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Fecundity and fitness in cross-compatible pollinations of tristylous North American *Lythrum salicaria* populations

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Abstract As heterostyly and self incompatibility are linked in tristylous *L. salicaria*, all cross-compatible pollinations – those between anthers and styles of the same level – should produce viable seed. The rigor of this theory was tested using seed set and germination of cross-compatible pollinations in 18 naturalized *L. salicaria* populations in Minnesota/Wisconsin (n=342 genotypes; n=86 shorts, n=127 mids, and n=129 longs). Seed set for short-styled genotypes ranged from zero to 135 (36±38); mids–0–156, (53±39), and longs–0–151 (59±39). Mean seed set per capsule was not significantly different for mids and longs, but both were significantly greater than that of shorts (F=14, P<0.0001). Zero seed set frequently occurred in most compatible crosses, in contrast with theoretical expectations. The high percentage of populations deviating from normality must be due to incompatibility. An incompatibility system independent of heterostyly could cause this, where failed crosses result from matches of incompatibility specificities in pollen and pistil. This independence is questionable, however, given the non-significant difference in failed outcrosses for pooled χ^2 comparing within and between populations (χ^2=0.395, P>0.5). A sporophytic incompatibility model is proposed with a minimum of three specificities. Zero seed set in compatible crosses is due to the addition of alleles from *L. alatum*, a distylous species that forms introgressive hybrids with *L. salicaria*. Reduced fecundity could increase the deficiency of shorts, and significantly greater seed germination of shorts could explain the continued presence of short-styled individuals in *L. salicaria* populations.

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Introduction

Several unusual attributes of *Lythrum salicaria* L., purple loosestrife, have intrigued students of plant-reproductive biology for more than 150 years (Vaucher 1841). The first of these to attract attention was heterostyly, a system to enhance outbreeding that involves macroscopic differences in style and stamen lengths such that a population of plants is split into two or three morphs (Darwin 1865, 1868). Purple loosestrife is tristylous, with short-, mid-, and long-styled plants, each plant having two whorls of stamens positioning anthers at the heights of the stigmas of the other two style morphs. Vaucher (1841) was the first to describe tristyly in *L. salicaria*; Wirtgen (1848) and Darwin (1865, 1868) added more exact observations. Tristyly may be the most evolutionarily advanced mechanism that promotes outcrossing (Ganders 1979). In *Lythrum*, the effects of heterostyly are enhanced by the linkage of self incompatibility (SI) to genes affecting flower morphology. SI is a system enhancing outbreeding through the control of pollen-tube growth after pollination. Matching specificities expressed in pollen and pistil after self pollinations or crosses between related individuals causes recognition, which prevents fertilization (Vuilleumier 1967; Ornduff 1975; O'Neil 1994; Hermann et al. 1999). For example, a mid-styled individual would set seed after pollination with pollen from mid anthers originating either from a short- or long-styled plant (Fig. 1). Legitimate pollinations of purple loosestrife set significantly more seed than illegitimate crosses or selfs (Mulcahy and Caporello 1970; O'Neil 1994). In cases of failure of the SI system, limited to full complements of seed from incompatible pollinations will be produced (pseudo-self compatibility or PSC). A complete and permanent breakdown of SI, conferring self compatibility, is rare (Ganders 1976).

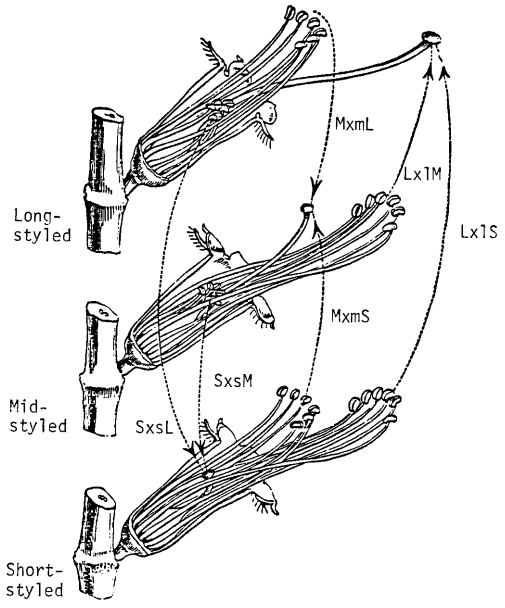

Fig. 1 Directions of compatible or legitimate pollinations producing full complements of seed in tristylous *Lythrum salicaria* (Anderson and Ascher 1993a; adapted from Darwin 1865, 1868). Arrows indicate direction of compatible pollen flow. There are six compatible combinations: *S*×*sM* Short styles×short anthers (from a mid-styled plant), *S*×*sL*, *M*×*mS*, M×*mL*, *L* ×*lS*, and *L*× *lM*. All other pollination combinations are incompatible or illegitimate

Darwin (1865) theorized that the three floral morphs should occur at equal frequencies (isoplethic equilibrium) in stable populations. While some populations observed by Darwin and subsequent European researchers can be statistically shown to be at isoplethic equilibrium (Eckert et al. 1996), pooled data from *n*=24,823 plants taken from more than 23 different European populations reveal a significant deficiency in short-styled plants, an occurrence also documented with *n*=11,819 plants originating in nine Minnesota populations (Anderson and Ascher 1995). Several hypotheses have been proposed to explain this consistent lack of isoplethic equilibrium (anisoplethy). One is that floral morphs differ in seed-setting potentials after compatible (legitimate) pollination (fecundity). For example, Darwin (1868) and Barlow (1913) found that seed set differed between the three stylar forms after legitimate pollinations: long-styled flowers averaged 93 seeds per capsule, mid-styled flowers averaged 130 seeds per capsule, and short-styled flowers averaged 84 seeds per capsule. Incidentally, the floral morphs of *L. salicaria* do not differ in the number of ovules per capsule (O'Neil 1991). Purple loosestrife populations in North America have remarkable fecundity for a polyploid (4x, 6x), perennial species. A conservative estimate of annual production for a plant with an average of 110 seeds per capsule, five capsules per whorl, 70 whorls per spike, and 15 spikes per plant would yield approximately 600,000 seeds per year (Cutright 1986; Ottenbreit 1991). Such seed production is unusually high for a perennial species, in which individuals must also direct energy to resources for the following season (Wiens 1984). In this respect, fecundity in purple loosestrife more closely resembles that of annuals.

Another hypothesis to explain anisoplethy is that seeds produced by the different floral morphs differ in germinability, a component of fitness (O'Neil 1992, 1994). For example, Nichols (1987) reported that seeds from mid-styled plants have significantly higher percent germination, although O'Neil (1994) found no significant differences between morphs. Seed germination in purple loosestrife has been a subject of interest to many researchers since Lehmann's (1918) initial observations concerning the effects of various environmental parameters, such as light intensity, duration of light, and temperature, on seed set and germination. He reported that as little as 1 minute of light would cause seed germination. Shamsi and Whitehead (1974) found that temperatures below 20°C began to inhibit germination and no germination occurred at temperatures under 14°C. Continuous darkness also greatly reduces seed germination, although other photoperiod variations do not significantly alter germination per se. Apparently, light eliminates an inhibitor in the seed coats of *L. salicaria* (Barton 1965). Welling and Becker (1990, 1993) demonstrated the effects of light on seedling emergence by experimentally burying seed. Germination decreased linearly from 90% at the soil surface to 0% at 2 cm. Seed is viable for several years even though it is thin-walled and lacks endosperm (Thompson et al. 1987). Stratification for 3 years at 3–4°C did not significantly alter germination compared to that of freshly harvested seed. Other researchers have delineated germination requirements in natural populations (Keddy and Constabel 1986; Isabelle et al. 1987).

Since heterostyly and SI are linked in tristylous *L. salicaria*, all cross-compatible pollinations – those between anthers and styles of the same level – should produce viable seed. The objectives of the study reported here were to test the rigor of this theory using fitness traits (seed set, germination) in naturalized *L. salicaria* populations. If the assumptions of heterostyly are correct, then all legitimate crosses between anthers and styles of the same height should be compatible and produce viable (germinable) seed, and the number of seeds would accurately measure fecundity. Seed set should follow a normal distribution, all crosses should have seed set greater than zero, with each respective mean occurring near the expectation for each morph, and mean seed set should differ significantly between style morphs (Darwin 1868; Barlow 1913). A secondary objective of this study was to determine whether deviations from theoretical expectations for fitness traits could account for anisoplethy.

Materials and methods

Seventeen naturalized purple loosestrife populations from Minnesota, all without any previous history of herbicide application or other eradication efforts, were analyzed. One Wisconsin popula-

tion (Green Bay), established at a similar latitude and reproductively isolated from the Minnesota populations, was included. Geographic locations of each population have been described elsewhere (Anderson and Ascher 1994a) and varied from emergent wetlands to dry, upland habitats. Populations were coded for ease in identification: S-01 (Fort Snelling State Park), S-02 (Taft Park), S-03 (Springbrook Nature Center), S-04 (Maplewood Nature Center), S-05 (Black Dog Prairie Scientific and Natural Area), S-06 (Ferndale Marsh), S-07 (Pig's Eye Lake), S-08 (Wirth Lake), S-10 (University of Minnesota Landscape Arboretum), S-11 (Jim McKee Prairie), S-12 (Oakdale Marsh), S-17 (Hayward), S-30 (Mississippi River), S-32 (Lavinia), S-33 (Green Bay, Wisconsin), S-34 (White Bear Lake), S-35 (Winona), and S-37 (Birch Pond). A total of n=342 genotypes, including n=86 short-styled (S) plants, n=127 mid-styled (M) plants, and n=129 long-styled (L) plants, were used in this study. Population (S-34) has been previously researched for seed germination and seed bank dynamics (Welling and Becker 1990, 1993), and all 18 populations have been analyzed for style-morph frequencies (Anderson and Ascher 1995). All three style morphs were present in each population with the exception of S-10, S-11, S-12, S-17, and S-30. One population, S-34, was at isoplethy; the remaining populations exhibited all three style morphs but had an excess of mids and longs and a deficiency of shorts. Most of the selected Minnesota populations were reproductively isolated from each other (allopatric), being separated by distances greater than those traveled by common pollinators, *Apis mellifera*, *Bombus vagans*, and *B. terricola* (Mulcahy and Caporello 1970; Levin and Kerster 1973; O'Neil 1992). However, populations S-01 and S-02; S-04, S-11, and S-12; S-08 and S-37 were sympatric and not reproductively isolated from each other.

Representative individuals from each population were cloned (vegetative cuttings) and raised under greenhouse conditions for hand pollination experiments. Rooted stem cuttings were potted in 15-cm standard pots using commercial potting medium (Baccto Prof. Planting Mix, Mich. Peat Co, Houston, Tex.) in greenhouses at St. Paul, Minnesota (lat. 45°N). Greenhouses were maintained at 24°±5°C days and 17°±2°C nights with long days for flower bud initiation and development (natural day lengths, 21 March to 21 September; artificial long days, 21 September to 21 March, supplemental lighting provided by high-intensity discharge lamps, 0600–2200 HR).

The uniform crossing environment minimized, as much as possible, potential environmental effects on fertility and seed set as well as enabling pollination between allopatric and sympatric populations. Relative trends for fecundity in studies between artificial (Anderson and Ascher 1993a, b) and natural (Lindgren and Clay 1993) settings have been similar with horticultural cultivars of loosestrife; Ottenbreit (1991) did not find any differences in fecundity between hand-pollinated (greenhouse) and open-pollinated flowers. O'Neil (1992) reported that hand-pollination did not result in higher seed set for mid and long morphs, although seed production in short morphs increased over control (natural) pollinations.

Capsules from the lower and mid regions of most inflorescences tend to produce more seeds than those of the top regions (Stout 1923; Ottenbreit 1991). Therefore, flowers from the lower regions of the inflorescence were used in all pollination experiments to maximize potential seed set. Unopened flowers (prior to anthesis) were emasculated and later pollinated with the appropriate pollen, when the stigmas were receptive (Anderson and Ascher 1993a). Pollinated flowers were tagged and the seed capsules harvested when brown in coloration. Capsules were dried for 3–4 weeks at 26°C prior to seed cleaning and counting.

Male fertility was evaluated, using pollen stainability, as previously described (Anderson and Ascher 1993a), with 0% stainable pollen indicating male sterility. Female fertility was determined by seed set per capsule from replicated, random, cross-compatible pollinations (n=3 crosses per genotype) within populations. This also provided fecundity information on an intrapopulational level. A 1:1:1 χ^2 was performed to test whether morphs (S:M:L) differed in the number of failed legitimate crosses.

Cross-compatible pollinations were also performed to assess seed set within and between populations of *L. salicaria* (n=3 reps per genotype). We have previously reported cross-compatible seed set of *L. salicaria* populations (as female) with fertile, commercial *L. salicaria* horticultural cultivars and interspecific *L. salicaria* × *L. alatum* Pursh. cultivars (Anderson and Ascher 1993a); this data will be compared with the cross-compatible pollinations performed in the current research. If all intraspecific, compatible pollinations on a given plant failed to produce seed, the plant was deemed female sterile.

Seed set data were analyzed for unbalanced analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) for mean separations. Distributions of seed set were tested for normality, as seed-set should follow a normal curve in cases where pollinations are compatible and environmental variation is minimized (hand pollination, with parents known to be cross-compatible, performed under uniform greenhouse environments), there is no genetic load or ovule abortion systems, and pollen load is not a factor (Liedl and Anderson 1993). Data for each population were fitted to a normal distribution and tested for skewness, kurtosis, goodness of fit (G), and Chi-square (using Yates' correction for continuity).

Seed germination, as a measurement of fitness, was performed in two experiments. The first measured percent germination of open-pollinated seeds collected from the parental plants used for fertility analyses. The second experiment used seeds from hand-pollinated compatible crosses (within and between populations).

Open-pollinated (OP) seeds were collected from genotypes in n=12 populations from the field (during the same year as they were clonally propagated): S-01 through S-08, S-10 through S-12, and S-17. OP seeds from n=360 plants (n=89 shorts, n=137 mids, and n=134 longs) were included. Since not all OP parents could be clonally propagated and established in the greenhouse, the number of OP parents tested for seed germination varied from those included in the hand pollination experiments.

Conditions for seed germination were adapted from previously described protocols (Lehmann 1918; Shamsi and Whitehead 1974; Nicholls 1987; Anderson and Ascher 1993a). Replicated samples of seeds were placed on Whatman filter paper (7 cm) and kept moistened with deionized, distilled water inside sterile, plastic petri dishes (Fisher Scientific, 15×100 mm). The germination environment was 21°C with light (short days, cool-white fluorescent lamps, 0800–1700 HR).

Prior to conducting OP germination experiments, we conducted a test to determine sample size. Bulked, OP seeds from four populations (S-32, S-33, S-34, S-35) were used. Sample sizes (n=30, 60, 90, 120 seeds) were not significantly different, based on a t-test (t=0.1, P>0.1). Thus, only a sample size of n=30 seeds per genotype was used for seed germination trials. Seeds were considered germinated when the radicle emerged through the outer seed coat. Percent seed germination values were arcsine square-root transformed prior to performing ANOVA, if the assumptions for ANOVA were not met.

Seeds from hand pollinations were also tested for germination. The experimental design was completely randomized, with n=30 seeds per cross. Seeds from the six compatible crossing groups were randomly selected from the fecundity studies, based on seed set (n≥30/capsule per cross) and common male/female parents. Four crosses (within or between populations) were included in each compatible crossing group: n=2 (each) with a common male and female parent.

Results

Pollen stainability ranged from 0% to 100% in *L. salicaria* populations, although the average was more than 50%. A few genotypes exhibited either partial (one stamen length) or complete (both stamen lengths) male sterility. For instance, long stamens in short- or mid-styled individuals from populations S-03 (n=3), S-07 (n=2), and S-34 (n=1) failed to mature and anthers did not de-

Table 1 Descriptive statistics and ANOVA for seed set per capsule from cross-compatible pollinations (n=3 per genotype) within Minnesota and Wisconsin *Lythrum salicaria* populations

Population[b]	Short styles				Mid styles				Long styles				ANOVA		
	n	Range	Mean	SD	*n*	Range	Mean	SD	*n*	Range	Mean	SD	*F* values[a]		
													G	S	G(S)
S-01	17	0–102	33.0 a[c]	35.5	17	0–156	48.5 b[c]	41.7	13	0–109	52.9 b[c]	33.1	1.6	4.3*	1.2
S-02	3	0–135	66.6	42.3	25	0–147	57.9	67.5	19	0–147	63.9	40.2	0.5	1.2	0.9
S-03	12	0–113	33.9 a	36.5	11	0–128	56.4 b	33.6	10	0–128	41.9 a	37.4	2.2*	4.7*	1.3
S-04	3	0–96	36.7	43.6	22	0–107	50.3	31.2	21	0–140	51.3	38.0	1.6	0.5	0.9
S-05									4	0–117	59.0	36.2	1.4		
S-06	1	52–78	64.3	13.0					2	0–81	45.8	29.7	0.2	1.4	−0.2
S-07	5	0–106	35.8 a	40.5	12	0–118	55.9 ab	40.7	13	0–146	66.2 b	40.7	2.3*	3.9*	0.8
S-08	5	0–92	53.3	25.0	5	31–82	60.9	16.1	5	0–130	64.4	43.9	2.1	0.4	5.4***
S-10					1	0–92	53.0	26.1							
S-11					4	0–96	63.0	26.3	5	0–116	50.6	44.9	3.4*	1.1	1.6
S-12					3	0–130	79.9	42.4					11.9**		
S-17					1	43–74	63.3	17.6							
S-30					5	0–151	56.7	39.7	1	0–151	52.6	38.5	1.0	0.9	0.6
S-32	3	16–82	57.6	24.9	2	0–136	47.6	59.4	2	13–92	50.3	31.6	23.8***	0.5	3.6*
S-33	6	0–99	32.6	37.2	3	0–70	27.2	27.8	5	0–126	63.6	45.6	1.1	0.1	0.7
S-34	12	0–112	32.6 a	37.4	1	38–78	60.7 ab	20.5	10	0–151	63.2 b	40.3	1.2	4.6*	0.5
S-35	2	0–127	84.2	58.8	4	25–98	69.3	23.6	11	0–127	62.1	35.7	0.7	0.9	0.3
S-37	8	0–120	64.6	39.0	7	0–110	66.0	29.4	2	25–87	55.3	26.1	2.3	0.2	0.4
Pooled	77	0–135	36.2 a	38.3	123	0–156	53.1 b	38.6	123	0–151	59.2 b	38.6	1.3*	14.0***	0.6

[a] Male Genotypes (G), Female Style Morphs (S), and nested G(S) effects. *,**,*** denote significance at the 5%, 1%, and <0.1% level, respectively. All other *F* values are not significant

[b] See text for population code descriptions

[c] Mean separations between style morphs within populations, Fisher's least significant difference (LSD) test, $P \leq 0.05$. Means not followed by a letter are not significantly different from each other for the three style morphs

hisce. The long stamens in 1 S-07 genotype (short-styled) did not grow beyond the length of the mid stamens. This aberration in stamen length was the only one observed in all of the populations. In addition to the long anthers failing to dehisce, the mid anthers for 1 S-03 genotype had low pollen stainability (<50%), making it the closest that any genotype with dehiscent anthers came to complete male sterility.

Eight genotypes (2% of the total number of individuals) exhibited complete female sterility. That is, they failed to produce any seed from intra- and inter-population, cross-compatible matings. These individuals were from 7 populations: S-01 (*n*=1), S-02 (*n*=1), S-03 (*n*=2), S-07 (*n*=1), S-11 (*n*=1), S-32 (*n*=1), and S-34 (n=1). This low percentage of sterility is surprising, given polyploidy and the perenniality of the species (Wiens 1984). All female- and/or male-sterile individuals were eliminated from subsequent analyses.

Seed set for short-styled genotypes ranged from zero to 135 and averaged 36±38, a mean that was significantly lower than those of mid- or long-style morphs (Table 1). Mid-styled flowers produced seed numbers ranging from zero to 156 seeds per capsule, with a mean of 53±39, while long-styled flowers averaged 59±39 seeds per capsule, with seed numbers ranging from zero to 151. Mean seed set per capsule (pooled) was not significantly different for mids and longs, although both were significantly higher than the mean from short-styled plants (F=14.0, P<0.0001). Genotypes were also significantly different (F=1.3, P=0.0405), but not when nested with style morphs (F=0.6, P=1).

Only populations S-01 (F=4.3, P=0.05), S-03 (F=4.7, P=0.05), S-07 (F=3.9, P=0.05), and S-34 (F=4.6, P=0.05) had significant differences in fecundity between style morphs (Table 1). In all of these populations, seed set of short-styled individuals was the lowest, although this either overlapped with mids and longs (S-07 and S-34), was the same as longs (S-03), or was significantly different from both mids and longs (S-01).

The minimum seed set for each style morph was zero, even though all pollinations were legitimate and, therefore, compatible (Table 1). The frequency of fertile individuals exhibiting zero seed set from one or more "compatible" pollinations ranged from 0% to 100% for all style morphs (Table 2). Only population S-17 had no pollinations with zero seed set, but this is likely attributable to the small sample size (*n*=1, mid style only). Likewise, population S-10, with 100% of the pollinations having zero seed set, had a sample size of 1 (mid style). When these 2 populations are eliminated, the average frequency of fertile individuals per population ranged from 14% to 80%. Short-styled plants exhibited the highest failure rate, with 57% of the "compatible" crosses producing no seeds. Mid- and long-styled plants were similar, averaging 41% and 40% failures, respectively. A 1:1:1 χ^2, performed to determine whether there were significant differences between the morphs in the number of failed legitimate crosses, was not significant at the 5% level (χ^2=3.9, df=2; Dulberger 1970a, b). However, the same test – applied to each of the 11 populations exhibiting all three morphs – was significant for all except S-02 and S-33 (Table 2).

Table 2 Frequency (%) and 1:1:1 χ^2 of fertile individuals within style morphs (short, mid, long) of Minnesota and Wisconsin *Lythrum salicaria* populations that, on occasion, produced zero seed set (cross-incompatible) following cross-compatible pollinations (*n*=3 reps per genotype) within populations (*ns* non-significant)

Population	Style morph			Mean	1:1:1	χ^2
	Short	Mid	Long			
S-01	59	71	39	57	9.2	*
S-02	33	32	21	28	3.1	ns
S-03	86	36	50	48	23.1	***
S-04	100	18	48	37	62.3	***
S-05	–	–	50	50	–	
S-06	0	–	100	67	–	
S-07	60	58	31	47	10.6	**
S-08	80	0	60	80	74.3	***
S-10	–	100	–	100	–	
S-11	–	25	60	44	–	
S-12	–	33	–	33	–	
S-17	–	0	–	0	–	
S-30	–	40	100	50	–	
S-32	0	50	0	14	100.1	***
S-33	67	67	60	64	0.5	ns
S-34	67	0	40	52	63.7	***
S-35	50	0	27	24	48.9	***
S-37	38	29	0	29	35.3	***
Pooled	57	41	40	44	3.9	ns

*, **, *** denote significance at the 5%, 1%, and <0.1% level, respectively

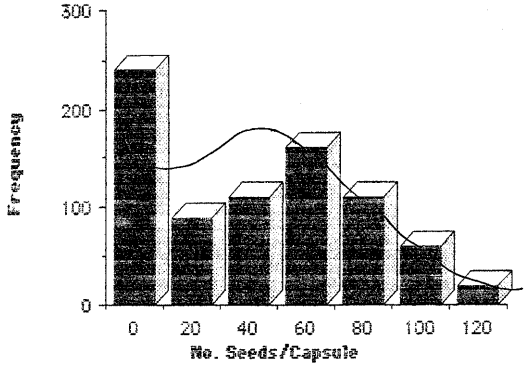

Fig. 2 Histogram of cross-compatible (legitimate) seed set, pooled across style morphs (short, mid, long) and Minnesota and Wisconsin *Lythrum salicaria* populations. A normal curve is fit to the data (*solid line*)

Thirteen populations were tested for normality, as 5 populations (S-01, S-02, S-05, S-06, S-10) had excessive failed "compatible" crosses (zero seed set) for normality tests to be performed. For data pooled across populations, the largest class was zero seed set (Fig. 2), and all tests revealed highly significant deviation from normality (Table 3). At the population level, t-tests for skewness were significant for S-03 (t=0.5, P=0.02) and S-30 (t=1.4, P=0.01). This skewness is attributable to the high

Table 3 Tests for normality in seed set distributions from cross-compatible pollinations in sample populations of *Lythrum salicaria*

Population	Two-tailed *t*-test for skewness		Two-tailed *t*-test for kurtosis		Likelihood ratio test[a]		Chi-square test[a]	
	t	*P*[b]	*t*	*P*[b]	G	*P*[b]	χ^2	*P*[b]
S-03	0.5	0.02*	–0.9	0.03*	23.0	<0.001***	22.2	0.001***
S-04	9.2	0.48	–1.3	0.001**	20.9	0.004**	21.0	0.004***
S-07	–0.1	0.33	–1.6	0.001**	32.9	<0.001***	31.3	<0.001***
S-08	0.3	0.23	0.2	0.42	5.3	0.022*	5.3	0.02*
S-11	–0.4	0.22	–1.3	0.07	3.0	0.15	4.2	0.1
S-12	–0.3	0.34	–0.4	0.4	8.0	0.01*	8.0	0.02*
S-17	–0.9	0.13	–1.7	0.13	3.6	0.17	4.1	0.1
S-30	1.4	0.01*	2.7	0.008**	0.07	–	0.07	–[c]
S-32	0.4	0.21	–0.9	0.16	3.1	0.15	4.1	0.1
S-33	0.5	0.09	–1.1	0.07	12.1	0.0024**	11.5	0.003**
S-34	0.3	0.15	–1.5	0.01*	19.0	<0.001***	16.8	<0.002**
S-35	–0.3	0.19	–0.7	0.15	8.4	0.01*	7.8	0.02*
S-37	–0.4	0.12	–0.7	0.15	3.6	0.17	4.4	0.11
Pooled	0.2	0.002**	–1.1	<0.0001***	114.2	<0.001***	116.7	<0.001***

[a] Yates' correction for continuity was performed if $n \leq 200$. The expected frequency was adjusted to reduce observed minus expected by 0.5

[b] *,**,*** Denote significance at the 5%, 1%, and <0.1% level, respectively. All other *P* values are not significant

[c] Insufficient degrees of freedom

Table 4 Percentage failed (zero seed set) compatible crosses after legitimate pollinations between *Lythrum salicaria* populations (as female)×(a) *L. salicaria* horticultural cultivars[a] (male) or (b) interspecific *L. salicaria*×*L. alatum* horticultural cultivars[b] (male) (Anderson and Ascher 1993a)

Female-style morph	Male parents						
	Short-styled Anther morph		Mid-styled Anther morph		Long-styled Anther morph		1:1
	Mid (mS)[c]	Long (lS)[c]	Short (sM)[c]	Long (lM)[c]	Short (sL)[c]	Mid (mL)[c]	χ^2
L. salicaria cultivars							
S (short)	–	–	38.0	–	47.0	–	1.0*
M (mid)	12.0	–	–	–	–	20.0	2.0*
L (long)	–	8.0	–	19.0	–	–	4.5**
Interspecific *L. salicaria*×*L. alatum* cultivars							
S (short)	–	–	26.0	–	–	–	
L (long)	–	–	–	18.0	–	–	

*, ** Denotes significance at the 5%, 1% levels, respectively

[a] Cultivars used as male included: short-styled The Beacon; mid-styled Morden Pink, Robert, Roseum Superbum, Purple Spires; long-styled Dropmore Purple, Feuer Kerze, Happy, Rose Queen

[b] Interspecific cultivars used as male included mid-styled Morden Gleam and Morden Rose

[c] Uppercase letters denote skyle morph; lowercase letters denote anther morph

frequency of zeroes, as, without the class of zeroes, seed set for these populations did not deviate significantly from a normal distribution (data not shown). These 2 populations, as well as 3 others (S-04, S-07, and S-34), had significant *t*-tests for kurtosis. Likelihood ratio and Chi-square tests for normality were significant for 8 of the 13 populations (Table 3). Clearly, all "legitimate" crosses in Minnesota and Wisconsin populations of purple loosestrife are not "compatible." Comparative results from crossing with commercial *L. salicaria* and *L. salicaria*×*L. alatum* cultivars (Anderson and Ascher 1993a) support the idea that floral morphology and SI are not tightly linked, since 8–47% of cross-compatible combinations failed to set seed (Table 4). Individuals with the same style morph within each population often differed in incompatibility specificity: one succeeding and the other failing with the same male parent.

Intra- and inter-population crosses also revealed individuals with the same style morph but different incompatibility specificity. In some combinations both individuals failed to set seeds but, more often, one cross succeeded and the other failed. Because the spread of purple loosestrife involves the founding of new populations, one might expect fewer incompatibility specificities (*S* alleles) and, therefore, more failed cross combinations within populations than between populations (due to genetic drift from the founder effect). However, a χ^2 comparing the frequency of failed compatible crosses within and between populations for the six compatible cross combinations revealed significance in only three of the

Table 5 Percentage failed (zero seed set) compatible crosses within and between Minnesota and Wisconsin populations of *Lythrum salicaria* (*ns* non-significant)

Female[a]	Male[a]	Intra-populational	Inter-populational	1:1χ2	
S	sM	18.2	17.7	0.007	ns[a]
	sL	29.2	14.6	4.86	**
M	mS	20.8	16.8	0.42	ns
	mL	3.8	20.0	11.02	***
L	lS	16.7	19.1	0.16	ns
	lM	38.5	15.5	9.78	***
Pooled		21.2	17.3	0.395	ns

, * Denote significance at the 1% and <0.1% level, respectively. All other *P* values are not significant

[a] Uppercase letters denote style morph, lowercase letters denote anther morph. S,s=short; M,m=mid; L,l=long

six combinations, two of these having an excess of failures in intra-population crosses, as might be expected, but the third having a significant excess in inter-population crosses (Table 5).

Average seed germination for half-sib families ranged from 0% to 89% within style morphs (Table 6). Genotypes (F=1.7, P=0.016) and style morphs (F=3.03, P=0.054) were significant. Short styles had the highest mean percent germination (51%), significantly different than that of the mids (46%) and longs (42%). Pooled averages within populations were somewhat lower in range (0–69%) than within style morphs, although they were also highly significant (F=14.04, P<0.0001).

Percent seed germination of full-sib families from hand pollinations was higher than that of half-sib families from open pollination for all style morphs, with shorts ranging from 93% to 100%, and both mids and

Table 6 Percentage germination (mean±SD) of open-pollinated *Lythrum salicaria* half-sib families, grouped by style morph of female parent (short, mid, or long)

[a] Mean separations over style morphs between populations; Fisher's least significant difference (LSD) test, $P \leq 0.05$ (LSD=0.4)

[b] Mean separations between style morphs across populations; Fisher's least significant difference (LSD) test, $P \leq 0.05$ (LSD=1.2)

Population	Short styles			Mid styles			Long styles			Pooled mean[a]
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	
S-01	20	54	32	20	61	32	15	60	29	57 c
S-02	4	61	28	27	75	23	19	62	37	69 a
S-03	15	85	21	16	89	8	19	56	42	69 a
S-04	3	0	0	25	10	12	22	15	21	12 j
S-05							7	0	0	0 k
S-06	16	58	38	19	49	41	15	56	38	54 d
S-07	11	32	32	14	15	25	16	25	28	23 h
S-08	20	68	28	10	42	32	20	44	36	53 e
S-10				1	64	0				64 b
S-11				1	35	0	1	65	0	50 f
S-12				1	40	0				40 g
S-17				3	22	22				22 i
Pooled[b]	89	51 a	25	137	46 b	24	134	42 c	22	43

Table 7 Average±SD percentage germination of selected cross-compatible pollinations (full-sib families) performed between and within *Lythrum salicaria* populations from Minnesota and Wisconsin (*ns* not significant)

Cross	Common male parents			Common female parents			Pooled	
	Type[a]	Mean	SD	Type[a]	Mean	SD	Mean	SD
L×lM[b]	II	90.0	±0.0	II	73.3	±2.7		
	II	60.0	8.2**[c]	II	63.3	5.4 ns[c]	71.7 c[d]	±4.4
L×lS	I	70.0	4.7	II	76.7	2.7		
	I	96.7	2.7***	II	66.7	2.7 ns	77.5 b, c	3.8
M×mL	II	83.3	13.6	II	63.3	11.8		
	II	93.3	5.4 ns	II	60.0	4.7 ns	75.0 b, c	6.3
M×mS	I	96.7	2.7	II	66.7	2.7		
	I	96.7	2.7 ns	II	76.7	2.8 ns	84.2 b	4.0
S×sM	I	93.3	5.4	I	96.7	2.9		
	I	100.0	0.0 ns	I	93.3	2.8 ns	95.8 a	1.9
S×sL	II	100.0	0.0	II	100.0	0.0		
	II	93.3	2.6 ns	I	96.7	2.7 ns	97.5 a	1.2

[a] Type of cross: I, within populations; II, between populations

[b] Upper-case letters denote style morph; lower-case letters denote anther morphs. *S*,*s*=short; M,m=mid; L,l=long

[c] Paired *t*-test for differences between crosses within common parental groups. **,*** denote significance at the 1%, and <0.1% level, respectively. All other values are not significant

[d] Mean separations between style morphs within populations; Fisher's least significant difference (LSD) test, $P \leq 0.05$ (LSD=6.7). Means followed by a common letter are not significantly different

longs ranging from 60% to 97% (Table 7). In three cases involving short-styled female parents, all replications had 100% germination. Variances were also less than those of half-sib families (Table 6). Paired t-tests performed between full-sib families within common male or female parents were not significant except for L×lM and L×lS, with common male parents (Table 7). As with half-sib families, mean fitness (seed germination) of short-styled individuals was significantly higher than that of mids or longs (F=9.9, P<0.0001, Table 7). Reciprocal differences (F=15.4, P=0.003) and crosses within legitimate pollinations (F=0.2, P=0.007) were highly significant.

Populations exhibiting all three style morphs and having no significant difference in fecundity between morphs (S-02, S-04, S-08, S-32, S-33, S-35, and S-37) might be expected to be at isoplethic equilibrium. This is not the case, however, since analysis of these populations revealed that all deviated significantly from the 1:1:1 χ^2 ratio of floral morphs (Anderson and Ascher 1995). In fact, the only population at isoplethic equilibrium was S-34 (White Bear Lake), and this population had significant differences between floral morphs for seed production (Table 1). Therefore, seed germination for the S-34 population was tested for significance between style morphs (n=3) and compatibility groups (n=6). Percent germination averaged 20.4 for S×sM pollinations, 43.8 for S×sL, 64.8 for M×mS, 47.9 for M×mL, 75 for L×lS, and 73.8 for L×lM. Style morphs were highly significant (F=12.5, P=0.0001), although anthers (F=0.09, P=0.77) and their interaction with styles (F=2.8, P=0.08) were not.

Discussion

The idea that the deficiency of short-styled individuals in populations of purple loosestrife (Anderson and Ascher 1995) could involve differences in fecundity of the floral morphs is supported by our finding that mean seed set of short-styled plants pooled across populations is significantly less than that of mids or longs (Table 1). Darwin (1868) and Barlow (1913, 1923) also reported that short-styled plants of European populations averaged fewer seeds per capsule than mids or longs. Similarly, crosses involving horticultural cultivars as female with the Minnesota-Wisconsin populations of *L. salicaria* as males resulted in lower seed set from short-styled plants, although the reciprocal crosses did not (Anderson and Ascher 1993a). In contrast, Ågren and Ericson (1996) found that in Swedish populations the long-styled morph produced approximately 20% fewer seed/fruit than either short- or mid-styled plants.

While the pooled means and those for populations S-01, S-03, S-07, and S-34 exhibited significantly lower seed set from short-styled morphs, the means of 6 other populations containing all three floral morphs were not significantly different (Table 1). Ottenbreit (1991) and O'Neil (1992) observed significant differences in fecundity of floral morphs of North American populations, but the relative rankings of mean seed set for shorts, mids, and longs were not consistent and appeared to be population-dependent. Therefore, differences in fecundity, with the short-styled morph being less fecund, may account for the shortage of short-styled morphs in some but not all purple loosestrife populations.

How the short-style morphs remain a part of *L. salicaria* populations becomes an interesting question, considering both the lower fecundity of short-styled morphs and the lower frequency of this morph in populations, whether or not fecundity is the basis for the lowered frequency. The significantly higher percent germination found for seeds of short-styled plants (Tables 6, 7) would indicate that increased fitness, as measured by seed germination, might compensate for a lowered fecundity in short-styled individuals. Schoch-Bodmer (1938) suggested that germination differences might exist between style morphs. Anderson and Ascher (1993a) found style morphs (compatibility groups) to differ significantly for seed germination in *L. salicaria*×cultivar pollinations, although the reciprocal crosses (cultivars×*L. salicaria*), while occasionally differing for mean percent germination, did not vary significantly between style morphs or legitimate crossing groups. Ottenbreit (1991), O'Neil (1992), and Ågren and Ericson (1996) reported a similar lack of difference in germinability between style morphs for *L. salicaria* populations. Nichols (1987) observed significant differences in seed germination between morphs; seeds from mids germinated more effectively than those of longs, with shorts having the lowest level of germination. In summation, increased fecundity of short-styled morphs may contribute to the continuation of the morph in some, but, again, not all populations.

Given the significantly lower frequency of short-styled morphs in European and Minnesota-Wisconsin populations of purple loosestrife (Anderson and Ascher 1995), the trend toward reduced fecundity of the short-styled morph (Darwin 1868; Barlow 1913; Table 1) and the mixed results of germination tests (Anderson and Ascher 1993a; Ottenbreit 1991; O'Neil 1992; Tables 6, 7), the persistence of the short-styled morph, even at low frequencies in populations, becomes more intriguing. Some insight may be gained by an examination of the literature on the inheritance of this system of tristyly. Two loci are involved, with *S* producing short styles and being epistatic to *M*, which confers mid styles. Genotypes for the three morphs would be *ssmm* for long-, *ssM-* for mid-, and *S–* for short-styled plants (Barlow 1923; Fisher and Mather 1943). Since the short-styled phenotype requires one dominant, epistatic allele, crosses between mids and longs will not generate shorts, but rather a 1:1 segregation of mids to longs if the mid parent is heterozygous for *M*, or all mids if the mid parent is homozygous for *M*. Therefore, at least one *S* must be in a population for the appearance of short phenotypes. Once *S* is in the population, however, all legitimate crosses involving short-styled plants will result in progenies that segregate for at least half short phenotypes, regardless of

potential segregations of mids and longs. In crosses with heterozygous mids (*ssMm*), the short phenotype will be in the majority (1 long: 1 mid: 2 shorts). This segregation advantage would be enhanced by even a small increase in seed germinability, as the progenies containing short phenotypes would contribute a greater proportion of seedlings for each succeeding generation, and these would be the progenies highest in the short phenotype. Significantly lower fecundity, higher seed germination, and the segregation advantage could keep short-styled morphs in populations. Actually, with this scenario for inheritance of tristyly, it is surprising that long-styled plants are not the deficient morph in most populations. Perhaps the segregation disadvantage is overcome by an increased vigor of long-styled morphs (Bodmer 1927).

The accepted theory concerning heterostylous plants with an SI system (heteromorphic incompatibility) is that the locus(-i) controlling recognition between pollen and pistil is/are tightly linked to the locus(-i) governing floral morphology (Lewis 1954). Therefore, in the distylous "Primula" system, for example, a sporophytic SI system with two alleles, one dominant to the other in pistil and pollen, is linked to a sequence of tightly linked loci governing floral morphology. As with the tristylous system of *Lythrum*, the short-styled morph (thrum) carries the dominant alleles (*Ss*– traditionally noted as a single locus because of tight linkage), with the long-styled morph (pin) being homozygous recessive (*ss*). The incompatibility allele expressing dominance is linked to the dominant floral morph alleles, forming a "supergene" of linked loci with limited recombination (Lewis 1954; Richards 1986). Although the two "loci" (*S* and *M*) responsible for tristyly in *L. salicaria* are not linked and segregate independently (Lewis 1954; Ganders 1979), each is considered to be a "supergene", with linked alleles conferring the compatibility group and associated morphological traits.

Complete compatibility of legitimate crosses is implied in tristylous *L. salicaria* when SI is linked to genes controlling floral morphology. However, 40–57% of "legitimate" crosses, expected to be compatible, failed in Minnesota-Wisconsin populations of purple loosestrife (Table 2, Fig. 2), resulting in significant deviations from normality (Table 3). Since all plants exhibiting male or female sterility were eliminated from analysis, the failures observed must be due to incompatibility rather than sterility. A possible explanation could be that the SI system in *L. salicaria* is not linked to floral morphology. Dulberger (1970b) reported independent segregation of a multiple-allelic, sporophytic SI system and distyly in *Anchusa hybrida* (Boraginaceae). Eckert and Barrett (1993) also reported unlinked *S* and *M* loci in tristylous *Decodon verticillatus* (Lythraceae). With an independently inherited SI system, failed crosses in *L. salicaria* would result from matching incompatibility specificities in pollen and pistil, regardless of legitimacy of pollination. Further, individuals of a given style morph could differ in incompatibility phenotype, such that one would succeed and another fail in a cross with the same plant, as was evident in test crosses with commercial cultivars and crosses within and between Minnesota-Wisconsin purple loosestrife populations (Table 4).

With an independent SI system, the frequency of incompatible outcrosses within and between populations would be solely a function of the number of incompatibility alleles in the population (or system). The high level of cross incompatibility apparent in Minnesota-Wisconsin populations of purple loosestrife would suggest rather extreme limitations in numbers of incompatibility alleles. Since purple loosestrife is relatively new to Minnesota and is expanding rapidly (Rendall 1989), limited numbers of incompatibility alleles within populations might be expected from genetic drift, as a consequence of the founder effect. Each new population would be limited to the number of incompatibility alleles present in the founder(s). However, by chance, independently founded populations would not be expected to share incompatibility alleles unless the total number of alleles in the system is severely limited. Therefore, the percentage of failed outcrosses should be higher within than between populations. Considering the rapid expansion of *L. salicaria* to new areas in Canada and the United States, the founder effect could account for the high numbers of failed legitimate crosses. Although χ^2 tests for 1:1 distributions of failed crosses within versus between populations were significant for three crossing combinations, tests for the other three crossing combinations and, most importantly, the pooled χ^2, were not (Table 5). Non-significance of the pooled χ^2 means that matching incompatibility phenotypes are as likely between as within populations and suggests that the founder effect, with a multi-allelic-incompatibility system, is not the cause of failed compatible crosses. Rather, the SI system in *L. salicaria* is extremely limited in total number of incompatibility alleles.

The significant χ^2 tests for equal frequencies of failed, legitimate crosses within and between populations for three of the six crossing combinations (Table 5) and cultivars (Table 4) are difficult to rationalize if the SI system is, indeed, multi-allelic and independent of floral morphology. These "crossing combinations" are based on compatibilities predicted by linkage of incompatibility and morphology (Fig. 1) rather than independence. If the SI system was truly independent of floral morphology, no relationship should exist between floral morphology and compatibility in any population, and the only regularly failing pollination should be selfs (Dulberger 1970b). Clearly, such a scenario does not exist in *L. salicaria* (Darwin 1865, 1868; Stout 1923; Mulcahy and Caporello 1970; Ottenbreit 1991; O'Neil 1994).

Because a model for the mode of action of a pollen-pistil-incompatibility system linked with genes conferring tristyly has not been published, interpretation of the failed "legitimate" crosses with Minnesota-Wisconsin purple loosestrife is speculative. An SI system linked to floral morphology in *L. salicaria* must be based on a sporophytic incompatibility system. Each of the three floral morphs has pollen of two incompatibility classes.

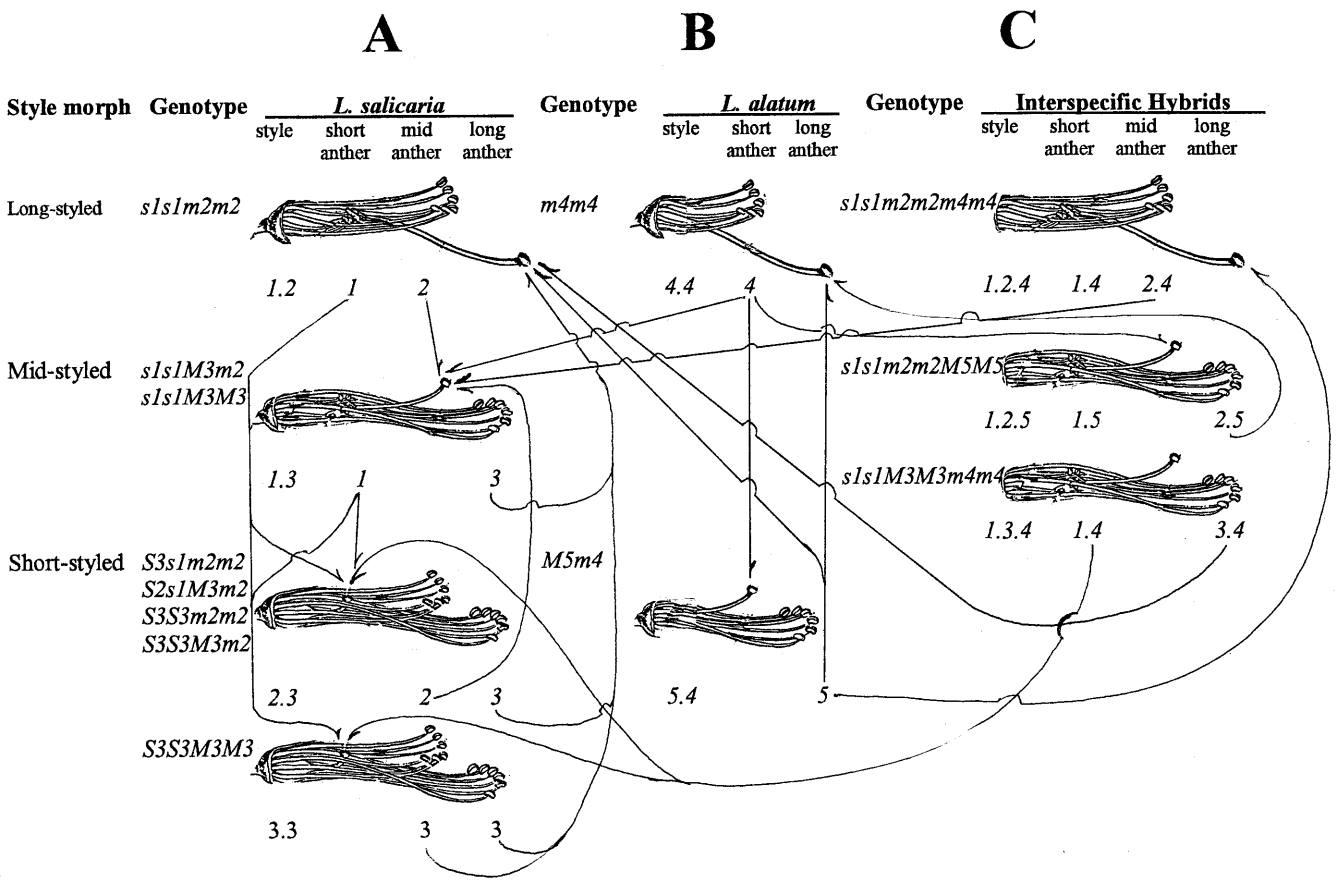

Fig. 3 Proposed *Lythrum salicaria* (**A**), *L. alatum* (**B**), and interspecific *L. salicaria*×*L. alatum* (**C**) hybrid genotypes/alleles for heterostyly and self incompatibility. *Arrows* indicate direction of compatible pollen flow under the proposed model (adapted from Darwin 1865, 1868). See text for pistil/anther allelic dominance relationships

While the homomorphic, gametophytic system produces two pollen phenotypes (as well as genotypes) in a diploid, these occur as a result of meiotic segregation within a single anther, not in different anthers of a single plant (Ascher 1976; Liedl and Anderson 1993). In purple loosestrife, not only does the phenotype of the pollen grain depend on the genotype of the plant bearing the pollen, as with homomorphic sporophytic systems, but it also depends on the position in the flower in which the anther bearing the pollen grain occurs. The fact that genes can be expressed differently in different whorls of anthers of *L. salicaria* is proven by pollen color. Pollen from anthers on long stamens is green, while that on mid or short stamens of the same flower is yellow (Barlow 1923). These observations suggest that plants with long stamens are heterozygous for pollen color, with the allele for green dominant in the anthers of long stamens and the allele for yellow dominant in anthers of mid or short stamens. Differential expression of the incompatibility gene(s) in different floral organs is not unusual in homomorphic, sporophytic SI systems, where one *S* allele may be dominant to another in the pistil, in pollen, in both, or in neither (Sampson 1967; Ascher 1976; Liedl and Anderson 1993). Heteromorphic, sporophytic SI systems such as the "Primula" system of distyly depend on one *S* allele dominating the other in the pistil and pollen (Lewis 1954).

We propose the following model for the pollen-pistil-incompatibility system linked with tristyly for *L. salicaria.* Since the ploidy level varies (4x, 6x) within the species, it is unknown whether inheritance is tetrasomic, hexasomic, or diploid-like, and whether this is an auto- or allo-polyploid species. Our proposed model will be based at the diploid level (for subsequent modification pending additional genetic research). Assuming linkage between incompatibility-specificity genes and floral-morph genes, a minimum of three incompatibility specificities is necessary, each of which must be dominant to the other two in anthers of one of the stamen lengths and one of which must be dominant to the other two in the pistil. Following published genetic notations (Barlow 1923; Fisher and Mather 1943), the long-styled, recessive genotype *ssmm* would carry only two incompatibility specificities, one linked to *s* and the other to *m*, a two-loci-sporophytic system, to give the genotype *s1s1m2m2* (Fig. 3a). The italicized numbers represent alleles, with different specificities, at an incompatibility locus that is linked to each of the floral-morph sets of loci, rather than variations of the linked floral-morph alleles (the standard notation for the SI locus, *S*, followed by numbers to specify different alleles, is preempted by the use of *S* to

designate the linked set of loci conferring the short-style morph). Incompatibility results when the incompatibility allele (italicized number) expressed in the pollen matches an allele expressed in the pistil, regardless of whether the allele is attached to the *S* or *M* locus. For *s1s1m2m2* (Fig. 3A), both specificities would be expressed in the pistil, giving the long-style phenotype of *1.2*; the *1* phenotype would be expressed in the whorl of short stamens (*1>2*, *3* in short stamens); and the *2* phenotype would be expressed in the whorl of mid stamens (*2>1*, *3* in mid stamens). The third incompatibility specificity (*3*) would be linked to the dominant allele at both the *S* and *M* loci and would be dominant to both *1* and *2* in anthers of the long whorl of stamens but recessive to each in mid and short stamens (Fig. 3A). In the pistil, the incompatibility specificity linked to *S* would be dominant to the one linked to *s*; the specificity linked to *M* would be dominant to the one linked to *m*; and, as proposed in the genetic model for the inheritance of tristyly (Barlow 1923; Fisher and Mather 1943), *S* would be dominant to *M*. With this scenario, a mid-styled morph with the genotype *s1s1M3m2* (Fig. 3A) would have a pistil phenotype of *1.3* and produce pollen with the incompatibility phenotype *1* in anthers on short stamens (*1>2* and *3*) and *3* in anthers on long stamens (*3>1* and *2*). A short-styled morph with the genotype *S3s1m2m2* (Fig. 3A) would have a pistil phenotype of *2.3* and produce pollen with the incompatibility phenotype *2* in the anthers on mid stamens (*2>1* and *3*) and *3* in anthers on long stamens (*3>1* and *2*). Because of legitimate crosses between mids and shorts, a second genotype is possible for short-styled morphs, namely *S3s1M3m2* (Fig. 3A). Since *S3* is dominant to *M3*, *S3s1M3m2* would exhibit the same phenotype as *S3s1m2m2*. This second genotype for short-styled morphs leads to a second genotype for mid-styled morphs, *s1s1M3M3*, with a phenotype identical to *s1s1M3m2* (*M3* is dominant to *m2* in the pistil). Each morph would be SI and all ”legitimate” pollinations, that is pollinations involving anthers and stigmas of the same height, would be compatible.

With a fully-expressed SI system, plants homozygous for the dominant set of linked alleles, *S*, should not occur. However, incompatible pollinations sometimes produce seeds because of less than complete expression of the SI reaction, a phenomenon termed pseudo-self compatibility or PSC. PSC is documented in *L. salicaria*, as illegitimate pollinations occasionally produce seed (Darwin 1865; Stout 1923; Mulcahy and Caporello 1970; Ottenbreit 1991). The effect of PSC in purple loosestrife would be the possibility for homozygosity of the dominant set of alleles, *S*, which would reduce the number of incompatibility specificities in such short-styled individuals. While *S3S3m2m2* would express a phenotype identical to that of *S3s1m2m2* (*S3* is dominant to *s1* but not *m2* in the pistil), as would *S3S3M3m2* (*S3* is dominant to *M3* in the pistil), *S3S3M3M3* would produce short-styled individuals with pollen from anthers on mid stamens bearing incompatibility phenotype *3* rather than *2*. Such pollen would be incompatible on mid-styled females; pollen from both mid and long anthers would be compatible on long-styled plants (Fig. 3A). Short-styled individuals, *S3S3M3M3*, would also be compatible (as female) with pollen from both mid- and short-stamens, giving rise to compatible “illegitimate” pollinations as well as incompatible “legitimate” pollinations. This model provides a basis for failed “legitimate” crosses but does not explain the distribution of failed crosses in Minnesota-Wisconsin purple loosestrife where incompatible “legitimate” crosses were not restricted to M×mS pollinations but occurred in all legitimate crosses (Tables 4, 5).

Although the pooled χ^2 testing for equal frequency across style morphs of individuals with at least one failed compatible cross was not significant, 9 of 12 populations containing all three style morphs exhibited significant deviations (Table 2). Apparently, populations differ significantly, but the differences between populations are random. In an effort to clarify this point, we randomly selected n=66 compatible crosses within populations and n=66 compatible crosses between populations for each style morph and subjected these data to χ^2 tests for zero versus non-zero seed set, using the expected ratio 0:1 for tests within or between populations and the expected ratio of 1:1 for tests comparing within-population and between-population seed set. Within-population crosses deviated significantly from expected for all three floral morphs, with the deviation highly significant for long styles and the pooled value; crosses between populations differed significantly only for pooled values; and comparisons of seed set within and between populations deviated, with high significance, for both long-styled and pooled values (Table 8). More compatible crosses fail within than between populations, and long-styled females are the most likely to match incompatibility specificity with legitimate pollen. Long-styled plants also figure prominently in differences within and between populations for percent failures of compatible crossing combinations, as all three combinations exhibiting significant differences involved long-styled plants (Table 5).

The addition of incompatibility alleles that are not responsive to the dominance-recessive relationships in pollen and pistil required for linked tristyly could explain the high frequency of failed legitimate crosses in a tristyled system. A likely source of such incompatibility alleles for Minnesota-Wisconsin populations of purple loosestrife would be sympatric *L. alatum* or interspecific *L. salicaria*×*L. alatum* hybrid cultivars (Anderson and Ascher 1994a). *L. alatum* is a cosmopolitan North American species whose native habitat is frequently sympatric with naturalized *L. salicaria*. Natural or artificial hybridization between the two species can occur (Harp 1957; Levin 1970; Anderson and Ascher 1994a; Ottenbreit and Staniforth 1994; Anderson et al. 1995), notwithstanding the fact that *L. salicaria* is a tristyled polyploid (4x, 6x) and *L. alatum* is a distyled diploid (2x, producing 2n gametes). In addition, interspecific *L. salicaria*×*L. alatum* hybrids, widely cultivated as garden flowers in the Midwest, are fertile (Anderson and Ascher 1994a, 1995a)

Table 8 Frequency and χ^2 test of zero: non-zero seed set for randomly selected (*n*=66 per style morph) *Lythrum salicaria* cross-compatible pollinations (within and between populations) (*ns*=not significant)

Populations	Style morph	Number of zeroes	Number of non-zeroes	χ^2 ratio tested	χ^2	*P* value[a]
Within	Short	18	48	0:1	4.9	0.02*
	Mid	17	49	0:1	4.4	0.02*
	Long	32	34	0:1	15.5	<0.01***
	Pooled	67	131	0:1	22.7	<0.01***
Between	Short	16	50	0:1	3.9	0.06 ns
	Mid	12	54	0:1	2.2	0.13 ns
	Long	10	56	0:1	1.5	0.2 ns
	Pooled	38	160	0:1	7.3	<0.01***
Within:between	Short	18:16		1:1	0.12	0.72 ns
	Mid	17:12		1:1	0.86	0.4 ns
	Long	32:10		1:1	11.52	<0.01***
	Pooled	67:38		1:1	8.0	<0.01***

[a] *, *** denote significance at the 5% and <0.1% level, respectively. All other *P* values are not significant

Table 9 Ratios of mean style and anther length measurements (mm) in representative Minnesota populations of distylous *Lythrum alatum* (Anderson and Ascher 1993b), tristylous *L. salicaria*, and tristylous interspecific *L. salcaria*×*L. alatum* hybrid cultivars (Anderson and Ascher 1993a)

Species population	*n*	Styles			Anthers		
		Long:mid	Long:short	Mid:short	Long:mid	Long:short	Mid:short
L. alatum							
Ottawa	37		1.4:1			1.4:1	
Iron Horse	14		1.4:1			1.2:1	
Redwood	10		1.1:1			1.0:1	
Expandere	13		1.4:1			0.9:1	
Pooled	74		1.3:1			1.3:1	
L. salicaria							
S-01	52	1.5:1	3.3:1	2.2:1	1.6:1	2.4:1	1.8:1
S-02	48	1.5:1	3.0:1	2.0:1	1.6:1	2.3:1	1.6:1
S.03	39	1.7:1	3.0:1	1.8:1	1.4:1	2.0:1	1.7:1
S-04	48	1.4:1	2.8:1	2.0:1	1.5:1	2.3:1	1.6:1
S-07	31	1.5:1	3.0:1	2.0:1	1.4:1	2.3:1	1.6:1
S-08	15	1.5:1	2.9:1	1.9:1	1.6:1	2.9:1	1.5:1
S-32	8	1.5:1	3.0:1	2.0:1	1.5:1	2.7:1	1.8:1
S-34	24	1.6:1	2.9:1	1.7:1	1.5:1	2.5:1	1.5:1
S-35	17	1.5:1	3.7:1	2.5:1	1.6:1	2.4:1	1.7:1
S-37	17	1.6:1	2.6:1	1.6:1	1.5:1	2.2:1	1.7:1
Pooled	299	1.5:1	3.0:1	2.0:1	1.5:1	2.3:1	1.6:1
L. salicaria×*L. alatum*							
Morden Gleam	1					1.8:1	
Morden Rose	1					2.0:1	

and enhance introgression between the species. Throughout Minnesota-Wisconsin, *L. salicaria* populations are frequently sympatric with the interspecific cultivars, including the following populations in the present study: S-01, S-02, S-06, S-07, S-08, S-10, S-12, S-30, S-33, S-34, and S-37. Indeed, many individuals from the Minnesota and Wisconsin populations possessed diagnostic traits characteristic of *L. alatum*, for example, alternate rather than opposite or whorled leaves, single flowers per node rather than multiple, and glabrous rather than pubescent leaves/stems (Anderson and Ascher 1994a, b; Anderson et al. 1995).

Since distylous *L. alatum* possesses SI (Ornduff 1978) and occurs at isoplethic equilibrium in Minnesota populations, that is a 1:1 ratio of pin and thrum plants (Anderson and Ascher 1993b), the "Primula" system for control of incompatibility can be applied. However, comparisons of distylous North American *Lythrum* species, including *L. alatum*, with tristyled *L. junceum* revealed that anther and stigma positions in the distylous species were equivalent to the mid and long rather than short and long positions of the tristyled species (Ornduff 1979). To ensure that the *L. alatum* populations in Minnesota had the same positional relationship, we measured style/anther lengths (Dulberger 1970a) for *n*=299 *L. salicaria*, *n*=74 *L. alatum* (Anderson and Ascher 1993b), and *n*=2 *L. salicaria*×*L. alatum* (Anderson and Ascher 1993a) individuals (*n*=10 reps per genotype). The ratios of long:mid, long:short, and mid:short for styles and anthers indicate that for *L. alatum* individuals the pin/thrum lengths were equivalent to the mids and longs of *L. salicaria* (Table 9). Since the data agree with Ornduff (1979), the notation used in the "Primula" system, that is, *Ss* for thrum and *ss* for pin (Lewis 1954), was

changed to *Mm* for thrum (equivalent to mid-styled *L. salicaria*) and *mm* for pin (equivalent to long-styled *L. salicaria*). According to the "primrose" system, the incompatibility allele linked to the dominant *M* (for example, *5*) would be dominant in both pollen and pistil to the one linked to *m* (for example, *4*) (Fig. 3B). Since *L. salicaria* and *L. alatum* evolved independently on different continents, dominance relationships in one should not coincide with those of the other. This would be particularly true of pollen, as function in the tristyled system depends on dominance of one allele over two others at each anther height. Since only two incompatibility alleles exist in the distyled system, selection for dominance-recessive relationships involving three alleles in pollen would be precluded. To fix floral morphology after introgression, the *S* sequence of linked loci in *L. salicaria* must dominate the *M* sequence from *L. alatum*, just as it does that of the *M* loci from *L. salicaria*. With these assumptions, adding *M5* and *m4* to *L. salicaria* would produce long-styled plants expressing *1.2.4* pistils, *1.4* pollen from short stamens, and *2.4* pollen from mid stamens (Fig. 3C). Mid-styled hybrids would have two genotypes, one with *1.2.5* expressed in the pistil, *1.5* in pollen from short stamens, and *2.5* pollen from long stamens, and the other with *1.3.4* expressed in the pistil, *1.4* in pollen from short stamens, and *3.4* in pollen from long stamens (Fig. 3C). Interestingly, the one long- and two mid-styled phenotypes noted above would result from legitimate crosses between mid- and long-styled purple loosestrife and the pin and thrum of *L. alatum*, and all legitimate crosses between the hybrids would be cross-incompatible. This cross-incompatibility has been confirmed, as predicted by the long:short ratio differences between interspecific cultivars and *L. salicaria* populations in Table 9 (Anderson and Ascher, unpublished data). In addition, the *1.2.5*, mid-styled hybrid would be incompatible as female with legitimate crosses of *L. salicaria* and as male on long-styled morphs of *L. salicaria* (Fig. 3c). Placing the alien alleles in short-style morphs, either by illegitimate direct crosses between the species or through legitimate intercrossing of hybrids with short-styled *L. salicaria*, would increase the number of incompatible legitimate crosses, both because of an extra incompatibility allele (*M5* or *m4*) expressed in the pollen of each whorl and because of one or two extra alleles expressed in the pistil (two if the dominance of loci at *S* do not include dominance of *S3* over *M5*).

Using our model for an SI system linked to the genes controlling tristyly (Fig. 3) and the proposed outcome of hybridization of individuals with this reproductive system to individuals possessing SI linked to distyly (the "Primula" system), all of the unexpected phenomena observed in this study can be explained. Individuals of the same style morph exhibiting different incompatibility specificities and reciprocal differences in "legitimate" crosses would both result from expression of alien incompatibility specificities not controlled by the dominance relationships integral to the tristyled system. Differences between populations in frequencies of failed "legitimate" crosses, and failed crosses to style morph or crossing combination would be a function of the founder effect, the opportunity for hybridization with *L. alatum* or cultivated hybrids involving *L. alatum*, and the history of the populations since the founding events.

Addition of incompatibility alleles to tristylous *L. salicaria* by means of hybridization with sympatric, distylous *L. alatum* or cultivated *L. salicaria*-*L. alatum* hybrids and the resulting disruption of the reproductive system is a classic case of incongruity, the failure of normal function because of mis-matching genetic information in hybrids from parents which have been sexually isolated (Hogenboom 1973, 1975). Thus, seed set from a "legitimate" crossing of Minnesota-Wisconsin purple loosestrife may measure incongruity between two SI systems, rather than fecundity. Likewise, our seed germination data may be estimating the degree of interspecific hybridization and subsequent incongruity in various populations, since hybrid breakdown is a symptom of incongruity often expressed as germination failure, rather than fitness of the various floral morphs in Minnesota-Wisconsin populations of purple loosestrife. This occurrence of incongruity and the model for *L. salicaria* proposed here are unique to North American populations of purple loosestrife. It would not be expected that European populations would possess the high failure rate (zero seed set) from cross-compatible matings, since cross-hybridization with distylous North American species is undocumented.

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