mid \sum_i F_{i,t,j} > 0\}$
Duration of flowering	$\Omega_i - \alpha_i$	$\Omega_j^{\Sigma} - \alpha_j^{\Sigma}$
Day of mean flowering	$\mu_i = \sum_t t\, P_{i,t}$	$\mu_j^{\Sigma} = \sum_t t\, P_{t,j}^{\Sigma}$
Flowering schedule variance	$\sigma_i^2 = \sum_t (t-\mu)^2\, P_t$	$\sigma_j^{2\Sigma} = \sum_t \left(t - \mu_j^{\Sigma}\right)^2 P_{t,j}^{\Sigma}$
Flowering schedule coefficient of skew	$CS_i = \frac{\sum_t (t-\mu_i)^3\, P_{i,t}}{\sigma_i^3}$	$CS_j = \frac{\sum_t \left(t-\mu_j^{\Sigma}\right)^3 P_{t,j}^{\Sigma}}{\sigma_j^{3\Sigma}}$
Flowering schedule coefficient of kurtosis	$CK_i = \frac{\sum_t (t-\mu_i)^4\, P_{i,t}}{\sigma_i^4} - 3$	$CK_j = \frac{\sum_t \left(t-\mu_j^{\Sigma}\right)^4 P_{t,j}^{\Sigma}}{\sigma_j^{4\Sigma}}$

Table 2. Summary of the first five eigenvector axes of a Principal Coordinates Analysis (PCoA) using Kolmogorov-Smirnov distances between flowering schedules of 369 plants from 13 populations of *Lythrum salicaria* sampled along a latitudinal gradient and grown in a common garden at the Koffler Scientific Reserve in Newmarket, ON. Percent variation is calculated as the eigenvalue over the sum of all positive eigenvalues.

Axis	**Eigenvalue**	**Variation explained (%)**
PCoA1	13.4	23.5
PCoA2	6.6	11.5
PCoA3	3.9	6.9
PCoA4	3.2	5.6
PCoA5	2.6	4.7

Table 3. Standardized slope and test statistics of univariate linear regressions testing latitude as a predictor of the bootstrapped means of average and aggregate population flowering schedule traits, and the emergent property (i.e. population aggregate minus average) in populations of *Lythrum salicaria*. Bold text indicates significant values.

			Linear term		**Quadratic term**	
Traits		β	$F_{(1,11)}$	P	X^2	P
Start	Average	-0.856	30.0	**< 0.001**	1.73	0.189
	Aggregate	-0.591	5.92	**< 0.05**	0.01	0.907
	Emergent	0.857	30.52	**< 0.001**	2.50	0.114
Duration	Average	-0.369	1.73	0.215	0.11	0.738
	Aggregate	-0.849	28.37	**< 0.001**	0.01	0.904
	Emergent	-0.470	3.13	0.105	2.96	0.085
Mean	Average	-0.881	38.0	**< 0.001**	0.47	0.494
	Aggregate	-0.845	27.5	**< 0.001**	0.12	0.724
	Emergent	0.368	1.66	0.224	0	0.948
Variance	Average	0.031	0.01	0.920	0.07	0.791
	Aggregate	-0.214	0.53	0.483	0	0.977
	Emergent	0.156	0.276	0.610	6.83	**< 0.01**
Skew	Average	0.745	13.7	**< 0.01**	1.03	0.310
	Aggregate	0.382	1.87	0.198	0.07	0.797
	Emergent	0.227	0.60	0.456	0.12	0.741
Kurtosis	Average	-0.590	5.88	**< 0.05**	0.20	0.659
	Aggregate	-0.592	5.94	**< 0.05**	0.88	0.347
	Emergent	-0.133	0.19	0.664	1.18	0.279

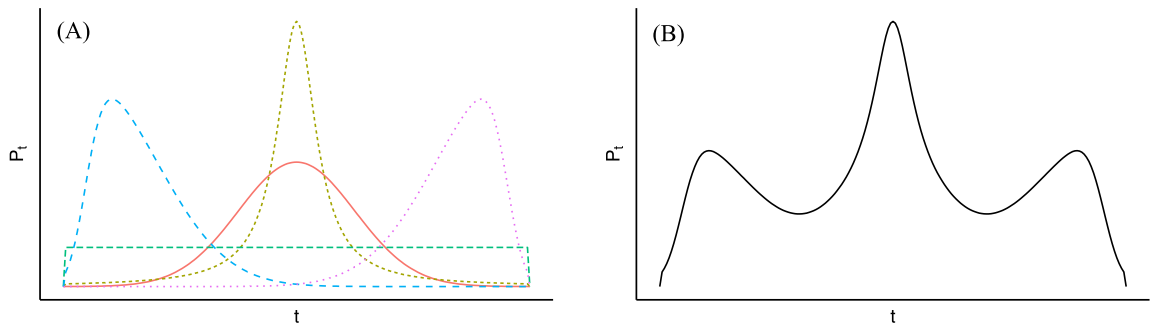

Figure 1. Hypothetical flowering schedules of (A) five individuals with different characteristics and (B) their pooled aggregate flowering schedule. Flowering schedules are scaled to the proportion of total flower production (P_t) over time (t).

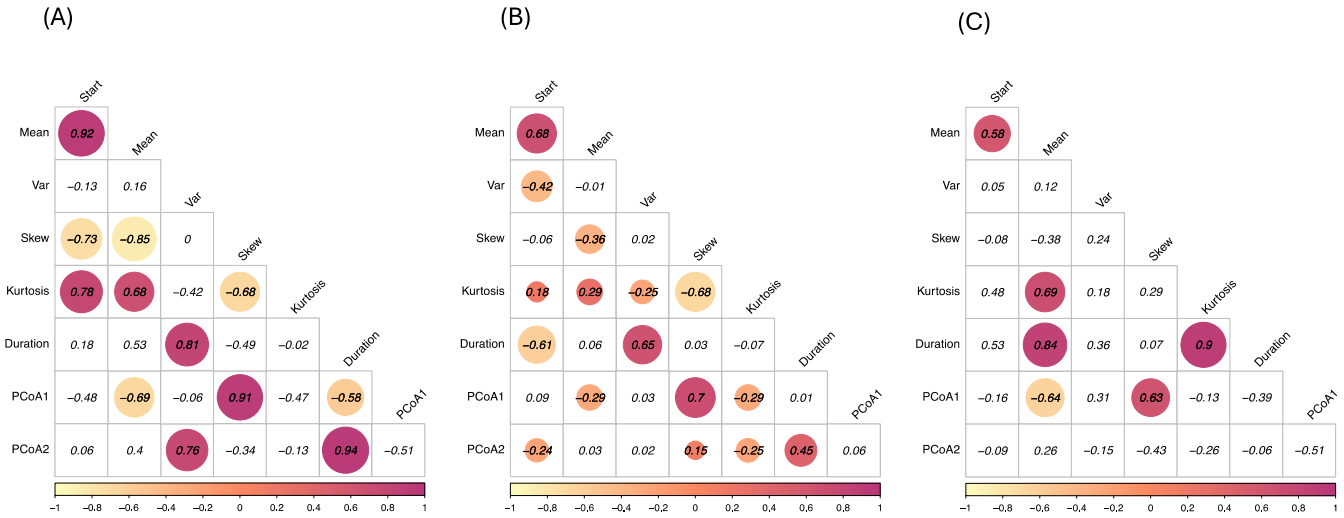

Figure 2. Matrices showing pairwise Pearson correlation coefficients among the start, duration, central moments (i.e., variance, skew and kurtosis), and two the first two eigenvectors of a principal coordinates analysis (PCoA) characterizing flowering schedules in populations of *Lythrum salicaria* grown in a common garden. The metrics were calculated using either (A) flowering schedule characteristics *averaged* across individuals within each population (n = 13), (B) the *residual* correlations calculated on deviations of individuals from their population means (n = 369), or (C) *aggregated* schedules for individuals pooled within populations (n = 13). 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).

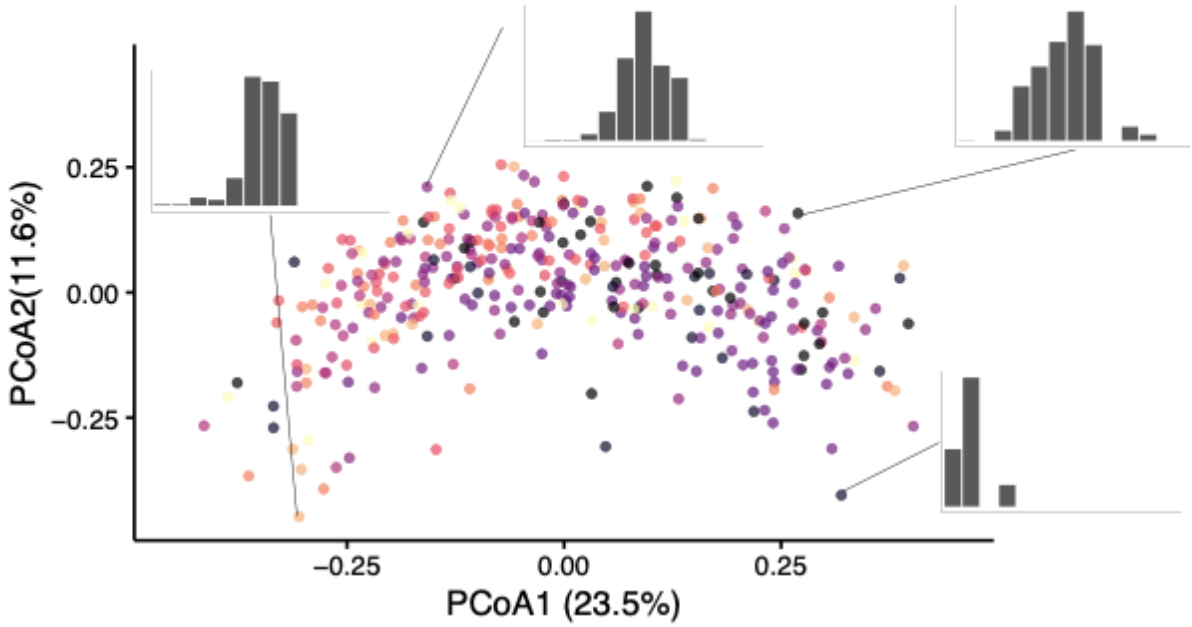

Figure 3. Principal Coordinates Analysis (PCoA) of Kolmogorov-Smirnov distances between flowering schedules of 369 plants from 13 populations of *Lythrum salicaria* sampled along a latitudinal gradient and grown in a common garden at the Koffler Scientific Reserve in Newmarket, ON. Point colors correspond to latitude of origin, ranging from dark (48° N) to light (38° N). Bar plots show representative flowering schedules of four individual plants. PCoA1 is inversely related to the central moments of variance and mean such that points on the right represent individuals with lower flowering schedule variance and an earlier peak.

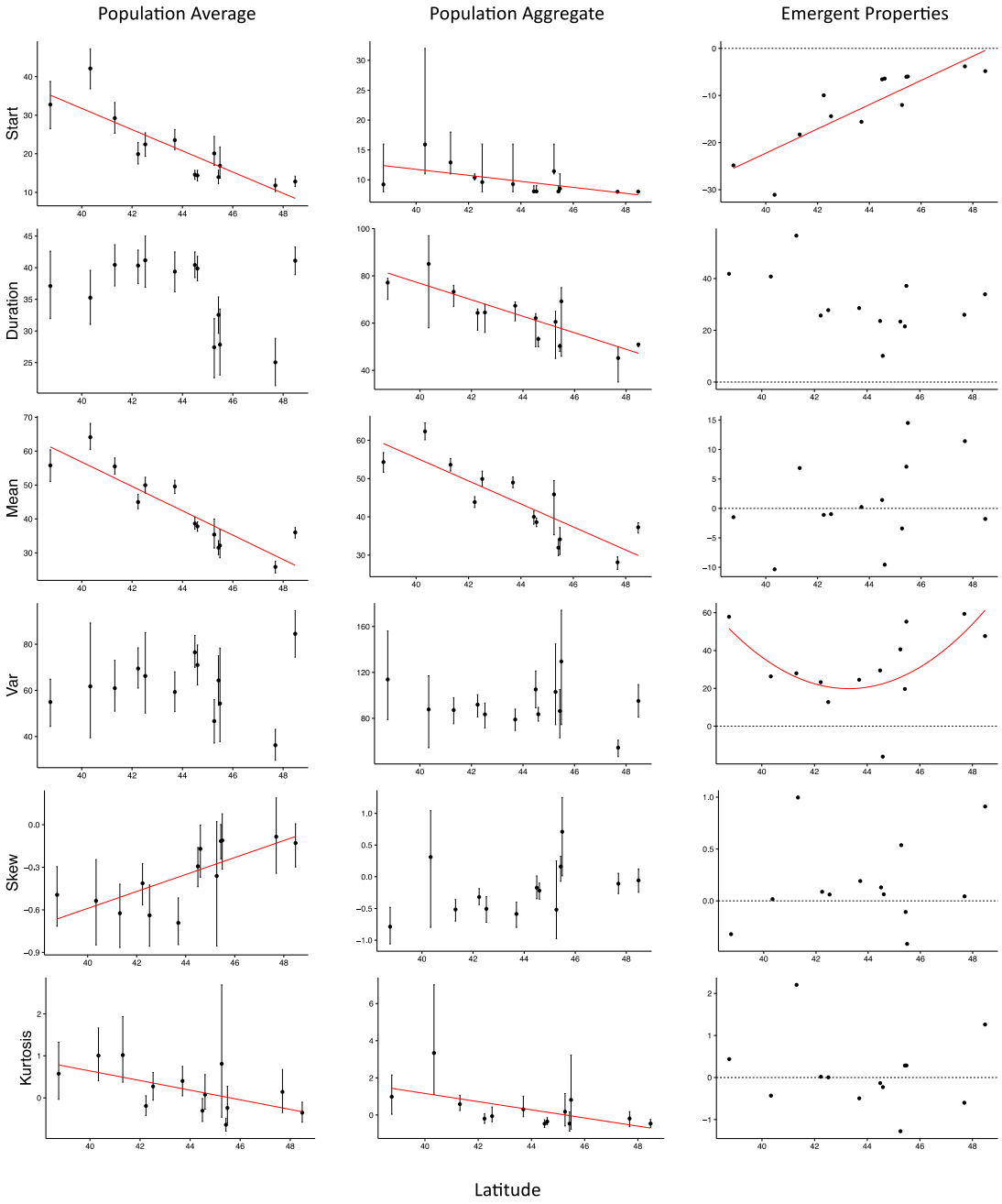

Figure 4. Bivariate plots showing relations between latitude and flowering schedule characteristics of 13 populations of *Lythrum salicaria* grown in a common garden field experiment. The bootstrapped estimate and 95% confidence intervals of *average* individual flowering schedule characteristics are shown for each population along the left column. The bootstrapped estimate and 95% confidence intervals of the *aggregate* flowering schedules for all flowers within each population are shown in the middle column. The right column indicates the 'emergent properties' of each population, defined as the aggregate minus the average.

Appendix S1. The distribution of 369 individuals and 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. Point colors correspond to latitude of origin ranging from dark (48° N) to light (38° N).

Latitude (°)	**Number of seed families**	**Number of 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

FIGURE LEGENDS

Figure 1. Hypothetical flowering schedules of (A) five individuals with different characteristics and (B) their pooled aggregate flowering schedule. Flowering schedules are scaled to the proportion of total flower production (P_t) over time (t).

Figure 2. Matrices showing pairwise Pearson correlation coefficients among the start, duration, central moments (i.e., variance, skew and kurtosis), and two the first two eigenvectors of a principal coordinates analysis (PCoA) characterizing flowering schedules in populations of *Lythrum salicaria* grown in a common garden. The metrics were calculated using either (A) flowering schedule characteristics *averaged* across individuals within each population (n = 13), (B) the *residual* correlations calculated on deviations of individuals from their population means (n = 369), or (C) *aggregated* schedules for individuals pooled within populations (n = 13). 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).

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