

## VARIATION OF SELF-INCOMPATIBILITY WITHIN INVASIVE POPULATIONS OF PURPLE LOOSESTRIPE (*LYTHRUM SALICARIA* L.) FROM EASTERN NORTH AMERICA

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Colonization may favor self-compatibility (SC) in invasive plants, a process consistent with Baker's law. We investigated this hypothesis in invasive eastern North American populations of tristylous *Lythrum salicaria* L. (purple loosestrife) by controlled self- and cross-pollinations of 124 plants sampled from 12 populations grown under uniform glasshouse conditions. We evaluated whether populations at the northern front of the invasion exhibited higher levels of SC than southern populations, which are closer to source populations for the North American invasion. We also sought evidence for morph-specific differences in the strength of trimorphic incompatibility. We used the ASTER statistical program to jointly model three measures of SC: (1) the probability of producing at least one seed following self-pollination, (2) the number of fruits produced by self-pollination, and (3) the average number of seeds per fruit resulting from self-pollination. Just over one-quarter of plants set at least one fruit after self-pollination, and there was a significant effect of style morph on variation in SC, with the mid-styled morph most compatible. Although variation for SC was detected in 11 of the 12 populations, there was no evidence that SC increased toward the northern range limit as a result of the invasion process.

**Keywords:** Baker's law, biological invasion, heteromorphic incompatibility, *Lythrum salicaria*, partial self-incompatibility, pollen limitation.

### Introduction

Plants at the margins of an expanding range may be isolated or occur in small populations, resulting in pollen limitation of seed set and Allee effects (Allee 1931; Baker 1955; Lloyd 1980; Davis et al. 2004; Elam et al. 2007). Stochastic forces and inbreeding can also reduce the frequency of deleterious recessive alleles in colonizing populations (Kimura et al. 1963; Bataillon and Kirkpatrick 2000; Pujol et al. 2009). Under these demographic and genetic conditions, selection may favor the breakdown of self-incompatibility (SI) because self-compatible (SC) individuals have the potential to maintain fertility under conditions of low density and to establish new populations following dispersal. This general principle, known as Baker's law (Baker 1955, 1967; Stebbins 1957), was initially used to explain the rarity of SI species on oceanic islands. However, recent theory suggests that frequent colonization episodes and metapopulation dynamics in many biogeographical contexts may also favor SC in colonizing plants (Pannell and Barrett 1998; Cheptou and Dieckmann 2002; Dornier et al. 2008).

Evidence supporting Baker's law and the importance of self-fertilization for colonizing success has largely involved comparative studies documenting a higher proportion of SC than SI species on islands (Baker 1967; McMullen 1987; Webb and Kelly 1993; but see Carr et al. 1986; Miller et al. 2008) or among introduced species (Brown and Marshall 1981; Price

and Jain 1981; Brown and Burdon 1987; Rambuda and Johnson 2004; but see Sutherland 2004). However, interpretation of the association between SC and colonizing success in introduced species is complicated by the absence of data on the relative rates of introduction of SC versus SI species (but see van Kleunen et al. 2008). The higher proportion of SC species among successful invaders may simply represent differences in importation (Colautti et al. 2006; Gravuer et al. 2008). Comparisons of conspecific populations that differ only in colonization history or demography could provide better tests of Baker's law (Lloyd 1980; Barrett and Shore 1987; Barrett et al. 1989; Cheptou et al. 2002; Schueller 2004; Busch 2005; Moeller and Geber 2005; Randle et al. 2009). However, such studies are rare for introduced species (but see Brennan et al. 2005; Lafuma and Maurice 2007), even though in principle biological invasions may provide opportunities to study the evolution of SC over contemporary timescales.

The breakdown of SI to SC is well documented in numerous flowering plant taxa and results from qualitative and/or quantitative changes to pollen-pistil interactions (de Nettancourt 1977; Barrett 1988; Stone 2002; Mable et al. 2005; Igic et al. 2008). SC can arise through diverse genetic mechanisms involving both major and minor genes (Levin 1996; Mable 2008). The transition can be rapid, for example, through the origin of homostyles in heterostylous species by recombination (reviewed in Barrett and Shore 2009) or by gradual changes to the strength of SI through intermediate stages that include partial or leaky SI, pseudo SC, and cryptic SI (Cruzan and Barrett 1996; Levin 1996). There is also evidence of a significant nongenetic component to the expression of SC in

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some species (Shore and Barrett 1986) and phenotypic plasticity for SI expression (Stephenson et al. 2000, 2003). Depending on the genetic mechanisms governing the breakdown of SI, the role of environmental factors in modulating the expression of SC, and the evolutionary cost of partial SI (Vallejo-Marín and Uyenoyama 2004), the timescales for transitions to complete SC may be quite variable. Short-term adaptive responses to pollen limitation under low-density conditions may occur if colonizing populations contain individuals with partial SI, enabling some selfed seed to be produced.

We investigated variation in SI in invasive populations of the perennial, animal-pollinated, tristylous species *Lythrum salicaria* L. (Lythraceae) from eastern North America to determine whether breakdown in SI expression was evident in recently colonized populations. *Lythrum salicaria* possesses a trimorphic self-incompatibility (TSI) system in which plants are usually self- and intramorph incompatible and fully compatible pollinations are those between anthers and stigmas of equivalent height (termed “legitimate pollinations”). Earlier controlled pollination studies of *L. salicaria* have detected low levels of SC, but they generally involved a small sample of individuals and populations (Darwin 1877; Stout 1923; Ottenbreit and Staniforth 1994; O’Neil 1994; Mal et al. 1999). Our goal in this study was to survey a larger sample of plants collected from populations along a latitudinal transect representing a colonization gradient in eastern North America. We assessed variation in the level of SI in 12 populations of *L. salicaria* sampled from Easton, Maryland, to Timmins, Ontario. The populations span  $\sim 10^\circ$  of latitude covering  $\sim 80\%$  of the latitudinal range of *L. salicaria* in eastern North America. Previous work on these populations identified latitudinal clines in flowering phenology and several other life-history traits implicating adaptive responses to local environmental conditions (Montague et al. 2008).

In this study, we address the following specific questions: (1) Is there evidence of variation in expression of SI among individuals, and if so, do the three style morphs (long-, mid-, and short-styled, hereafter L-, M-, and S-morphs) differ? Studies of tristylous species, including *L. salicaria*, commonly report weaker TSI in the M-morph than in the remaining style morphs (reviewed in Barrett and Anderson 1985; Barrett and Cruzan 1994). (2) Do populations differ in the strength of trimorphic incompatibility, and is there a correlation between latitude and SC? Specifically, we were interested in exploring whether populations at the northern front of the invasion exhibited higher levels of SC than southern populations, which are closer to putative source populations for the North American invasion.

## Material and Methods

### Study Species

*Lythrum salicaria* is native to Eurasia and was introduced to North America at the end of the eighteenth century, where it is now widespread, with populations in most U.S. states and Canadian provinces (Thompson et al. 1987). Herbarium records show a northern progression of the colonization front, beginning in the early twentieth century, from southeast New York near New York City, along the eastern seaboard, and

northwest into central Ontario, near the city of Timmins (Thompson et al. 1987). Although the number and locations of introductions to North America from Europe are not known with any certainty (see Houghton-Thompson et al. 2005; Chun et al. 2009), the spread of *L. salicaria* into southern and especially central Ontario, where the majority of our populations were sampled, is relatively recent (S. C. H. Barrett, personal observation). Colonization of new sites by *L. salicaria* occurs exclusively by seed dispersal because clonal growth is limited to ramet production from a common rhizomatous genet (Yakimowski et al. 2005). Studies of native populations have demonstrated that individuals in small populations receive both less total pollen and a higher proportion of incompatible pollen, resulting in reduced seed set (Ågren 1996; Waites and Ågren 2004). Surveys of style morph variation in Ontario populations indicate that stochastic processes (genetic drift and founder events) associated with small population size play an important role in influencing variation in mate availability (Eckert and Barrett 1992; Eckert et al. 1996a, 1996b).

### Seed Collection and Pollinations

We collected family-stratified, open-pollinated seed in the early autumn of 2003 from 12 populations in eastern North America using methods described by Montague et al. (2008). Sampling locations and population demographic parameters are provided in table 1. In summer of 2004, we grew 8 seedlings from 20 families from each population in  $2 \times 2$ -cm plug trays in glasshouses at the University of Toronto. After flowering, we randomly selected from each population 15 plants, from different families where possible, representing 6 L-, 6 M-, and 3 S-morphs. Plants were transplanted into plastic pots of 10-cm diameter after 3 wk and 12.5-cm pots 6 mo after germination, and then plants were allowed to grow for 2 yr in a common glasshouse environment maintained at  $20^\circ$ – $25^\circ\text{C}$ . In winter 2005 and 2006, we cut back plants to encourage vegetative growth and allowed them to regrow to flowering before pollination studies commenced in May 2006.

In controlled pollinations, we used three plants of the L-morph only as pollen donors, whereas the remaining 12 plants acted as both pollen donors and recipients for each population. Thus, 3 L-, 6 M-, and 3 S-morphs were used as maternal plants. We doubled the sample size of the M-morph because we were interested in comparing SC among populations, and previous studies found that it was the most self-compatible morph (Darwin 1877; Stout 1923; Ottenbreit and Staniforth 1994; O’Neil 1994; Mal et al. 1999). On the first day of anthesis, we pollinated an equal number of flowers with either self- or nonself pollen, and we continued pollinating “first-day flowers” on subsequent days until we had pollinated 10 self- and 10 cross-pollinated flowers per plant. We excluded 20 plants that did not produce enough flowers, resulting in a sample of 4–12 plants (median = 11) per population (table 1). Analyses (not shown) that excluded the two populations with the fewest plants (ONEC and NYLV in table 1) had no significant effects on the results presented.

In tristylous flowers, there are two stamen levels within each flower. For cross-pollinations, we chose stamen levels equivalent in height to the recipient stigmas (legitimate pollina-

Table 1

Geographic and Demographic Features of 12 Populations of *Lythrum salicaria* Sampled along a Latitudinal Gradient in Eastern North America

Population code	Latitude (°N) <sup>a</sup>	Longitude (°W) <sup>a</sup>	Elevation (m) <sup>a</sup>	Density (stems/m <sup>2</sup> ) <sup>a</sup>	Population size (stems) <sup>a</sup>	N	Region	Proportion SC
ONTI	48.48	81.30	231	15	1800	10	North	.20
ONEC	47.69	80.28	260	16	5600	4	North	.75
ONWH	45.49	76.32	393	30	6000	12	North/mid	.42
ONNO	45.42	80.23	266	11	4950	11	North/mid	.27
ONPG	44.59	78.13	252	35	14,000	12	North/mid	.17
ONDM	44.49	77.64	232	10	10,000	13	North/mid	.00
NYLV	43.70	76.19	66	7	2800	8	South/mid	.38
NYSC	42.52	79.18	250	5	3000	10	South/mid	.10
NYCH	42.24	75.85	288	...	...	10	South/mid	.40
NJHP	41.31	74.68	389	5	7500	12	South/mid	.33
NJPT	40.34	74.65	9	5	1250	11	South	.36
MDDB	38.75	75.99	8	1	300	11	South	.18

Note. N = number of plants used in pollination study; region = latitudinal region assigned in this study to test for geographic variation in self-compatibility (SC); proportion SC = proportion of plants setting at least one seed through self-pollination.

<sup>a</sup> Source is Montague et al. (2008).

nations) because these produce maximum seed set. Specifically, we cross-pollinated individuals of the L-morph with pollen from long-level anthers of the S-morph, M-morph individuals with pollen from midlevel anthers of the L-morph, and used pollen from short-level anthers of the L-morph to pollinate individuals of the S-morph. For self-pollinations of the L- and S-morphs, we used pollen from midlevel anthers, and pollen from long-level anthers was used to self-pollinate plants of the M-morph. These particular morph and stigma-anther combinations were chosen because previous studies of *L. salicaria* (Darwin 1877; O'Neil 1994) indicate that they are the most compatible self-pollinations. We used fine forceps for all pollinations and emasculated flowers that were cross-pollinated. In contrast to several previous studies of *L. salicaria* (O'Neil 1994; Mal et al. 1999), pollination of the S-morph did not involve cutting the calyx to apply pollen to stigmas.

Following controlled pollinations, we marked flowers with a small dab of colored paint at the base of the calyx to indicate treatments. We also used unpollinated flowers on each plant to assess inadvertent pollination, which was negligible. We left fruits to mature on plants for ~3 wk, after which they were harvested and placed into 0.5-mL centrifuge tubes for storage. We counted all plump, presumably viable seeds from up to five outcrossed and five selfed fruits. These were light brown in color and easily distinguished from aborted seeds, which tended to be smaller in size and black.

#### Statistical Analysis

Statistical analysis of variation in SC in species with partial SI presents several challenges. First, SC can be measured by either fruit or seed set, but these two measures are not necessarily independent. Second, the distributions of fruit and seed set are often highly skewed count data (i.e., Poisson distribution) and therefore violate assumptions of multivariate normality. Finally, in a species with relatively strong SI, a large number of individuals may set zero fruit through self-pollination, weakening the fit of the Poisson distribution. To address these problems, we used the ASTER statistical program (Geyer et al. 2007) designed for the analysis of demographic data. In

contrast to standard statistical methods (e.g., ANOVA), ASTER uses a maximum likelihood algorithm that accounts for dependencies among different components of the response variable, in our case SC, which have different probability distributions (Geyer et al. 2007).

We used ASTER models to test whether SC differed by style morph, geographic region, or the interaction between morph and region. We simultaneously modeled three measures of SC: (1) whether a plant was able to produce any seeds following self-pollination (Bernoulli variable); (2) the number of fruits produced by self-pollination (Poisson variable, truncated at 0), given that a plant produced at least one fruit through selfing; and (3) the number of seeds per fruit produced through self-pollination (Poisson variable, truncated at 0), given that a plant produced one fruit through selfing. We averaged seed set across five fruits (or all fruits if less than five) and rounded to the nearest whole number. Plants may differ in vigor, resource allocation to reproduction, patterns of sterility, and inbreeding depression, and any of these factors could influence our measurements of SC. To account for these potential sources of variation, we also included as covariates total fruit set and average seeds per fruit following cross-pollination. All plants produced at least one fruit through cross-pollination.

We used likelihood ratio tests in ASTER to test the significance of each factor: style morph, latitudinal region, and their interaction, as well as outcrossed fruit set and seeds per fruit. To test the significance of each factor, we removed it from the model and calculated a  $\chi^2$  statistic for each factor as  $-2\ln(\text{model}_2/\text{model}_1)$ , where  $\text{model}_2$  includes all factors and  $\text{model}_1$  excludes the factor of interest. However, the morph and region factors were tested against a model that did not include the region  $\times$  morph interaction. Because conventional calculations of the mean and standard error are not accurate measures of central tendency for overdispersed and skewed data, we report unconditional expectations ( $E_x$ ) and their standard errors, calculated using ASTER (details provided in Geyer et al. 2007).

As an additional test for geographical variation in SC, we calculated means for each of our three measures of SC and used these in separate least squares regressions against lati-

tude. In addition to latitude, we also investigated whether population size (ln transformed, estimated as the total number of vegetative stems) and population density (stem/m<sup>2</sup>) from data by Montague et al. (2008) influenced variation in SC. We used conventional means for these regressions because ASTER models would not converge with population as a factor, even after removing the two populations with the lowest replication. This was most likely an effect of the high variance in SC relative to our level of replication (i.e.,  $\leq 12$  individuals per population).

Finally, we compared our estimates of SC for the style morphs of *L. salicaria* with previously published studies (Darwin 1877; Stout 1923; Ottenbreit and Staniforth 1994; O'Neil 1994; Mal et al. 1999). We used seeds per pollination as a standard metric, rather than seeds per fruit or fruits per plant, because it was the only metric that we could calculate for all studies. Although not all pollination studies of *L. salicaria* have employed exactly the same anther-stigma combinations, this is unlikely to influence an assessment of the general patterns of morph-specific expression in SC.

## Results

### General Patterns

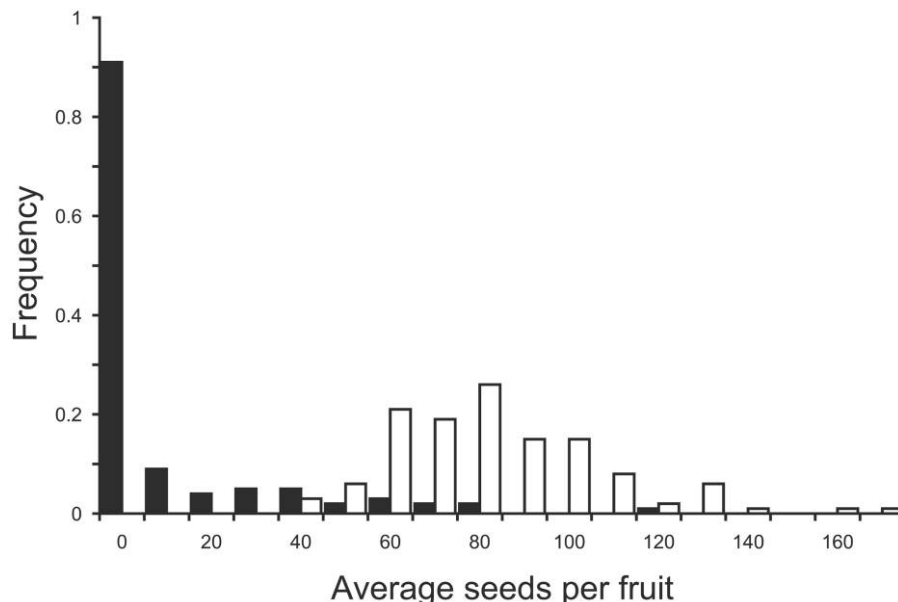
The results of experimental pollinations of *Lythrum salicaria* indicated that the species possesses a relatively strong TSI system. However, we detected some evidence for partial SI within most populations (table 1). The average proportion fruit set from cross- and self-pollination of all plants ( $N = 124$ ) in our experiment was 89.4% and 6.6%, with an average of 78.0 (SE = 2.13; range 31–161) and 33.3 (SE = 4.48; range 5–111) seeds per fruit, respectively. Despite the low level

of SC overall, there was significant variation among plants in their response to self-pollination. About one-quarter of the plants (33) produced seeds following self-pollination (fig. 1).

Mean values for fruit and seed set were overdispersed and highly skewed relative to a normal distribution, making means and standard errors unreliable estimates of central tendency. We therefore used ASTER to calculate global expectations of SC. Expectations from ASTER are analogous to parametric means and standard errors but account for the nonnormal distribution of the data. In this case, global expectations were calculated from a basic model containing a single intercept (i.e., no effect of morph, region, etc.). The expected proportion of plants setting at least one fruit through self-pollination was 0.266 ( $N = 124$ , SE = 0.039), the expected percent of fruits per self-pollination was 6.61% ( $N = 124$ , SE = 1.17%), and the expected number of seeds per self-pollination was 8.86 ( $N = 124$ , SE = 1.35). The discrepancy in seed set between the mean and the ASTER expectation can be explained by the large proportion of plants that produce no seed through self-pollination, which is included in the ASTER expectation.

### Absence of Geographical or Demographic Patterns

Despite the observed variation among *L. salicaria* plants in the strength of TSI expression, SC did not vary significantly by latitude or geographic region. In addition, SC was not correlated with the number of fruits or seeds per fruit resulting from cross-pollination (table 2). Regressions with latitude were nonsignificant for all the three measures of SC: (1) the proportion of individuals producing at least one fruit through self-pollination ( $N = 12$ ,  $R^2 = 0.061$ ,  $P = 0.442$ ), (2) the average number of self-pollinated fruits ( $N = 12$ ,  $R^2 = 0.122$ ,



**Fig. 1** Frequency distribution of individuals of tristylous *Lythrum salicaria* exhibiting variation in mean seed set per fruit (averaged across five fruits or across all fruits for plants producing fewer than five fruits) from either cross-pollination (open bars) or self-pollination (solid bars).

Table 2

Likelihood Ratio Tests of Floral Morph, Geographic Region, and Morph  $\times$  Region Interaction on Variation in Self-Compatibility (SC) of *Lythrum salicaria*

Factor	df	$\chi^2$	P
Categorical variables:			
Floral morph	2	9.1	.011
Geographic region	3	5.9	.117
Region $\times$ morph	7	26.6	<.001
Continuous variables:			
Fruits (crossed)	1	.8	.371
Seeds per fruit (crossed)	1	2.3	.129

Note. Significance estimates are from likelihood ratio tests of nested, unconditional ASTER models of 124 individuals, sampled from 12 populations in eastern North America. Two covariates—fruit set and seed set from cross-pollinations—are included to account for variation in fertility. SC is a composite measure based on fruit set and average seeds per fruit produced through self-pollinations.

$P = 0.267$ ), and (3) the average number of seeds per self-pollinated fruit ( $N = 12$ ,  $R^2 = 0.167$ ,  $P = 0.188$ ). When populations were grouped by geographic region, the full ASTER model, which simultaneously included all three measures of SC as response variables, also failed to approach significant levels (table 2). The lack of any geographical pattern in SC is the result of a relatively even but low distribution of plants with partial TSI among the populations sampled. Eleven of the 12 populations contained at least one plant capable of producing seed through self-pollination, and plants with high SC values occurred in populations from both northern and southern regions (table 1). Also, there was no significant effect of population size (ln transformed:  $N = 12$ ,  $P > 0.646$ ) or density ( $N = 12$ ,  $P > 0.656$ ) on variation in any of the three measures of SC.

#### Morph-Specific Differences in SC

There was a significant effect of floral morph on seed set after self-pollination and a significant interaction between morph and geographic region with respect to variation in SC (table 2). Morph-specific effects on SC were evident for the overall ASTER model (table 2) and also for specific SC measurements: fruit set (morph  $P = 0.014$ ; morph  $\times$  region  $P < 0.001$ ) and seeds per fruit (morph  $P = 0.012$ ; morph  $\times$  region  $P < 0.001$ ). On average, the M-morph was most self-compatible, followed by the S- and L-morphs. This pattern occurred for both the proportion of SC plants in a population sample and the average number of seeds per fruit (fig. 2). As shown in figure 3, this result is consistent with several earlier studies of *L. salicaria* (Darwin 1877; Stout 1923; Ottenbreit and Staniforth 1994; O'Neil 1994; Mal et al. 1999). Higher SC was also reported for the M-morph by Barlow (1913), but fruit and seed counts were not provided. However, the SC detected in some individuals of the S-morph in our study contrasts with the results of O'Neil (1994) and Mal et al. (1999), who found no evidence for SC in this morph. The significant morph  $\times$  region interaction was driven largely by variability in SC of the S-morph among regions (fig. 3).

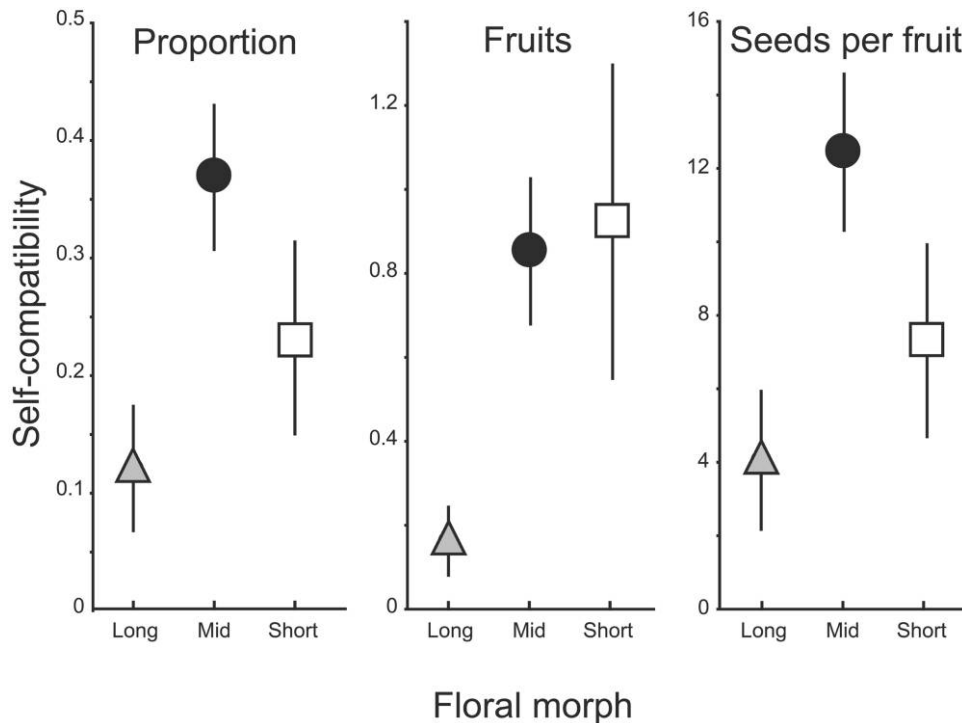
## Discussion

In this study, we sought evidence that the invasion process may be associated with selection for SC in plants of tristylous *Lythrum salicaria* sampled from 12 populations spanning much of the latitudinal range in eastern North America. Although our controlled self- and cross-pollinations revealed considerable individual variation in the strength of TSI, there was no evidence that this variation was geographically structured and associated with the likely historical pattern of migration. We consider why our analysis may have failed to reveal geographical patterns in the distribution of SC and discuss the possible causes and consequences of our finding of morph-specific variation in the strength of TSI.

#### Colonization and Selection for Self-Compatibility

There is considerable theoretical and comparative evidence to indicate that recurrent colonizing episodes favor SC individuals, particularly those that are capable of autonomous self-pollination (Baker 1955, 1967; Brown and Burdon 1987; Pannell and Barrett 1998; Rambuda and Johnson 2004; Cheptou and Schoen 2007; van Kleunen et al. 2008). Under low-density conditions when ovules may be pollen limited, selection should favor individuals with some level of SC over those that are strongly SI. This of course does not preclude SI species from becoming invasive, and there are numerous examples of successful colonizers that maintain SI systems (Friedman and Barrett 2008 and references cited therein), particularly among species with perennial life histories (Barrett, forthcoming). Nevertheless, several features of *L. salicaria* led us to suspect that the invasion process in eastern North America may have been accompanied by erosion in the strength of TSI and the evolution of SC, at least in some populations.

First, because *L. salicaria* is heterostylous and possesses TSI, we would expect that the strength of selection for SC should be stronger than in species possessing homomorphic SI systems. This is because heterostylous species have only two or three floral morphs, whereas the number of compatible mating types in homomorphic SI systems is potentially much larger (but see Byers and Meagher 1992; Bechsgaard et al. 2004). Second, previous studies of *L. salicaria* have consistently reported the occurrence of individuals capable of setting abundant seed on self-pollination (fig. 3). Indeed, partial SI is a ubiquitous feature of most heterostylous groups (reviewed in Cruzan and Barrett 1996). This variation is a prerequisite for selection response and the evolution of SC, assuming that it has a heritable basis (Shore and Barrett 1986; Barrett and Anderson 1985). Finally, there is considerable evidence from other heterostylous species for the breakdown of heteromorphic incompatibility and the evolution of self-compatible forms (reviewed in Barrett 1989; Weller 1992; Cruzan and Barrett 1996), and in several cases, this is associated with colonization processes and conditions expected under Baker's law (Baker 1966; Ornduff 1972; Shore and Barrett 1986; but see Larson and Barrett 1999). However, contrary to our predictions, we found no obvious patterns in the geographical distribution of SC individuals. There was no significant effect of region on variation in SC and no



**Fig. 2** Unconditional expected mean estimates ( $E_x$ ) and standard errors of self-compatibility measures for each floral morph of tristylous *Lythrum salicaria* estimated from ASTER models and the experimental pollination data. Proportion = expected proportion of individuals setting at least one fruit following self-pollination; fruits = expected number of fruits set following self-pollination; seeds per fruit = expected number of seeds per fruit following self-pollination.

effect of latitude on the average level of SC in a population. Although variation in SC was evident in most populations, SC plants were distributed evenly across the sampled range.

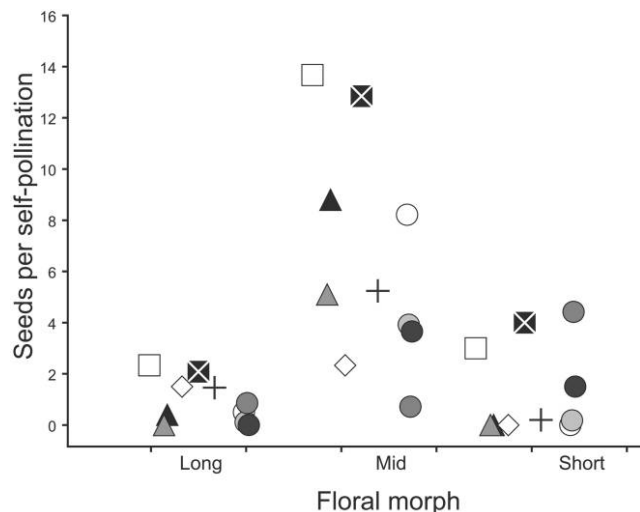
There are several possible explanations for our failure to find evidence for Baker's law among invasive populations of *L. salicaria*. Although herbarium records and our field studies over the past 30 yr generally support a south to north colonization gradient at a large geographical scale (Thompson et al. 1987; S. C. H. Barrett, personal observation), populations are still spreading locally throughout this region regardless of latitude. As a result, the latitude of a given population probably represents only a rough correlate of time since North American colonization. Moreover, since the number and locations of *L. salicaria* introductions to North America are unknown, it is possible that the migration of the species in eastern North America is more complicated than our simple latitudinal transect implies. In this regard, it is important to note that we also found that no measure of SC was correlated with population size or density, and we also failed to detect any significant difference among regions, regardless of the latitude of populations.

Another explanation for our failure to show geographical differentiation in SC among populations of *L. salicaria* could involve the genetic and environmental basis of SC variation in this species. The heritability of SC in *L. salicaria* has not been investigated in detail; however, the pattern of weak TSI in the M-morph commonly observed in studies of tristylous plants, including *L. salicaria* (reviewed in Barrett and Cruzan

1994), is unlikely to be explained solely by environmental effects, especially in our work where plants were grown under uniform glasshouse conditions for several years. However, SC in some species has been shown to have transient expression, often disappearing in the offspring of SC plants (de Nettancourt 1977; Shore and Barrett 1986), and it is possible that if there is a significant nongenetic component to SC expression, selection for high SC may be constrained. Alternatively, partial SC may represent the best of both worlds (sensu Becerra and Lloyd 1992), insofar as it allows reproduction by early colonists but thereafter becomes effectively nonfunctional if compatible mates arrive in populations and outcross pollen delivery is sufficient to maintain fertility. In a perennial such as *L. salicaria*, initial SC colonizers, and therefore alleles for SC, may persist for a considerable time unless inbreeding depression is particularly strong. Under this scenario, selection for full SC may be quite weak in *L. salicaria* populations, especially if population growth is rapid following colonization and outcross pollen is favored over self-pollen when mates are available (Cruzan and Barrett 1996; Levin 1996). All 12 populations that we investigated in this study were tristylous, and it is therefore unlikely that there would have been a deficit of cross-compatible pollen delivered to stigmas.

#### *The Role of the Mid-Styled Morph in Colonization*

Pollination studies on *L. salicaria* have shown that individuals of the M-morph often have weak TSI expression, espe-



**Fig. 3** Comparison of morph-specific variation in self-compatibility (SC) from five controlled pollination studies of *Lythrum salicaria*. SC is measured as the average number of seeds per self-pollinated flower (i.e., total seeds/N pollinations). Studies are represented by squares (Darwin 1877), squares with an X (Stout 1923), triangles (O'Neil 1994), diamonds (Mal et al. 1999), crosses (Ottenbreit and Staniforth 1994), and circles (this study). O'Neil's (1994) data includes two populations: Barrington (black) and Tiverton (gray). Shading of circles distinguishes geographic regions for the data from this study: south (black), mid/south (dark gray), mid/north (light gray), and north (white). One outlier was excluded from the S-morph from the mid/south region.

cially when self-pollinated with pollen from long-level anthers. Curiously, this morph-specific pattern is also a feature of species in other tristylous families (reviewed in Barrett and Anderson 1985; Barrett and Cruzan 1994). Although the proximate genetic and physiological mechanisms responsible for weak TSI in the M-morph are not understood, the ecological consequences of partial SI expression are worth considering. Both theoretical and experimental evidence indicates that the M-morph of tristylous species is more susceptible to self-pollination and displays higher selfing rates than the L- and S-morphs (Charlesworth 1979; Kohn and Barrett 1992). This probably occurs because the M-morph possesses two stamen levels that are "one level" away from the stigma, whereas the other morphs have only one stamen level closer to stigmas. Significantly, in addition to this architectural feature of tristylous, there is evidence in *L. salicaria* for genetic variation for herkogamy, with the M-morph characterized by a significantly smaller stigma-anther separation than the other morphs (Mal and Lovett-Doust 2005). The combination of partial TSI expression and lower herkogamy in the M-morph could increase the colonizing potential of this morph by allowing it to establish colonies through autonomous self-pollination following dispersal.

What evidence is there that in *L. salicaria* there is polymorphism for colonizing ability because of morph-specific differences in compatibility and herkogamy? A large-scale survey of style morph frequencies in 102 populations of *L. salicaria*

in Ontario revealed that 20 were dimorphic, and of these, 75% had lost the S-morph (Eckert and Barrett 1992). This specific pattern was predicted on the basis of stochastic modeling of the two-locus diallelic genetic system controlling the inheritance of tristylous in *L. salicaria*. Because the dominant S allele governing the expression of the S-morph occurs at a lower frequency than the remaining alleles (*s*, *M*, *m*) at the tristylous loci and is only carried by the S-morph, it is more vulnerable to stochastic loss (see Fisher and Mather 1943; Heuch 1980). A likely mechanism for this process would be founder events involving the M-morph. Isolated plants of the M-morph that have the capacity for autonomous or pollinator-mediated selfing could found dimorphic populations composed of the L- and M-morphs if they were heterozygous at the M-locus. Alternatively, dimorphism could result from bottlenecks and genetic drift as a result of changes in population size. Although the relative importance of founder events versus genetic drift in invasive populations of *L. salicaria* in eastern North America is not known, it seems likely that partial TSI expression in individuals of the M-morph could account for the origin of some dimorphic populations. Comparisons of seed set and rates of colony establishment in isolated individuals of the three floral morphs could be used to evaluate this hypothesis. In addition, it would be crucial to determine whether inbreeding depression influences this scenario by either preventing or at least limiting population growth after colony establishment from isolated founders.

In conclusion, our study has documented variation in the expression of TSI within invasive populations of *L. salicaria* in eastern North America. Our results are not sufficient to reject Baker's law and a role for SC during colonization. This is because the morph-specific differences in SC that our pollination studies revealed could account for the high frequency of dimorphic populations of *L. salicaria* composed of the L- and M-morph in this region. Theoretical models often treat the compatibility systems of plants as a simple dichotomy involving SI and SC individuals. Our studies raise the possibility that partial SI may allow a degree of flexibility in the regulation of mating, depending on local demographic conditions and the availability of mates. If context-dependent mating related to density is a feature of *L. salicaria* populations, it may serve to limit the selective advantage of fully SC genotypes. Future models of mating system evolution in colonizing species could usefully consider the role of partial SI in population establishment.

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## Literature Cited

- Ågren J 1996 Population size, pollinator limitation and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology* 77:1779–1790.
- Allee WC 1931 Animal aggregations: a study in general sociology. University of Chicago Press, Chicago.
- Baker HG 1955 Self-compatibility and establishment after “long-distance” dispersal. *Evolution* 9:347–349.
- 1966 Evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. *Evolution* 20:349–368.
- 1967 Support for Baker’s law: as a rule. *Evolution* 21:853–856.
- Barlow N 1913 Preliminary note on heterostylism in *Oxalis* and *Lythrum*. *J Genet* 6:53–65.
- Barrett SCH 1988 The evolution, maintenance and loss of self-incompatibility systems. Pages 98–124 in J Lovett-Doust, L Lovett-Doust, eds. Reproductive strategies of plants: patterns and strategies. Oxford University Press, New York.
- 1989 The evolutionary breakdown of heterostyly. Pages 151–169 in Y Linhart, J Bock, eds. The evolutionary ecology of plants. Westview, Boulder, CO.
- Forthcoming Reproductive systems (plants). In D. Simberloff, M Rejmánek, eds. Encyclopedia of invasive introduced species. University of California Press, Berkeley.
- Barrett SCH, JM Anderson 1985 Variation in expression of trimorphic incompatibility in *Pontederia cordata* L. (Pontederiaceae). *Theor Appl Genet* 70:355–362.
- Barrett SCH, MB Cruzan 1994 Incompatibility in heterostylous plants. Pages 189–219 in EG Williams, AE Clarke, RB Knox, eds. Genetic control of self-incompatibility and reproductive development in flowering plants. Kluwer, Dordrecht.
- 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* 41:1398–1416.
- Barrett SCH, JS Shore 1987 Variation and evolution of breeding systems in the *Turnera ulmifolia* L. complex (Turneraceae). *Evolution* 41:340–354.
- 2009 New insights on heterostyly: comparative biology, ecology and genetics. Pages 3–32 in V Franklin-Tong, ed. Self-incompatibility in flowering plants: evolution, diversity and mechanisms. Springer, Berlin.
- Bataillon T, M Kirkpatrick 2000 Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet Res* 75:75–81.
- Becerra JX, DG Lloyd 1992 Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level? *Evolution* 46:458–469.
- Bechsgaard J, T Bataillon, MH Schierup 2004 Uneven segregation of sporophytic self-incompatibility alleles in *Arabidopsis lyrata*. *J Evol Biol* 17:554–561.
- Brennan AC, SA Harris, SJ Hiscock 2005 Modes and rates of selfing and associated inbreeding depression in the self-incompatible plant *Senecio squalidus* (Asteraceae): a successful colonizing species in the British Isles. *New Phytol* 168:475–486.
- Brown AHD, JJ Burdon 1987 Mating systems and colonizing success in plants. Pages 115–131 in AJ Gray, MJ Crawley, PJ Edwards, eds. Colonization, succession and stability. Blackwell, Oxford.
- Brown AHD, DR Marshall 1981 Evolutionary changes accompanying colonization in plants. Pages 351–363 in GGT Scudder, JL Reveal, eds. Evolution today. Proceedings of the Second International Congress of Systematic and Evolutionary Biology. Carnegie Mellon University, Pittsburgh.
- Busch JW 2005 The evolution of self-compatibility in geographically peripheral populations of *Leavenworthia alabamica* (Brassicaceae). *Am J Bot* 92:1503–1512.
- Byers DL, TR Meagher 1992 Mate availability in small populations of plant species with homomorphic sporophytic self-incompatibility. *Heredity* 68:353–359.
- Carr GD, EA Powell, DW Kyhos 1986 Self-incompatibility in the Hawaiian Madiinae (Compositae): an exception to Baker’s rule. *Evolution* 40:430–434.
- Charlesworth D 1979 The evolution and breakdown of tristylous. *Evolution* 33:486–498.
- Cheptou PO, U Dieckmann 2002 The evolution of self-fertilization in density-regulated populations. *Proc R Soc B* 269:1177–1186.
- Cheptou PO, J Lepart, J Escarre 2002 Mating system variation along a successional gradient in the allogamous and colonizing plant *Crepis sancta*. *J Evol Biol* 15:753–762.
- Cheptou PO, DJ Schoen 2007 Combining population genetics and demographical approaches in evolutionary studies of plant mating systems. *Oikos* 116:271–279.
- Chun YJ, JD Nason, KA Moloney 2009 Comparison of quantitative and molecular genetic variation of native vs. invasive populations of purple loosestrife (*Lythrum salicaria* L. Lythraceae). *Mol Ecol* 18:3020–3035.
- Colautti RI, IA Grigorovich, HJ MacIsaac 2006 Propagule pressure: a null model for biological invasions. *Biol Invasions* 8:1023–1037.
- Cruzan MB, SCH Barrett 1996 Post-pollination mechanisms influencing mating patterns and fecundity: an example from *Eichhornia paniculata*. *Am Nat* 147:576–598.
- Darwin CR 1877 The different forms of flowers on plants of the same species. J Murray, London.
- Davis HG, CM Taylor, JG Lambrinos, DR Strong 2004 Pollen limitation causes an Allee effect in a wind-pollinated invasive grass (*Spartina alterniflora*). *Proc Natl Acad Sci USA* 101:13804–13807.
- de Nettancourt D 1977 Incompatibility in angiosperms. Springer, Berlin.
- Dornier A, F Munoz, PO Cheptou 2008 Allee effect and self-fertilization in hermaphrodites: reproductive assurance in a structured metapopulation. *Evolution* 62:2558–2569.
- Eckert CG, SCH Barrett 1992 Stochastic loss of style morphs from tristylous populations of *Lythrum salicaria* and *Decodon verticillatus* (Lythraceae). *Evolution* 46:1014–1029.
- Eckert CG, D Manicacci, SCH Barrett 1996a Frequency-dependant selection on morph ratios in tristylous *Lythrum salicaria* (Lythraceae). *Heredity* 78:581–588.
- 1996b Genetic drift and founder effect in native versus introduced populations of an invading plant, *Lythrum salicaria* (Lythraceae). *Evolution* 50:1512–1519.
- Elam DR, CE Ridley, K Goodell, NC Ellstrand 2007 Population size and relatedness affect plant fitness in a self-incompatible invasive plant. *Proc Natl Acad Sci USA* 104:549–552.
- Fisher RA, K Mather 1943 The inheritance of style length in *Lythrum salicaria*. *Ann Eugen* 12:1–23.
- Friedman J, SCH Barrett 2008 High outcrossing in the annual colonizing species *Ambrosia artemisiifolia*. *Ann Bot* 101:1303–1309.
- Geyer CJ, S Wagenius, RG Shaw 2007 Aster models for life history analysis. *Biometrika* 94:415–426.
- Gravuer K, JJ Sullivan, PA Williams, RP Duncan 2008 Strong human association with plant invasion success for trifolium introductions to New Zealand. *Proc Natl Acad Sci USA* 105:6344–6349.
- Heuch I 1980 Loss of incompatibility types in finite populations of the heterostylous plant *Lythrum salicaria*. *Hereditas* 92:53–57.



- Houghton-Thompson J, HH Prince, JJ Smith, JF Hancock 2005 Evidence of hybridization between *Lythrum salicaria* (purple loosestrife) and *L. alatum* (winged loosestrife) in North America. *Ann Bot* 96:877–885.
- Igic B, R Lande, JR Kohn 2008 Loss of self-incompatibility and its evolutionary consequences. *Int J Plant Sci* 169:93–104.
- Kimura M, T Maruyama, JF Crow 1963 The mutation load in small populations. *Genetics* 48:1303–1312.
- Kohn JR, SCH Barrett 1992 Experimental studies on the functional significance of heterostyly. *Evolution* 46:43–55.
- Lafuma L, S Maurice 2007 Increase in mate availability without loss of self-incompatibility in the invasive species *Senecio inaequidens* (Asteraceae). *Oikos* 116:201–208.
- Larson BMH, SCH Barrett 1999 Reproductive biology of island and mainland populations of *Primula mistassinica* (Primulaceae) on Lake Huron shorelines. *Can J Bot* 76:1819–1827.
- Levin DA 1996 The evolutionary significance of pseudo-self-fertility. *Am Nat* 148:321–332.
- Lloyd DG 1980 Demographic factors and mating patterns in angiosperms. Pages 67–88 in OT Solbrig, ed. *Demography and evolution in plant populations*. Blackwell, Oxford.
- Mable BK 2008 Genetic causes and consequences of the breakdown of self-compatibility: case studies in the Brassicaceae. *Genet Res* 121:47–60.
- Mable BK, AV Roberson, S Dart, C Di Bernardo, L Witham 2005 Breakdown of self-incompatibility in the perennial *Arabidopsis lyrata* (Brassicaceae) and its genetic consequences. *Evolution* 59:1437–1448.
- Mal TK, J Lovett-Doust 2005 Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil moisture. *Am J Bot* 92:819–825.
- Mal TK, J Lovett-Doust, L Lovett-Doust 1999 Maternal and paternal success among flower morphs in tristylous *Lythrum salicaria*. *Aquat Bot* 63:229–239.
- McMullen CK 1987 Breeding systems of selected Galápagos Islands angiosperms. *Am J Bot* 74:1694–1705.
- Miller JS, RA Levin, NM Feliciano 2008 A tale of two continents: Baker's rule and the maintenance of self-incompatibility in *Lycium* (Solanaceae). *Evolution* 62:1052–1065.
- Moeller DA, MA Geber 2005 Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities and reproductive assurance. *Evolution* 59:786–799.
- 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.
- O'Neil P 1994 Genetic incompatibility and offspring quality in the tristylous plant *Lythrum salicaria* (Lythraceae). *Am J Bot* 81:76–84.
- Ornduff R 1972 Breakdown of trimorphic incompatibility in *Oxalis* section *Corniculatae*. *Evolution* 26:52–65.
- Ottenbreit KA, RJ Staniforth 1994 Crossability of naturalized and cultivated *Lythrum* taxa. *Can J Bot* 72:337–341.
- Pannell JR, SCH Barrett 1998 Baker's law revisited: reproductive assurance in a metapopulation. *Evolution* 52:657–668.
- Price SC, SK Jain 1981 Are inbreeders better colonizers? *Oecologia* 49:283–286.
- Pujol B, S-R Zhou, JS Vilas, JR Pannell 2009 Reduced inbreeding depression after species range expansion. *Proc Nat Acad Sci USA* 106:15379–15383.
- Rambuda TD, SD Johnson 2004 Breeding systems of invasive alien plants in South Africa: does Baker's rule apply? *Divers Distrib* 10:409–416.
- Randle AM, JB Snyder, S Kalisz 2009 Can differences in autonomous selfing ability explain differences in range size among sister-taxa pairs of *Collinsia* (Plantaginaceae)? an extension of Baker's law. *New Phytol* 183:618–629.
- Schueller SK 2004 Self-pollination in island and mainland populations of the introduced hummingbird-pollinated plant, *Nicotiana glauca* (Solanaceae). *Am J Bot* 91:672–681.
- Shore JS, SCH Barrett 1986 Genetic modifications of dimorphic incompatibility in the *Turnera ulmifolia* L. complex (Turneraceae). *Can J Genet Cytol* 28:796–807.
- Stebbins GL 1957 Self-fertilization and population variability in the higher plants. *Am Nat* 91:337–354.
- Stephenson AG, SV Good, DW Volger 2000 Interrelationships among inbreeding depression, plasticity in the self-incompatibility system, and the breeding system of *Campanula rapunculoides* L. (Campanulaceae). *Ann Bot* 85(suppl A):211–219.
- Stephenson AG, SE Travers, JI Mena-Ali, JA Winsor 2003 Pollen performance before and during the autotrophic-heterotrophic transition of pollen tube growth. *Philos Trans R Soc B* 358:1009–1017.
- Stone JL 2002 Molecular mechanisms underlying the breakdown of gametophytic self-incompatibility. *Q Rev Biol* 77:17–32.
- Stout AB 1923 Studies of *Lythrum salicaria*. I. The efficiency of self-pollination. *Am J Bot* 10:440–449.
- 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. *Fish and Wildlife Research* 2. U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC.
- Vallejo-Marín M, MK Uyenoyama 2004 On the evolutionary costs of self-incompatibility: incomplete reproductive compensation due to pollen limitation. *Evolution* 58:1924–1935.
- van Kleunen M, JC Manning, V Pasqualetto, SD Johnson 2008 Phylogenetically independent associations between autonomous self-fertilization and plant invasiveness. *Am Nat* 171:195–201.
- Waites AR, J Ågren 2004 Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *J Ecol* 92:512–526.
- Webb CJ, D Kelly 1993 The reproductive biology of the New Zealand flora. *Trends Ecol Evol* 8:442–447.
- Weller SG 1992 Evolutionary modifications of tristylous breeding systems. Pages 247–272 in SCH Barrett, ed. *Evolution and function of heterostyly*. Springer, Berlin.
- 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.