

STOCHASTIC PROCESSES DURING INVASION: THE INFLUENCE OF POPULATION SIZE ON STYLE-MORPH FREQUENCY VARIATION IN *LYTHRUM SALICARIA* (PURPLE LOOSESTRIFE)

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Premise of research. During biological invasion, the genetic diversity of populations may be reduced by founder events and genetic drift. The floral polymorphism tristily provides an exceptional opportunity to investigate the influence of stochastic forces on the maintenance of genetic polymorphism, because small population size leads to a characteristic signature of morph loss from populations. Here, we investigate the relations between population size and morph-frequency variation in invasive populations of tristylous *Lythrum salicaria* in Ontario, Canada. We also compare our results to a similar survey conducted 25 years ago in the same region.

Methodology. We surveyed the size and morph ratios of 114 *L. salicaria* populations in 2013. We calculated the relations between population size and style morph absence, population size and style morph evenness, and the number of populations lacking particular style morphs. For comparison of the patterns of morph-frequency variation between surveys, we used a sample of populations (1988/1989: 51 populations; 2013: 101 populations) with similar size distributions.

Pivotal results. Our survey confirmed that smaller populations were more likely to lack a style morph than larger populations and that morph ratios were less even in smaller populations. In dimorphic populations, the short-styled morph (S-morph) was absent most often, and the long-styled morph (L-morph) was least often missing, a pattern consistent with the stochastic theory of asymmetrical morph loss for tristylous species. There were no significant differences between the 1988/1989 and 2013 surveys in the frequency of populations missing style morphs or the relations between population size and style morph evenness.

Conclusions. Despite an increase in abundance of *L. salicaria* in Ontario during the past 25 years, genetic drift and founder events still play a dominant role in governing patterns of morph-frequency variation.

Keywords: biological invasion, floral polymorphism, founder events, frequency-dependent selection, genetic drift, population size, stochastic processes, tristily.

Introduction

Founder events and genetic drift are pervasive forces during the repeated episodes of colonization that characterize biological invasions. These stochastic processes have the potential to both limit and reduce genetic diversity in colonizing populations. The diversity of genetically based mating types in small populations of self-incompatible species is especially relevant because when mating types are lost through founder events and genetic drift, populations may experience pollen limitation of fertility or fail to reproduce sexually (Allee 1931; Byers and Meagher 1992; Vekemans et al. 1998; Ashman et al. 2004; Young and Pickup 2010; Barrett 2011). Though mate limitation may favor self-fertile individuals during establishment following long-distance dispersal (Baker 1955; Stebbins 1957), to date, there is mixed evidence that self-fertilization is commonly selected to

relieve mate limitation during biological invasion (Sutherland 2004; van Kleunen and Johnson 2007; Pannell 2015). Other landscape-level processes may aid in overcoming mate limitation, particularly as invasions mature in a region and populations become more abundant and less reproductively isolated. The increased connectivity resulting from pollen and seed dispersal may often serve to restore missing mating types and increase the genetic diversity of populations. Evidence for temporal changes in genetic diversity as biological invasions mature has not been investigated in colonizing species.

The genetic polymorphism tristily provides a valuable system for investigating the role of stochastic processes in colonizing populations. Populations of tristylous species are generally composed of three mating types or morphs (long-, mid-, and short-styled morphs, hereafter L-, M-, and S-morphs) distinguished primarily by style length and the position of anthers within a flower (Darwin 1877; Ganders 1979; Barrett 1993). Floral trimorphism is commonly associated with a trimorphic incompatibility system in which the style morphs are self- and intramorph-incompatible; the only compatible pollinations are intermorph and occur between anthers and stigmas of equivalent height. Large

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tristylous populations at equilibrium are expected to contain equal frequencies of the style morphs (isoplethy) when all three morphs are completely outcrossing and possess equal fitness (Fisher 1941; Fisher and Mather 1943; Heuch 1979*a*, 1979*b*). The principal mechanism maintaining 1:1:1 morph ratios in tristylous populations is negative frequency-dependent selection resulting from phenotypic disassortative mating (Barrett et al. 1987; Eckert et al. 1996*a*). However, surveys of style-morph frequencies in tristylous species frequently indicate deviations from this equilibrium expectation. Biased morph ratios (anisoplethy) or morph absence from populations of tristylous species can result from a variety of stochastic (e.g., Barrett et al. 1989; Husband and Barrett 1992; Eckert et al. 1996*b*) and deterministic (e.g., Barrett et al. 1983, 2004; Weller 1986) forces. Colonizing events and periods of small population size are commonly associated with stochastic processes, and therefore, successful tristylous invaders provide useful model systems for investigating the influence of demographic factors on morph-frequency variation.

Over the past century, purple loosestrife (*Lythrum salicaria*, Lythraceae), a tristylous wetland perennial, has become one of the most widespread invasive species in North America. The species is native to Eurasia but was introduced to the Eastern Seaboard during the past 150 yr, spreading over much of the northern regions of eastern North America and more recently to central and western North America (Thompson et al. 1987; Mal et al. 1992; Colautti and Barrett 2013). Studies of *L. salicaria* have a long and venerable history beginning with the seminal work of Darwin (1865, 1877), who characterized the general morphological features of tristily and performed controlled pollinations to establish the compatibility relationships of style morphs. Later, Fisher and Mather (1943) determined the genetic basis of tristily in *L. salicaria* through controlled crosses, and subsequent surveys of style-morph frequencies in European populations found general support for Fisher's theoretical prediction of 1:1:1 style morph ratios in populations at equilibrium (Haldane 1936; Schoch-Bodmer 1938; Halkka and Halkka 1974; Ågren and Ericson 1996). More recently, investigations of invasive *L. salicaria* populations in eastern North America have sought to determine the influence of finite population size on morph-frequency variation (Eckert and Barrett 1992) and to compare morph-frequency variation in the introduced versus native range of the species (Eckert et al. 1996*b*). These surveys indicate striking differences between continents, with patterns of variation in introduced populations consistent with those expected if stochastic processes were playing a more important role in governing variation in style-morph frequencies in comparison with native populations.

A particularly attractive feature of the tristylous polymorphism is that theoretical studies demonstrate that stochastic processes produce a characteristic signature of asymmetric morph loss because of the inheritance of tristily (Heuch 1980; Barrett et al. 1989; Eckert and Barrett 1992). Genetic studies in the three most widely studied families (Lythraceae, Oxalidaceae, Pontederiaceae) indicate that the polymorphism is governed by two diallelic loci (*S*, *M*), with the *S* locus epistatic to the *M* locus (Fisher and Mather 1943; Weller 1976; Lewis and Jones 1992; Gettys and Wofford 2008). Because the dominant *S*-allele that governs the expression of short styles is carried only by the *S*-morph (*S*—), it is at a lower frequency in equilibrium populations than the

remaining other three alleles (*s*, *M*, *m*) at the loci governing tristily. As a result, when all morphs possess equal fitness, the *S*-morph is more susceptible to stochastic loss from populations than the other style morphs. In contrast, the *L*-morph (genotype *ssmm*) should be rarely lost from populations, because the recessive *s* and *m* alleles are commonly present in genotypes of all three style morphs and therefore exist at higher frequencies in equilibrium populations (Heuch and Lie 1985). Thus, if genetic bottlenecks and drift play prominent roles in the population biology of a tristylous species, as a result of repeated colonizing events, there should be a characteristic pattern in which the *S*-morph is absent most often, the *L*-morph is rarely absent, and the *M*-morph is absent at a rate intermediate between the *S*- and *L*-morphs (i.e., probability of morph absence: *S*-morph > *M*-morph > *L*-morph). Empirical support for these theoretical predictions has come from extensive surveys of invasive *L. salicaria* populations in Ontario, Canada (Eckert and Barrett 1992; Eckert et al. 1996*b*), and in populations of the annual colonizer *Eichhornia paniculata* (Barrett et al. 1989) in Brazil and the Caribbean. Both species exhibit dimorphic populations that are predominantly composed of the *L*- and *M*-morphs.

Here, we measure style-morph frequency variation in *L. salicaria* populations in Ontario, Canada, in relation to their size. We asked a series of questions related to the influence of stochastic processes on morph evenness and morph loss from populations: (1) Is morph absence in populations related to their size? We predicted that style morphs would be missing from small populations more often than from large populations. (2) Does the evenness of morph ratios change with population size? We predicted greater morph evenness in larger populations than in small populations. (3) Among the three style morphs, is the *S*-morph most commonly absent from nontrimorphic populations, as predicted because of stochastic loss of the *S*-allele governing the phenotype of this morph? (4) Are there differences in the overall patterns of morph-frequency variation in comparisons between the 1988/1989 and 2013 surveys? *Lythrum salicaria* has increased in abundance and expanded its range dramatically in Ontario since Eckert and Barrett (1992) measured morph frequencies ~25 yr ago (Thompson et al. 1987; Colautti and Barrett 2013; Warne 2016; S. C. H. Barrett, personal observation). The change in abundance led us to hypothesize that there may be a greater connectivity among populations and more opportunities for gene flow than in 1988/1989, and this could result in a reduced frequency of nontrimorphic populations and greater evenness in morph frequencies.

Material and Methods

Study Species

Lythrum salicaria is an outcrossing, showy, insect-pollinated herb that was introduced to eastern North America from its native Eurasian range during the late eighteenth century (Thompson et al. 1987). The species possesses a trimorphic incompatibility system limiting opportunities for self- and intramorph mating; however, occasional individuals, particularly of the *M*-morph, are pseudo-self-compatible, enabling fertile seed to be produced from self- and intramorph mating (Colautti et al. 2010). *Lythrum salicaria* colonizes a variety of wetland habitats, particularly roadside ditches, marshes, and low-lying pas-

tures, exclusively through seed dispersal (Mal et al. 1992; Yakimowski et al. 2005). Seedlings of *L. salicaria* can reach flowering within 8–10 wk after germination (Shamsi and Whitehead 1974), and individuals are perennial and can survive up to 12 yr and perhaps longer (S. C. H. Barrett, personal observation). The species forms an extensive seed bank, which allows populations to regenerate quickly after disturbance (Yakimowski et al. 2005). Herbarium specimens indicate population expansion north and south along the North American Eastern Seaboard and northwest into southern Ontario during the twentieth century (Thompson et al. 1987; Blossey et al. 2001). Subsequently, rapid northward expansion of the geographical range of *L. salicaria* in Ontario has occurred over the last 50 yr (Colautti and Barrett 2013; S. C. H. Barrett, personal observation). The species is commonly associated with active transportation corridors and human settlement, with most of the dispersal northward in Ontario associated with human activities. In Ontario, populations flower between late June and mid-September, with peak flowering in most populations occurring in early to mid-August (Montague et al. 2008).

Population Surveys

In 2013, we estimated style morph frequencies in 114 populations of *L. salicaria* in Ontario (fig. 1). Our sampling area largely overlapped the geographic region sampled by Eckert and Barrett (1992) in their 1988/1989 survey of 102 populations;

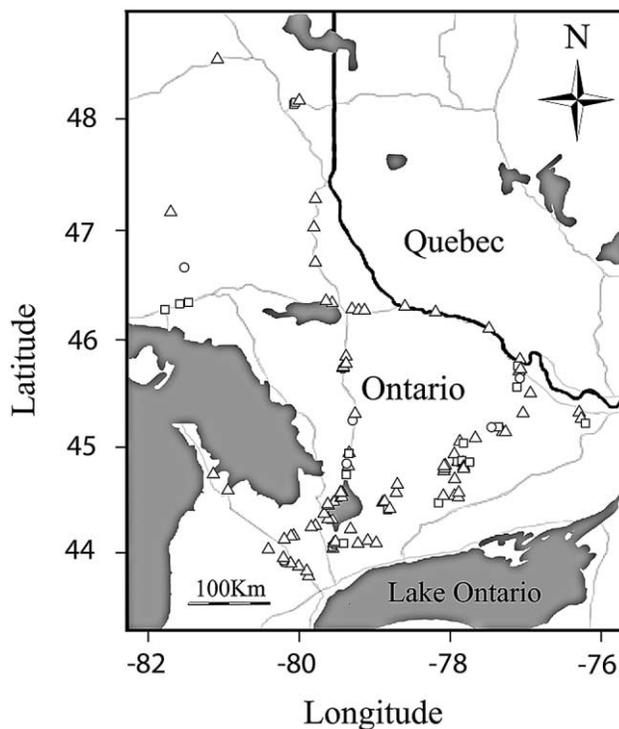


Fig. 1 Location of *Lythrum salicaria* populations sampled for style morph ratios in Ontario, Canada, during summer 2013. Trimorphic, dimorphic, and monomorphic populations are depicted as triangles, squares, and circles, respectively. Light gray lines represent major roadways, whereas black lines represent provincial borders.

however, it was not possible to resample the specific populations investigated by Eckert and Barrett (1992), because geographic coordinates were not available, and many easily identified populations were extirpated. However, we made a particular effort in our sampling to cover the main areas represented in their study. We defined a population as a group of three or more plants separated from other such groups by at least ~0.5 km; the vast majority of populations that we sampled were separated by more than 1 km. Because our study focused primarily on the role of stochastic forces on morph-frequency variation, we intentionally sampled populations mostly of ~100 or fewer individuals, because these are likely to be most susceptible to genetic drift, and populations above this size are predominantly trimorphic (see Eckert and Barrett 1992). The identification of genets in *L. salicaria* is straightforward because they develop into clumps with the root stock being the main organ of perennation (Shamsi and Whitehead 1974). In populations smaller than 100 individuals, we counted all flowering individuals in the population, whereas in populations larger than 100 individuals, we randomly sampled ~100 plants. We estimated census size in populations larger than 100 individuals by counting the number of individuals in two transects that intersected at 90° near the center of the population. We estimated the area covered by each population and calculated census size from the area and approximate density.

After comparing the 1988/1989 and 2013 data with a Wilcoxon sum-rank test, as expected, we found that our sampling scheme resulted in a smaller median population size in the 2013 survey than in the 1988/1989 survey ($W = 3341$, $P < 0.001$; 1988/1989 median = 125.5; 2013 median = 29.5; fig. 2A, 2B). To obtain comparable population size distributions for comparisons between years, we selected a subset of populations containing <130 individuals from each survey. This procedure resulted in no significant difference in median population size according to a Wilcoxon sum-rank test ($W = 2909$, $P > 0.25$; 1988/1989 median = 31, $n = 52$ populations; 2013 median = 28, $n = 101$ populations; fig. 2C, 2D). In most cases, subsequent comparisons between the two surveys used the subset of populations with these equivalent size distributions.

Style Morph Absence and Population Size

We examined data from each survey and survey subset for differences in morph-frequency distribution or evenness using de Finetti diagrams plotted with the R package ggtern (Hamilton 2015). Points close to the center of these plots represent populations with equal morph ratios, points along edges of the triangle are dimorphic and missing the morph labeled at that edge, and points at the apices of the triangle are monomorphic for style morph (see Eckert and Barrett 1992).

We tested the association between population size and morph absence in the 2013 survey using a 2×3 heterogeneity G-test. Our test compared the number of populations with three morphs (trimorphic populations) to the number of populations lacking at least one morph (nontrimorphic populations) in each of three size classes (3–25, 26–50, and >50 individuals). To investigate whether the likelihood of morph absence differed between the 1988/1989 and 2013 surveys, the population size classes, or the combination of survey and population size class, we used a 2×4 heterogeneity G-test that compared the number

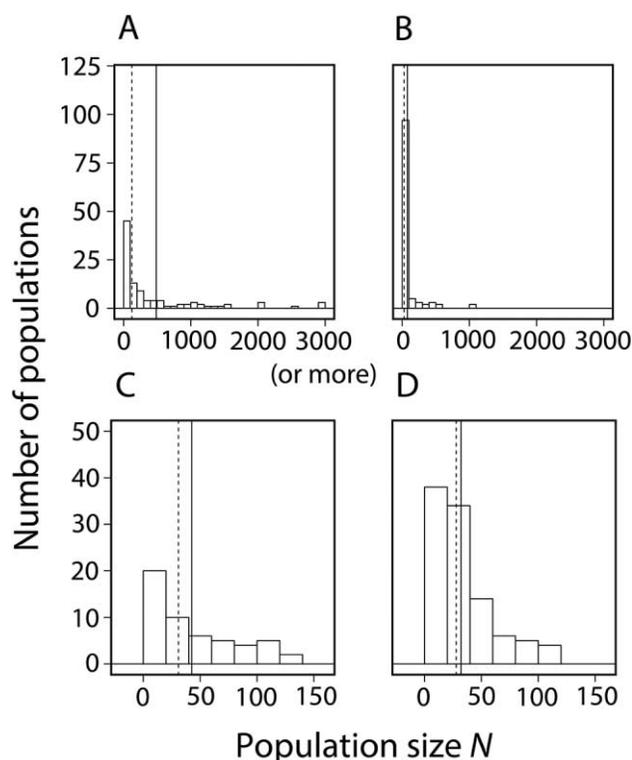


Fig. 2 Histograms of population sizes in *Lythrum salicaria* in Ontario, Canada. *A*, 1988/1989, complete survey. *B*, 2013, complete survey. *C*, 1988/1989, populations containing <130 individuals. *D*, 2013, populations containing <130 individuals. Solid lines represent mean population size, whereas dashed lines represent median population size. *A* and *B* possess different average population sizes ($W = 3341$, $P < 0.001$; estimated difference between survey medians = 76.0; 95% confidence interval [CI]: lower = 40, upper = 135), whereas *C* and *D* are similar in average population size ($W = 2909$, $P > 0.25$; estimated difference between survey medians = 4.0; 95% CI: lower = -3.0, upper = 15.0). Neither survey exhibits a normal distribution for population size.

of trimorphic and nontrimorphic populations present in each of two population size classes (3–25 and 26–130 individuals) from the 1988/1989 and 2013 surveys. In these analyses, we used two population size classes rather than three, because the 1988/1989 survey contained only nine populations in the 26–50 size class, and inclusion of this category reduced statistical power. We also conducted a series of 2×2 heterogeneity G -tests comparing the number of trimorphic and nontrimorphic populations across various combinations of size classes and survey years to investigate the individual effects of survey year and population size class on the frequency of trimorphic versus nontrimorphic populations.

Relation between Population Size and Evenness in 1988/1989 and 2013

We tested the effect of population size on morph evenness in the 2013 survey (all populations) using linear regression in R, version 3.1.1 (R Development Core Team 2014); R was also used for all further analyses. Population size was heavily skewed, and a few large populations overpowered the smaller populations.

Therefore, we used the natural logarithm of population size to mitigate the effects of large populations on the regression. We calculated a morph evenness index for each population:

$$E = \frac{1 - (f(L)^2 + f(M)^2 + f(S)^2)}{2/3}$$

where E represents morph evenness and $f(L)$, $f(M)$, and $f(S)$ are the frequencies of the L-, M-, and S-morphs, respectively (Barrett et al. 1989). This index equals 1 when the three style morphs are present at equal frequency in a population, 0 when only one morph is present, or a value between 0 and 1 when morph frequencies are uneven or one morph is absent.

We tested for differences in the relation between population size and morph evenness between the 1988/1989 and 2013 surveys using the combined data sets of the two subsamples. We constructed three linear regression models using the `lm` function in R. The most complex model explained the response variable (population evenness) as a function of the natural logarithm of population size, survey year, and the interaction of population size and survey year; the second model contained only the natural logarithm and survey year as terms; and the most simple model contained only the natural logarithm of population size as a predictor. We compared the three models by F -tests using the ANOVA function in R, which determined the significance of each survey year's intercept and the interaction between survey slopes relative to simpler models. These data possessed similar distribution shapes but also contained minor violations of model assumptions of normality and homoscedasticity. Therefore, we compared population evenness between years in the 3–25 and 26–130 individual size classes through a Kruskal-Wallis sum-rank test to validate our results.

Asymmetric Morph Loss

We identified the number of populations lacking each style morph in the two surveys and in the subsamples from each survey to determine whether the 2013 survey data exhibited a similar pattern of morph absence to the 1988/1989 survey. Formal statistical tests were not possible, because the absence of multiple morphs from some populations resulted in nonindependence and small sample sizes, thus preventing us from analyzing combinations of morph absences.

Results

Style Morph Absence and Population Size

Among the 114 populations that we sampled in 2013, 84 (74%) were trimorphic, 21 (18%) were dimorphic, and nine (8%) were monomorphic. Of 21 dimorphic populations, 12 contained only L- and M-morphs, six contained L- and S-morphs, and three contained M- and S-morphs. Of the nine monomorphic populations, eight contained only L-morph individuals, and one contained only M-morph individuals. Although our survey included a higher representation of small populations than the 1988/1989 survey, the proportion of populations that were trimorphic was not significantly different between the two complete surveys (1988/1989 = 77%; 2013 = 74%; $G = 0.414$, $df = 1$, $P > 0.50$). The majority of populations in the 1988/1989 and 2013 surveys clustered around the center of the de

Finetti diagram (fig. 3A and 3B, respectively). In the subsets of smaller populations from each survey, we observed, as expected, that a higher percentage of populations were missing a morph than in the full surveys and that points (populations) were also more scattered around the center of the de Finetti diagram (fig. 3C, 3D).

The proportion of nontrimorphic populations differed significantly between population size classes in the complete 2013 survey ($G = 7.97$, $df = 2$, $P < 0.02$; table 1). A lower proportion of large populations lacked a morph than smaller populations (3–25 individuals: 39% missing a morph; 26–50: 22%; >50: 12%), indicating a negative relation between population size and probability of morph absence. The 2×4 heterogeneity G -test, which investigated the differences between populations in each survey year and each population size category, revealed that the proportion of nontrimorphic populations in different population size classes and survey years was significantly different ($G = 10.77$, $df = 3$, $P < 0.02$; table 2, pt. A). However, the additional 2×2 heterogeneity G -tests, which investigated differences between the survey years or population size classes, did not detect significant differences

in the overall proportion of nontrimorphic populations between surveys ($G = 0.97$, $df = 1$, $P > 0.3$; table 2, pt. B) or between the surveys within each population size class ($G = 1.18$, $df = 1$, $P > 0.25$; table 2, pt. D; $G = 0.10$, $df = 1$, $P > 0.70$; table 2, pt. E). Further G -tests detected significant differences in the proportion of nontrimorphic populations present in the population size classes overall ($G = 9.5$, $df = 1$, $P < 0.01$; table 2, pt. C) and in the population size classes within each survey ($G = 5.05$, $df = 1$, $P < 0.03$; table 2, pt. F; $G = 4.76$, $df = 1$, $P < 0.03$; table 2, pt. G). G -tests comparing survey years (table 2, pts. B, D, E) do not support a difference between the surveys in the proportion of populations lacking a morph, but G -tests comparing population size classes (table 2, pts. C, F, G) demonstrated significant differences in the proportion of nontrimorphic populations in each size class. These results indicate that in both of the surveys, smaller populations are more likely to be nontrimorphic than larger populations, as predicted.

Relation between Population Size and Evenness in 1988/1989 and 2013

The linear regression of population evenness (E) over the natural logarithm of population size indicated a positive, significant relation between these two variables in the complete 2013 survey ($F = 12.7$, $df = 1, 112$, $P < 0.001$; fig. 4A). The small r^2 value from this relation (0.102) indicates that although greater style morph evenness tends to occur in larger populations, other unmeasured factors besides population size also contribute toward the evenness of morphs in populations.

In the reduced data sets excluding larger populations, we also detected a significant, positive relation between morph evenness and the natural logarithm of population size from the 1988/1989 ($F = 21.7$, $df = 1, 50$, $P < 0.0001$; fig. 4B) and 2013 ($F = 8.83$; $df = 1, 99$, $P < 0.01$; fig. 4C) surveys. The relations in each survey did not differ significantly in their slopes ($F = 0.775$, $df = 1$, $P > 0.35$) or intercepts ($F = 0.018$, $df = 1$, $P > 0.85$). The Kruskal-Wallis sum-rank test validated these findings, as there were no significant differences in morph evenness between surveys in either population size class (3–25 individuals: Kruskal-Wallis $\chi^2 = 0.40$, $df = 1$, $P > 0.50$; 26–130 individuals: Kruskal-Wallis $\chi^2 = 0.81$, $df = 1$, $P > 0.35$). These results indicate that for smaller populations of *Lythrum salicaria*, the relation between population size and morph evenness has not changed significantly between 1988/1989 and 2013.

Asymmetric Morph Loss

In 2013, data on morph representation from the complete survey of populations revealed that the S-morph was absent from populations most often (21 times), followed by the M-morph (14 times), and the L-morph was least often absent from populations (four times). Data from the restricted sample of smaller populations from 2013 was nearly identical, except that the S-morph was absent 20 times. These results parallel data from Eckert and Barrett (1992) in which the S-morph was absent 18 times and the M- and L-morphs were absent seven and one times, respectively. In the restricted 1988/1989 sample, the S-morph was absent 14 times, and frequencies

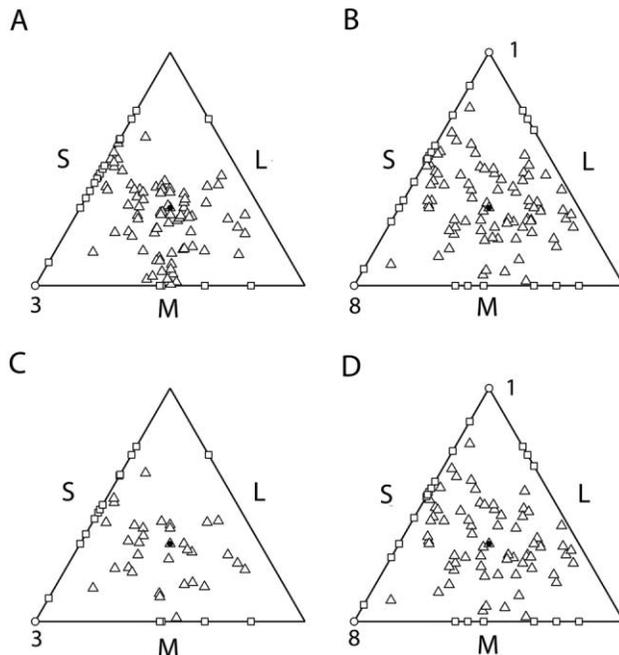


Fig. 3 De Finetti plots of population morph frequencies in populations of *Lythrum salicaria* sampled from Ontario, Canada. A, 1988/1989, complete survey. B, 2013, complete survey. C, 1988/1989, populations containing <130 individuals. D, 2013, populations containing <130 individuals. Triangles, squares, and circles represent trimorphic, dimorphic, and monomorphic populations, respectively. A point located farther from the L-, M-, or S-edge of the triangle contains a high frequency of the L-, M-, or S-morph, respectively, whereas a point on the edge of the triangle lacks the morph labeled on that edge. Numbers near the triangle apices represent the number of monomorphic populations with that morph present in the sample. In each study, the S-morph is absent more often than the M-morph, and the M-morph is absent more often than the L-morph.

Table 1

Heterogeneity G-Test Comparing the Number of Nontrimorphic and Trimorphic Populations between Size Classes in the 2013 Study of *Lythrum salicaria* in Ontario

Population size	Population count: observed (expected)		% Nontrimorphic	G	df	P
	Nontrimorphic	Trimorphic				
3–25	19 (13)	30 (36)	39	7.97	2	<u><.02</u>
25–50	7 (8)	25 (24)	22			
>50	4 (9)	29 (24)	12			

Note. Significant P value is underlined.

of absence of the L- and M-morphs were identical in both the complete and the restricted samples.

Discussion

The primary goal of our study was to investigate the role of stochastic forces during biological invasion using patterns of style-morph frequency variation in invasive populations of *Lythrum salicaria*. Theoretical models of the influence of finite population size on the maintenance of the tristylous genetic polymorphism make specific predictions about the expected patterns of morph-frequency variation and morph loss (Heuch 1980; Barrett et al. 1989; Eckert and Barrett 1992). Our results were generally consistent with the predictions of these models and indicate that repeated colonizing events and small pop-

ulation size play an important role in governing morph-frequency variation during invasion.

An earlier survey of invasive populations of *L. salicaria* in the same region (Eckert and Barrett 1992) provided us with an opportunity to examine whether patterns of morph-frequency variation have changed during 25 yr of ongoing invasion. The increased abundance of populations across the landscape has the potential to increase genetic connectivity among populations, which, in turn, may limit the intensity of stochastic processes acting on morph evenness and loss (see Eckert et al. 1996b). However, our comparisons of data collected in 1988/1989 and 2013 revealed few differences in the patterns of morph-frequency variation between the surveys. The similarity in results indicates that despite the overall differences in invasion age between the two surveys, stochastic processes continue to play an important role during this biological invasion, especially in small populations. We begin our discussion by considering

Table 2

Heterogeneity G-Tests Comparing the Proportion of Nontrimorphic and Trimorphic Populations of *Lythrum salicaria* in Ontario in Each Survey, Population Size Class, and Combination of Survey and Size Class in Each Survey Year

	Population count: observed (expected)			G	df	P
	Nontrimorphic	Trimorphic	% Nontrimorphic			
A. Complete model (year: population size):						
1988/1989: 3–25	13 (8)	12 (17)	52	10.77	3	<u><.02</u>
2013: 3–25	19 (15)	30 (34)	39			
1988/1989: 26–130	6 (8)	21 (19)	22			
2013: 26–130	10 (16)	42 (36)	19			
B. Compare years, pooled population size:						
1988/1989	19 (16)	33 (36)	37	.97	1	>.3
2013	29 (32)	72 (70)	29			
C. Compare population size, pool year:						
3–25	32 (32)	42 (50)	43	9.5	1	<u><.01</u>
26–130	16 (25)	63 (54)	20			
D. Compare years, populations of 3–25 individuals:						
1988/1989	13 (11)	12 (14)	52	1.18	1	>.25
2013	19 (21)	30 (28)	39			
E. Compare years, populations of 26–130 individuals:						
1988/1989	6 (5)	21 (22)	22	.10	1	>.70
2013	10 (11)	42 (41)	19			
F. 1988/1989 large vs. small populations:						
3–25	13 (9)	12 (16)	52	5.05	1	<u><.03</u>
26–130	6 (10)	21 (17)	22			
G. 2013 large vs. small populations:						
3–25	19 (14)	30 (35)	39	4.76	1	<u><.03</u>
26–130	10 (15)	42 (37)	19			

Note. Significant P values are underlined.

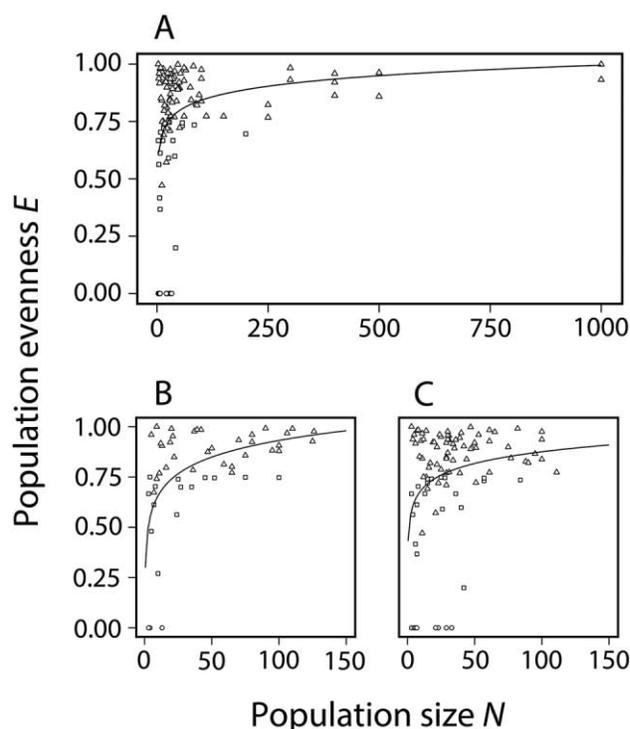


Fig. 4 Relation between morph evenness and population size in *Lythrum salicaria* populations sampled in Ontario, Canada, during summer 2013. A morph evenness index of 1 represents a population in which the three morphs are equally frequent, whereas values below 1 represent populations with unequal morph ratios or morph loss. A, All populations from the 2013 survey ($E = 0.54 + 0.006 \times \log(N)$). B, 1988/1989 survey for populations containing <130 individuals ($E = 0.382 + 0.119 \times \log(N)$). C, 2013 survey for populations containing <130 individuals ($E = 0.49 + 0.0836 \times \log(N)$). The triangles, squares, and circles represent trimorphic, dimorphic, and monomorphic populations, respectively. Lines represent a regression of the evenness index (E) as predicted by the natural logarithm of population size ($\log(N)$). Regressions for the complete 2013 survey and the restricted 1988/1989 and 2013 surveys are shown. All three regressions are significant; however, low r^2 values indicate that 90% of the variance in population evenness is not explained by population size (A: $r^2 = 0.102$, $P < 0.001$; B: $r^2 = 0.303$, $P < 0.0001$; C: $r^2 = 0.0819$, $P < 0.01$).

the diverse historical, demographic, and genetic factors that may explain the results of our study and then consider why the patterns of morph-frequency variation differ between native and introduced populations of *L. salicaria*.

The Pervasive Influence of Stochastic Forces in Invasive Populations

Despite 25 yr of ongoing invasion and an increase in the abundance of *L. salicaria* in many disturbed habitats of southern and central Ontario, the patterns of morph frequency observed in 1988/1989 and 2013 were remarkably similar. The relative frequencies of nontrimorphic populations in the two samples were not significantly different (1988/1989 = 23%; 2013 = 26%), and the specific style morphs that were absent

from dimorphic populations were essentially the same in both surveys, with the S-morph and L-morph most often and least often absent, respectively, results consistent with stochastic theory (Heuch 1980). In both surveys, we also found a positive relation between morph evenness and population size, with larger populations containing morph ratios closer to the isoplethic equilibrium. Despite the concordance between the findings of the two surveys, it is worth considering other explanations that may have caused the overall similarities in results.

The two independent surveys of morph-frequency variation in *L. salicaria* conducted 25 yr apart both involved a large number of populations distributed across the same parts of southern and central Ontario. A more optimal sampling strategy would have been to revisit the entire set of 102 populations originally surveyed by Eckert and Barrett (1992) in 1988/1989. However, GPS coordinates were not available from the earlier survey, and some easily located populations from the first survey were not located and were therefore presumed destroyed. Our sample, therefore, mainly involved *L. salicaria* populations that were not represented in the original survey. Because of the increased abundance of *L. salicaria* in Ontario, many of these new populations are likely to have been established since the 1988/1989 survey. Also, the survey we conducted in 2013 focused mainly on populations of <100 individuals because of our interest in looking for signatures of stochasticity, and as a result, the median population size was significantly smaller than the sample collected in 1988/1989. Because of this difference, we conducted comparisons of population samples from the two surveys, with similar population size distributions and median population sizes. It is possible that this subsampling procedure contributed to us not finding a difference between the samples, although comparisons involving the complete samples from both surveys gave similar results. Future work investigating the influence of invasion age on patterns of morph-frequency variation in *L. salicaria* should involve either the same sample of populations among years or a large random sample of populations across the region of interest.

Our initial assumption in comparing the two surveys was that the increased abundance of *L. salicaria* in Ontario during the 25 yr between samples should have reduced the influence of stochastic forces on morph-frequency variation, even in smaller populations. Higher overall morph evenness values and fewer populations missing style morphs may have been expected. Such patterns could potentially occur through two mechanisms. First, the degree of isolation of many populations along roadsides and other transport corridors should be reduced through time, with new populations colonizing areas that were previously unoccupied. This process could influence the genetic connectivity of populations through pollen- and seed-mediated gene flow and buffer populations against morph loss even in small populations, as has been observed in France (Eckert et al. 1996b) and Spain (Costa et al. 2016). Second, populations surviving during the 25-yr period might increase in size, and this would serve to reduce the influence of stochastic processes on morph loss. Indeed, our data on the relation between population size and morph representation in populations support this possibility.

However, both of these initial assumptions about the demography and genetics of populations may be false. Although there are undoubtedly more populations of *L. salicaria* occupying disturbed habitats in Ontario today than 25 yr ago, includ-

ing some that are very large, the majority of populations are still small, and many are relatively isolated. It is therefore unclear how frequently gene flow plays a role in converting populations from stylar dimorphism to trimorphism. Moreover, although we have not studied changes in population size of *L. salicaria* in Ontario over many years in detail, our field observations indicate that population growth is not an inevitable feature of invasive populations. Rather, many populations remain relatively small, owing to restrictions on suitable habitat, with some becoming reduced in size or locally extirpated through either control measures or chance environmental disturbance (e.g., urban development and road widening). Significantly, Eckert et al. (1996a) found no relation between the magnitude of the increase in population size and changes in morph evenness in a sample of 24 populations sampled over a 5-yr period, suggesting that morph-frequency change even with population growth may be a relatively slow process, owing to demographic factors associated with perenniality and variation in amounts of sexual recruitment. Therefore, the dynamics of the invasion process in a particular region can be highly heterogeneous, with ongoing colonization and population turnover taking place over many decades, so long as there are unoccupied sites across the landscape. In a relatively long-lived perennial such as *L. salicaria*, nonequilibrium morph frequencies may persist for long time periods (see Eckert et al. 1996a), and thus, a greater duration between sampling intervals than our 25-yr interval may be needed to observe significant changes in the patterns of morph-frequency variation in an invaded region.

Style Morph Absence from Populations in Ontario versus Europe

Several investigations of style-morph frequency variation in *L. salicaria* populations in Europe provide an opportunity to compare patterns between native and introduced populations. The overall results of European surveys generally indicate that populations differ significantly from those in Ontario. Approximately 95% of European populations that have been surveyed are tristylous (Haldane 1936; Schoch-Bodmer 1938; Halkka and Halkka 1974; Andersson 1994; Ågren and Ericson 1996; Eckert et al. 1996b; Costa et al. 2016), compared with only ~75% of populations in Ontario (Eckert and Barrett 1992; this study). Two large-scale surveys of style-morph frequency variation in France (Eckert et al. 1996b, $n = 102$ populations) and the Iberian Peninsula (Costa et al. 2016, $n = 96$ populations) are worth highlighting, as both used the similar sampling approaches used in the two Ontario surveys. In each European survey, only five populations of *L. salicaria* were found that lacked a style morph. The large difference (~20%) in frequency of nontrimorphic populations between the native and the introduced range of *L. salicaria*, therefore, raises the question of what factor(s) might account for these different geographical patterns.

The difference in the degree of nontrimorphism between Ontario and European populations of *L. salicaria* may arise from differences in metapopulation dynamics between the regions. Computer simulations suggest that gene flow on the order of $m \geq 0.05$ between populations in a metapopulation has the potential to maintain tristylous in small populations by restoring absent morphs to dimorphic populations (Eckert et al. 1996b).

Fossils of *L. salicaria* in Eurasia have been dated to 10,000 yr before present (Graham 2013), indicating that this species has had a much longer time to saturate suitable habitats in its European range relative to the much shorter time period that the species has been present in Ontario (Thompson et al. 1987). Thus, according to this argument, native European metapopulations have had a significantly longer time to reach drift-migration equilibrium. Some level of ongoing colonization is obviously a feature of all species (Lewontin 1965), regardless of whether one considers native or introduced populations; however, it seems probable that native populations of *L. salicaria* are largely tristylous, because gene flow limits the persistence of nontrimorphic populations over long timescales. Estimates of morph evenness are significantly higher for trimorphic populations in France compared to those in Ontario (Eckert et al. 1996b), further supporting the view that long-term gene flow in native populations reduces the influence of stochastic forces on morph-frequency variation.

Another factor that may contribute to the difference in frequency of nontrimorphic populations between native and introduced populations of *L. salicaria* concerns the types of landscapes in which *L. salicaria* occurs. Large areas of Europe, particularly in France and the Iberian Peninsula, are composed of agricultural land, thus providing *L. salicaria* with numerous dispersal routes along roads and drainage ditches and a high level of habitat connectivity (Eckert et al. 1996b; Costa et al. 2016). Although agriculture activities are a dominant feature of landscapes in southern Ontario, where the majority of our populations were sampled, a portion of the area we sampled was on the Canadian Shield, where mixed deciduous and coniferous forests dominate. In forested regions of central Ontario, *L. salicaria* populations are less abundant across the landscape and are largely restricted to open roadside ditches and disturbed wetlands. This pattern of distribution is less likely to permit gene flow among populations, potentially enhancing the significance of founder effects and genetic drift and contributing to the lower levels of evenness and higher percentage of nontrimorphism in Ontario populations.

It is often difficult to determine whether genetic drift or founder events are the specific cause of morph loss from tristylous populations. However, several of the patterns revealed in surveys of native versus introduced populations of *L. salicaria* suggest that the relative importance of these two stochastic processes may differ between Europe and Ontario. In their comparison of native versus introduced populations, Eckert et al. (1996b) found that the probability of morph evenness and morph loss was more strongly associated with population size in the native compared to the introduced range. Only very small populations of less than 10 individuals in France lost morphs, a pattern also found in the Iberian Peninsula by Costa et al. (2016), where all nontrimorphic populations were less than 15 individuals. These results are consistent with the operation of genetic drift. In contrast, nontrimorphic populations in the introduced range can occasionally be quite large (e.g., Ontario: $N = 100\text{--}500$; Eckert and Barrett 1992). In our survey, dimorphic populations in Ontario possessed a median size of 25 individuals, in contrast to seven in dimorphic populations from Sweden (Ågren and Ericson 1996). Moreover, among the eight monomorphic populations of the L-morph revealed

by our survey, four were relatively large ($N = 21\text{--}33$ individuals) and above the threshold value of 15, below which theoretical studies indicate that genetic drift will play a dominant role in causing morph loss (Heuch 1980). Indeed, these models demonstrate that tristily can be maintained for up to 150 generations if population sizes are above 20 individuals. The occurrence of relatively large nontrimorphic populations in Ontario suggests that unlike the native range, founder events may play a more important role than genetic drift in explaining morph absence from populations of *L. salicaria*.

During invasion, colonizing plants often lack compatible mates, and weak self-incompatibility (pseudo-self-fertility) may therefore provide reproductive assurance to founding individuals (Levin 1996). Variation in the expression of pseudo-self-fertility among floral morphs may favor some morphs during founder events, potentially contributing to morph absence from populations. Beginning with Darwin's early work on *L. salicaria* (summarized in Darwin 1877), controlled crosses have consistently revealed a small number of self-fertile individuals, especially of the M-morph and to a lesser extent the L-morph (reviewed in Colautti et al. 2010). These individuals are capable of setting seed from self- and intramorph pollinations. As a result of the inheritance of tristily (Barlow 1923; Fisher and Mather 1943), plants of the M-morph following selfing always segregate M-morph progeny and in most cases L-morph progeny, whereas L-morph plants produce only L-morph prog-

eny. S-morph progeny are not produced in either case, because the dominant S-allele is restricted to genotypes of the S-morph. Thus, it is quite plausible that genetic drift played no role in the origin of the 12 dimorphic L-M-morph populations and eight monomorphic L-morph populations in our survey. Rather, these could have arisen from founder events favoring individuals of the M- and L-morph with leaky self-incompatibility, a process consistent with Baker's law (Baker 1955; Pannell 2015). Future studies comparing native and introduced populations of *L. salicaria* would be valuable to determine whether the invasion process may have selected for an increased prevalence of pseudo-self-fertility in North American populations.

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Literature Cited

- Ågren J, L Ericson 1996 Population structure and morph-specific fitness differences in tristylous *Lythrum salicaria*. *Evolution* 50:126–139.
- Allee WC 1931 Animal aggregations: a study in general sociology. University of Chicago Press, Chicago.
- Andersson S 1994 Unequal morph frequencies in populations of tristylous *Lythrum salicaria* (Lythraceae) from southern Sweden. *Heredity* 72:81–85.
- Ashman TL, TM Knight, JA Steets, P Amarasekare, M Burd, DR Campbell, MR Dudash, et al 2004 Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology* 85:2408–2421.
- Baker HG 1955 Self-compatibility and establishment after “long-distance” dispersal. *Evolution* 9:347–349.
- Barlow N 1923 Inheritance of the three forms in trimorphic species. *J Genet* 13:133–146.
- Barrett SCH 1993 The evolutionary biology of tristily. Pages 283–326 in D Futuyma, J Antonovics, eds. *Oxford surveys in evolutionary biology*. Oxford University Press, Oxford.
- 2011 Why reproductive systems matter for the invasion biology of plants. Pages 195–210 in DM Richardson, ed. *Fifty years of invasion ecology: the legacy of Charles Elton*. Oxford University Press, Oxford.
- Barrett SCH, AHD Brown, JS Shore 1987 Disassortative mating in tristylous *Eichhornia paniculata* (Pontederiaceae). *Heredity* 58:49–55.
- Barrett SCH, LD Harder, WW Cole 2004 Correlated evolution of floral morphology and mating-type frequencies in a sexually polymorphic plant. *Evolution* 58:964–975.
- Barrett SCH, MT Morgan, BC Husband 1989 The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 43:1398–1416.
- Barrett SCH, SD Price, JS Shore 1983 Male fertility and anisoplethic population structure in tristylous *Pontederia cordata* (Pontederiaceae). *Evolution* 37:745–759.
- Blossey B, LC Skinner, J Taylor 2001 Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodivers Conserv* 10:1787–1807.
- Byers DL, TR Meagher 1992 Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. *Heredity* 68:353–359.
- Colautti RI, SCH Barrett 2013 Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science* 342:364–366.
- Colautti RI, NA White, SCH Barrett 2010 Variation of self-incompatibility within invasive populations of purple loosestrife (*Lythrum salicaria* L.) from eastern North America. *Int J Plant Sci* 171:158–166.
- Costa J, S Castro, J Loureiro, SCH Barrett 2016 Variation in style morph frequencies in tristylous *Lythrum salicaria* in the Iberian Peninsula: the role of geographical and demographic factors. *Ann Bot* 117:331–340. doi:10.1093/aob/mcv173.
- Darwin C 1865 On the sexual relations of the three forms of *Lythrum salicaria*. *J Linn Soc Bot* 8:169–196.
- 1877 The different forms of flowers on plants of the same species. J Murray, London.
- Eckert CG, SCH Barrett 1992 Stochastic loss of style morphs from populations of tristylous *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* 46:1014–1029.
- Eckert CG, D Manicacci, SCH Barrett 1996a Frequency-dependent selection on morph ratios in tristylous *Lythrum salicaria* (Lythraceae). *Heredity* 77:581–588.
- 1996b Genetic drift and founder effect in native versus introduced populations of an invading plant, *Lythrum salicaria* (Lythraceae). *Evolution* 50:1512–1519.
- Fisher RA 1941 The theoretical consequences of polyploid inheritance for the mid style form of *Lythrum salicaria*. *Ann Eugen* 11:31–38.

- Fisher RA, K Mather 1943 The inheritance of style length in *Lythrum salicaria*. *Ann Eugen* 12:1–23.
- Ganders FR 1979 The biology of heterostyly. *N Z J Bot* 17:607–635.
- Gettys LA, DS Wofford 2008 Genetic control of floral morph in tristylous pickerelweed (*Pontederia cordata* L.). *J Hered* 99:558–563.
- Graham SA 2013 Fossil records in the Lythraceae. *Bot Rev* 79:48–145.
- Haldane JBS 1936 Some natural populations of *Lythrum salicaria*. *J Genet* 32:393–397.
- Halkka O, L Halkka 1974 Polymorphic balance in small island populations of *Lythrum salicaria*. *Ann Bot Fenn* 11:267–270.
- Hamilton N 2015 ggtern: an extension to “ggplot2,” for the creation of ternary diagrams. R package version 1.0.6.0. <https://cran.r-project.org/web/packages/ggtern>.
- Heuch I 1979a Equilibrium populations of heterostylous plants. *Theor Popul Biol* 15:43–57.
- 1979b The effect of partial self-fertilization on type frequencies in heterostylous plants. *Ann Bot* 44:611–616.
- 1980 Loss of incompatibility types in finite populations of the heterostylous plant *Lythrum salicaria*. *Hereditas* 92:53–57.
- Heuch I, RT Lie 1985 Genotype frequencies associated with incompatibility systems in tristylous plants. *Theor Popul Biol* 27:318–336.
- Husband BC, SCH Barrett 1992 Effective population size and genetic drift in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 46:1875–1890.
- Levin DA 1996 The evolutionary significance of pseudo-self-fertility. *Am Nat* 148:321–332.
- Lewis D, DA Jones 1992 The genetics of heterostyly. Pages 129–150 in SCH Barrett, ed. *Evolution and function of heterostyly*. Springer, Berlin.
- Lewontin RC 1965 Selection for colonizing ability. Pages 77–94 in HG Baker, GL Stebbins, eds. *The genetics of colonizing species: proceedings of the first international union of biological sciences symposia on general biology*. Academic Press, New York.
- Mal TK, J Lovett-Doust, L Lovett-Doust, GA Mulligan 1992 The biology of Canadian weeds. 100. *Lythrum salicaria*. *Can J Plant Sci* 72:1305–1330.
- Montague JL, SCH Barrett, CG Eckert 2008 Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *J Evol Biol* 21:234–245.
- Pannell JR 2015 Evolution of the mating system in colonizing plants. *Mol Ecol* 24:2018–2037.
- R Development Core Team 2014 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org>.
- Schoch-Bodmer H 1938 The proportion of long-, mid- and short-styled plants in natural populations of *Lythrum salicaria* L. *J Genet* 36:39–43.
- Shamsi SRA, FH Whitehead 1974 Comparative eco-physiology of *Epilobium birsutum* L. and *Lythrum salicaria* L. I. General biology, distribution, and germination. *J Ecol* 62:279–290.
- Stebbins GL 1957 Self fertilization and population variability in the higher plants. *Am Nat* 91:337–354.
- Sutherland S 2004 What makes a weed a weed: life history traits of native and exotic plants in the USA. *Oecologia* 141:24–39.
- Thompson DQ, RL Stuckey, EB Thompson 1987 Spread, impact, and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. US Fish and Wildlife Service, Washington, DC.
- van Kleunen M, SD Johnson 2007 Effects of self-compatibility on the distribution range of invasive European plants in North America. *Conserv Biol* 21:1537–1544.
- Vekemans X, MH Schierup, FB Christiansen 1998 Mate availability and fecundity selection in multi-allelic self-incompatibility systems in plants. *Evolution* 52:19–29.
- Warne A 2016 Purple loosestrife (*Lythrum salicaria*). *Best Management Practices in Ontario*. Ontario Invasive Plant Council, Peterborough.
- Weller SG 1976 The genetic control of tristily in *Oxalis* section *Ionoxalis*. *Heredity* 37:387–393.
- 1986 Factors influencing frequency of the mid-styled morph in tristylous populations of *Oxalis alpina*. *Evolution* 40:279–289.
- Yakimowski SB, HA Hager, CG Eckert 2005 Limits and effects of invasion by the nonindigenous wetland plant *Lythrum salicaria* (purple loosestrife): a seed bank analysis. *Biol Invasions* 7:687–698.
- Young AG, M Pickup 2010 Low S-allele numbers limit mate availability, reduce seed set and skew fitness in small populations of a self-incompatible plant. *J Appl Ecol* 47:541–548.