

POLLEN LIMITATION AND THE EVOLUTION OF SELF-COMPATIBILITY IN *LINANTHUS* (POLEMONIACEAE)

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The magnitude of pollen limitation of reproduction was compared in three annual species of *Linanthus* with contrasting mating systems. In a 2-yr study, pollen limitation of total female reproductive success was measured in three populations of each species by comparing seed and fruit set in paired pollen-supplemented and open-pollinated plants. *Linanthus parviflorus*, a self-incompatible species, was found to be pollen limited for some measures of reproductive success in all populations in 1 yr and in two populations in the other. In contrast, no significant pollen limitation was detected in *Linanthus bicolor*, a highly selfing species, and in two populations of *Linanthus jepsonii*, a partially outcrossing but self-compatible species, indicating that autogamous selfing is providing reproductive assurance in most populations of these species. Significant spatial and temporal variation in pollen limitation was found in *L. parviflorus* and *L. jepsonii*. Although variable pollinator abundance is likely to have been one cause of the observed variation, it appears that a number of other factors, including resource availability, wind pollination, and pollen inviability, also contributed to variation in the magnitude of pollen limitation. Spatial variation in pollen limitation may explain why some lineages maintain outcrossing mechanisms whereas others evolve toward self-fertilization.

Keywords: pollen limitation, mating system evolution, wind pollination, self-compatibility, *Linanthus*, Polemoniaceae.

Introduction

Pollinator visitation may vary in time or space as a result of variation in pollinator abundance, competition between flowering species for pollinators, or ecological factors that result in asynchrony between flowering time and pollinator activity. In light of the potential for variability in visitation, it is not surprising that pollen limitation of reproduction has been demonstrated in numerous plant species. In a broad survey of pollination studies, 62% of species exhibited significant pollen limitation of reproduction at some time or place (Burd 1994). Hand-pollination of stigmas has been shown to increase seed or fruit set, by a more than tenfold value in some species (Burd 1994).

When female reproductive success in an obligately outcrossing species is strongly limited by pollen delivery, selection may favor mutations that confer self-fertilization, thereby assuring some reproduction without the service of pollinators. This “reproductive assurance hypothesis” has been put forth as an explanation for the evolution of self-fertilization from outcrossing (Baker 1955; Stebbins 1957), an evolutionary transition that has occurred repeatedly in the history of angiosperms (Stebbins 1974). Evidence for the hypothesis comes from the observation that selfing taxa often occupy habitats with low pollinator abundance, such as high altitudes, xeric sites, or species’ geographic range limits (reviewed by Lloyd 1980; Wyatt 1988).

If selfing confers reproductive assurance, selfing and obli-

gately outcrossing species are expected to differ in the magnitude of pollen limitation of female reproductive success, with little or no pollen limitation predicted in selfing species and higher and more variable levels expected in outcrossing species. A review of studies of pollen limitation in a broad range of selfing and outcrossing plant taxa (Burd 1994), as well as a recent comparative investigation using phylogenetically independent contrasts (Larson and Barrett 2000), has found associations between the magnitude of pollen limitation and the breeding system, lending support to the reproductive assurance hypothesis. Studies of closely related species with contrasting mating systems can provide greater understanding of the trends revealed in broad surveys because comparisons are drawn between taxa that are similar in other respects as a result of common ancestry. Although this approach has been useful in research on other aspects of plant mating systems (e.g., Solbrig and Rollins 1977; Wyatt 1988; Parker et al. 1995; Carr and Dudash 1996), very few studies have compared congeners or conspecifics to gain insights on pollen limitation and its role in mating system evolution (Piper et al. 1986). Here, I present data on pollen limitation for three annual species with contrasting mating systems in the genus *Linanthus* (Polemoniaceae).

Linanthus includes both self-compatible and self-incompatible species. Phylogenetic analyses of just one section of the genus indicate that there have been at least three independent origins of self-compatibility from outcrossing (Goodwillie 1999a). The current study was undertaken to provide insight into the selective factors that may have driven this frequent evolutionary transition in *Linanthus*. Three closely related, sympatric species—one self-incompatible, one highly selfing,

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and one self-compatible but partially outcrossing—provided an opportunity to compare the magnitude of pollen limitation and to investigate its role in mating system evolution in this genus. Because it is influenced by a number of ecological variables, pollen limitation is expected to vary both in space and time. Therefore, to characterize the range of pollen limitation experienced by a species requires replication in multiple populations and years (Baker et al. 2000). Of the large number of studies that report measures of pollen limitation, relatively few have taken this approach (reviewed by Burd 1994; Baker et al. 2000).

In a 2-yr study, I tested experimentally for pollen limitation of female reproductive success by comparing fruit and seed set in pollen-supplemented plants and open-pollinated plants in multiple populations of *Linanthus parviflorus*, *Linanthus bicolor*, and *Linanthus jepsonii*. The goals of the study were (1) to determine whether self-fertilization confers reproductive assurance and whether a relationship exists between the mating system and magnitude of pollen limitation in the three species and (2) to assess the extent of among-population and between-year variation in the magnitude of pollen limitation. The possible causes of this variation and its implications for mating system evolution are examined.

Material and Methods

Study Species and Sites

Linanthus is a genus of primarily annual species with a center of distribution in western North America. *Linanthus parviflorus* has a highly effective self-incompatibility system (Goodwillie 1997) that ensures obligate outcrossing, whereas *Linanthus jepsonii* and *Linanthus bicolor* are self-compatible and capable of autogamous selfing (Goodwillie 2000). Isozyme data for progeny arrays indicate that *L. jepsonii* has a mixed mating system, with population outcrossing rates in 1995 ranging from 0.10 to 0.30 (Goodwillie 2000), whereas *L. bicolor* is almost exclusively self-fertilizing. Self-fertilization in *L. jepsonii* usually does not occur until after the first day of flowering as a result, in part, of an initial separation of stigma and anthers, which creates an opportunity for outcrossing. Later in anthesis, stigma-anther separation is reduced, promoting delayed selfing (C. Goodwillie, unpublished data). In contrast, self-pollination in *L. bicolor* occurs immediately on flower opening and appears to promote early self-fertilization.

All three species have terminal, headlike inflorescences and five-lobed corollas with long, slender tubes. The flowers of *L. jepsonii* and *L. parviflorus* are morphologically very similar; their corolla tubes, corolla lobes, and stigma lobes are nearly twice as long as those of the diminutive selfing species *L. bicolor* (Schemske and Goodwillie 1996). *Linanthus jepsonii* and *L. parviflorus* are visited almost exclusively by long-tongued flies (e.g., *Bombylius* spp.) that probe the corolla tubes for nectar with their long proboscises (Grant and Grant 1965). Though Grant and Grant (1965) indicate that *L. bicolor* is visited by bees, I have observed only rare bee visits to the inconspicuous flowers of this small plant, and none were observed in the experimental populations.

Previous investigations have shown that in at least some populations of *L. parviflorus*, wind pollination also contrib-

utes to reproduction (Goodwillie 1999b). Substantial seed set occurred when insects, but not airborne pollen, were excluded and airborne pollen counts were high in *L. parviflorus* populations. In contrast, *L. jepsonii* populations were found to have low airborne pollen loads, indicating that wind pollination does not occur in this species. Data are not available for *L. bicolor*, but its diminutive height and low pollen production relative to the other species (C. Goodwillie, unpublished data) indicate that wind pollination does not occur.

Sequence data for the internal transcribed spacer (ITS) region of rDNA indicate that *L. bicolor* and *L. jepsonii* are sister species and that *L. parviflorus* shares a recent common ancestor with the two (Goodwillie 1999a). The range of *L. parviflorus* extends throughout much of California (Patterson 1993), and *L. bicolor* is distributed from central California to the Puget Sound region of Washington (Patterson 1993; Goodwillie and Stiller 2001). The distribution of *L. jepsonii* is restricted to three counties in the California North Coast Ranges, and there the three species co-occur (Schemske and Goodwillie 1996). Though populations of the three species may be found in close proximity where they are sympatric, their habitats are subtly distinct. *Linanthus jepsonii* occupies moist, often shady areas, *L. parviflorus* occurs on exposed hillsides and open woodlands, and *L. bicolor* is found in drier areas, sometimes extending onto serpentine soils.

Three populations of each species were studied in Napa County, California, in the spring of 1995 and 1996. Study populations of *L. jepsonii* and *L. parviflorus* were located at Mast Hill, the Wantrup Reserve, and the Litto Ranch. At each site, populations of the two species were separated by less than 100 m. A population of *L. bicolor* was also located at Mast Hill in a dry grassy area about 0.2 km from the *L. jepsonii* site. Two additional *L. bicolor* populations were studied at a site in Pope Canyon, one of these along the banks of Pope Creek and the other located about 0.5 km upland in a drier area referred to as Cedar Roughs.

Flowering Phenology

Flowering phenology was censused in populations of the three species at Mast Hill and in populations of *L. parviflorus* and *L. jepsonii* at Wantrup Reserve and Litto Ranch. In each population, four haphazardly chosen 0.25-m² quadrats were established and sampled in both years of the study. The number of open flowers in each plot was counted at 2- to 7-d intervals throughout the flowering season. Occasional periods of rain precluded a more regular sampling schedule, since flowers open only on sunny days.

Pollen Limitation

To assess the magnitude of pollen limitation, 60–90 pairs of adjacent plants (separated by less than 10 cm) of similar size were marked in each of the nine study populations in each year. One plant in each pair was randomly assigned to a pollen-supplementation treatment, the other served as an open-pollinated control. In pollen-supplementation treatments, pollen was applied to all open flowers by brushing the stigmas with the anthers of flowers from two donor plants. Since flowers remain open for 2 d or more (C. Goodwillie, unpublished data), pollen supplementation on alternate days was sufficient

to ensure that all flowers were treated. Plants were assigned to experimental treatments before the onset of flowering, and treatments were applied throughout the entire flowering season. Fruits were collected when mature. The total numbers of flowers, fruits, and seeds were counted for each plant.

Data Analysis

To test for pollen limitation in each population, open-pollinated and pollen-supplemented plants were compared using paired *t*-tests. Four measures of female reproductive success were considered: the numbers of fruits, seeds, fruits/flower, and seeds/fruit. Because the experiment was designed to test the hypothesis that pollen supplementation increases fruit or seed set, *t*-tests were one-tailed. Although flower number is not expected to be influenced directly by pollination treatment, this variable was examined in *t*-tests to investigate the possibility that investment in early fruits may affect later flower production, as has been shown in at least one other study (Stanton et al. 1987). Flower, fruit, and seed numbers were log transformed in *t*-tests to improve the fit to statistical models. Significance levels of *t*-tests for all species, populations, and years were adjusted to hold the experiment-wise Type I error rate at 0.05 using the sequential Bonferroni method (Rice 1989). This provides a conservative assessment of the significance of individual *t*-tests.

Two-way nested ANOVA was used to test for differences in the magnitude of pollen limitation, with species and year as main effects, population nested within species, and all possible interaction terms included. In all ANOVAs in this study, species was considered to be a fixed effect, and population and year were considered to be random effects. For each reproductive variable, pollen limitation was calculated for each pair of plants as the ratio of the open-pollinated plant to the pollen-supplemented plant. The ratio was log transformed to conform to the assumptions of the ANOVA. Error terms for mixed-model ANOVAs were calculated using a linear combination of variance components of the random effects and quadratic terms of the fixed effects (SPSS 1997). In separate analyses for each species, a two-way ANOVA was used to test for variation among populations and between years and for the interaction of the two factors.

The total number of seeds produced by pollen-supplemented plants provides a measure of reproduction when resources, not pollen, are limiting. This variable (log transformed) was used to test for differences among populations and years in resource availability. ANOVAs were carried out for each species, with population and year as main effects.

Results

Flowering Phenology

Linanthus bicolor at Mast Hill flowered earlier than populations of *Linanthus jepsonii* and *Linanthus parviflorus* in both years: peak flowering date was about 2 wk before that of the other two species (fig. 1). Flowering in *L. parviflorus* and *L. jepsonii* was approximately synchronous. Peak flowering dates for the two species were separated by no more than 4 d in any population in either year. Mean flowering densities,

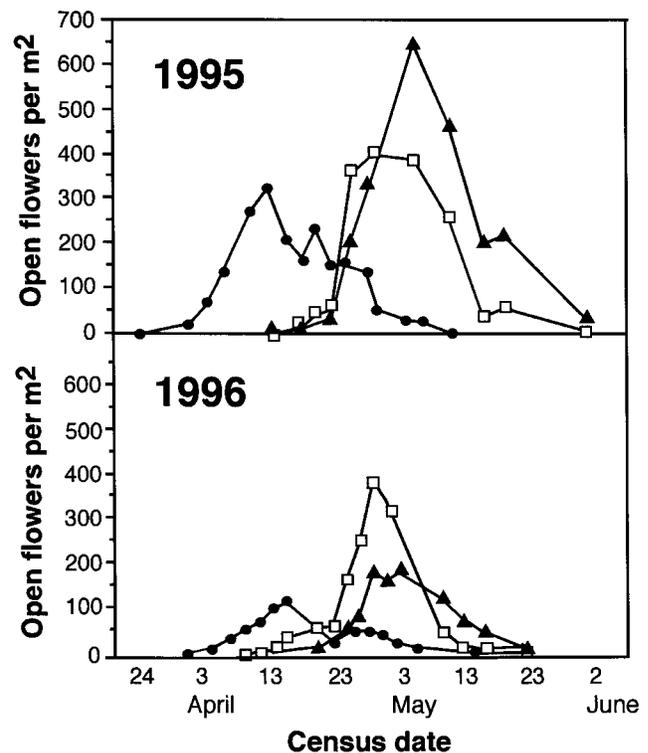


Fig. 1 Flowering phenology of *Linanthus bicolor* (filled circles), *Linanthus jepsonii* (filled triangles), and *Linanthus parviflorus* (open squares) at Mast Hill in 1995 and 1996. The number of open flowers per square meter is the sum of flowers in four 0.25-m² quadrats distributed throughout the population.

as indicated by quadrat flower counts, were higher in 1995 in all populations of all species.

Pollen Limitation Experiment

Final population sample sizes were reduced somewhat because of trampling by cattle (table 1, 42–88 treatment pairs, mean = 61.67). Population means for measures of reproductive success were higher for pollen-supplemented plants than for open-pollinated plants in all but eight of the 72 comparisons (three species \times three populations \times 2 yr \times four reproductive measures), and a number of these differences were significant at the individual test or experiment-wise level (table 1; fig. 2). In the presentation of results in the text below, I report significance at the experiment-wise level only. Although small increases in reproduction were conferred by pollen supplementation in the two self-compatible species, these differences were not significant in any *L. bicolor* population nor in two of three *L. jepsonii* populations. The Mast Hill population of *L. jepsonii* was found to be significantly pollen limited for seeds, fruits/flower, and seeds/fruit in 1995 and for fruits/flower in 1996, with significant increases attributable to pollen supplementation ranging from 14% to 50%.

In the self-incompatible species, significant pollen limitation was more frequently observed, and its magnitude was often higher. *Linanthus parviflorus* at the Litto Ranch was pollen limited for all measures of reproductive success in 1995 and

Table 1
Means for Pollen-Supplemented (Supp) and Open-Pollinated (Open) Plants for Four Measures of Female Reproductive Success with Sample Sizes and Significance Values from Paired *t*-Tests

Species/location/ reproductive variable	1995				1996			
	N (pairs)	Supp	Open	<i>P</i>	N (pairs)	Supp	Open	<i>P</i>
<i>Linanthus bicolor</i> :								
Cedar Roughs	42				55			
Fruit number		2.76	2.48	0.076		3.69	3.51	0.154
Seed number		23.91	19.98	0.027		35.04	31.86	0.122
Seeds per fruit		8.55	7.85	0.043		9.56	8.86	0.476
Fruits per flower		0.94	0.91	0.150		0.80	0.81	0.262
Mast Hill	59				56			
Fruit number		3.95	3.92	0.392		2.77	2.88	0.326
Seed number		40.80	38.10	0.104		26.07	26.22	0.305
Seeds per fruit		10.31	9.86	0.080		9.19	9.02	0.451
Fruits per flower		0.95	0.89	0.005		0.86	0.84	0.306
Pope Creek	56				67			
Fruit number		4.27	4.15	0.154		3.34	3.19	0.413
Seed number		36.38	34.91	0.122		31.39	28.67	0.473
Seeds per fruit		8.53	8.57	0.476		8.98	8.98	0.490
Fruits per flower		0.93	0.87	0.019		0.88	0.88	0.479
<i>Linanthus jepsonii</i> :								
Litto Ranch	52				57			
Fruit number		10.37	10.00	0.291		5.49	5.42	0.441
Seed number		77.42	70.04	0.146		34.16	32.51	0.178
Seeds per fruit		7.38	7.34	0.453		6.48	6.19	0.194
Fruits per flower		0.86	0.85	0.494		0.89	0.90	0.261
Mast Hill	55				56			
Fruit number		12.11	9.88	0.007		8.91	7.50	0.064
Seed number		76.84	51.28	<0.001**		53.84	41.00	0.017
Seeds per fruit		6.56	4.89	0.001**		6.18	5.20	0.016
Fruits per flower		0.91	0.72	<0.001**		0.82	0.72	0.001**
Wantrup Reserve	57				56			
Fruit number		11.26	10.73	0.461		7.09	7.02	0.262
Seed number		75.16	74.20	0.433		47.44	43.48	0.376
Seeds per fruit		6.99	6.95	0.431		6.47	6.09	0.246
Fruits per flower		0.91	0.85	0.007		0.80	0.77	0.243
<i>Linanthus parviflorus</i> :								
Litto Ranch	55				54			
Fruit number		9.69	6.71	<0.001**		10.34	10.34	0.427
Seed number		54.40	25.04	<0.001**		58.91	47.05	0.033
Seeds per fruit		5.61	3.79	<0.001**		5.58	4.35	<0.001**
Fruits per flower		0.84	0.65	<0.001**		0.84	0.74	0.003
Mast Hill	82				88			
Fruit number		10.92	11.24	0.443		7.07	6.74	0.071
Seed number		68.07	63.87	0.079		40.66	33.05	0.026
Seeds per fruit		6.20	5.52	0.012		5.76	5.17	0.015
Fruits per flower		0.92	0.89	0.162		0.78	0.73	0.058
Wantrup Reserve	77				86			
Fruit number		11.33	10.25	0.007		8.61	8.17	0.382
Seed number		70.94	49.56	<0.001**		54.69	46.16	0.087
Seeds per fruit		6.23	4.82	<0.001**		6.19	5.33	0.001**
Fruits per flower		0.89	0.82	0.001**		0.81	0.75	0.006

** Significant at the experiment-wise level.

for seeds/fruit in 1996. Significant increases with pollen supplementation ranged from 28% to 117%. In the Wantrup Reserve population of *L. parviflorus*, pollen supplementation produced significant increases ranging from 9% to 43% for seed number, seeds/fruit, and fruits/flower in 1995 and a significant increase of 16% in seeds/fruit in 1996. In contrast,

the Mast Hill population of *L. parviflorus* was not significantly pollen limited for any variable in either year.

Pollen supplementation did not appear to affect subsequent investment in flower production; the number of flowers in control and pollen-supplemented treatments was not significantly different in any population in any year, and the rank

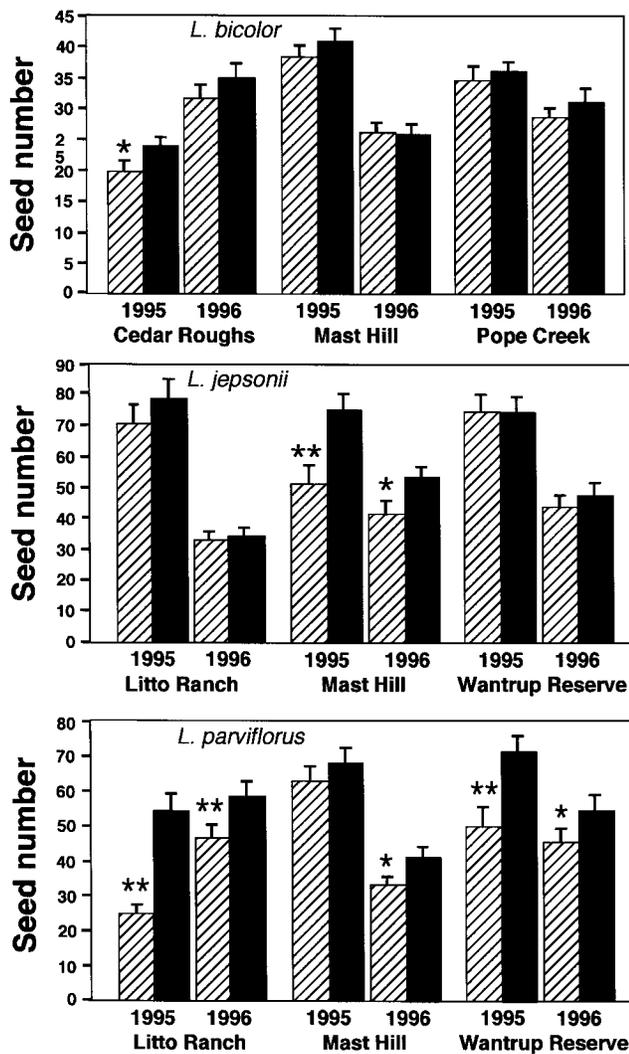


Fig. 2 Population means and standard errors for seed number in pollen-supplemented plants (filled bars) and open-pollinated plants (hatched bars). One asterisk denotes means that are significantly different at the level of individual tests; two asterisks denote means that are significantly different at the experiment-wise level.

ordering of the treatments varied among populations and years. This result also confirms that the assignment of plants to treatment groups was not biased.

ANOVA results for all four measures of female reproductive success were qualitatively similar to each other. Results of the full ANOVA (table 2) and the species-level ANOVAs (table 3) are presented only for seed number, a measure that encompasses all levels of pollen limitation. The full-model ANOVA indicates that, overall, pollen limitation was greater in 1995 with marginal significance. The interaction between year and population was also a significant source of variation. In contrast to the trend that emerges from the results of population-level *t*-tests, the difference among species in the magnitude of pollen limitation for seed number was not significant. However, in post hoc tests, *L. bicolor* had significantly lower pollen

limitation than *L. parviflorus* (Scheffé and Bonferroni tests, $P < 0.001$).

In species-level ANOVAs, pollen limitation in *L. bicolor* did not vary significantly between years or among populations (table 3). In *L. jepsonii*, among-population variation in pollen limitation was marginally significant. The interaction between year and population in pollen limitation was significant in *L. parviflorus*, but neither main effect was found to be significant.

Pollen deposition may limit either the proportion of flowers initiating fruits (fruits/flower) or the proportion of ovules fertilized in a fruit (seeds/fruit). In this study, pollen limitation was found to include effects at both levels. Pollen limitation was generally greater for seeds/fruit than for fruits/flower in *L. parviflorus*. Of the six comparisons for seeds/fruit in that species (three populations \times 2 yr), four were significant, and the mean increase resulting from pollen supplementation was 21.1%. In contrast, the mean increase in fruits/flower was 9.2%, and only two comparisons (Wantrup Reserve and Litto Ranch in 1995) showed significant pollen limitation for this variable. In *L. jepsonii*, the effect of pollen supplementation was more similar at the two levels, with mean increases of 8.6% and 7.4% in seeds/fruit and fruits/flower, respectively.

The number of seeds produced by pollen-supplemented plants was higher in 1995, and the difference was marginally significant (table 4), which indicates that, overall, resource availability was greater in that year. The interaction between years and populations within species was also significant, indicating that populations differed somewhat in their patterns of year-to-year variation in resource availability.

Discussion

Pollen Limitation and Mating Systems

If self-fertilization provides reproductive assurance in species of *Linanthus*, pollen limitation is expected to be lower in selfing than in outcrossing species. The analyses presented here indicate a relationship between the magnitude of pollen limitation and the mating system. In the highly selfing species, *Linanthus bicolor*, pollen limitation was never found to be significant, and the increases with pollen supplementation did not exceed 20% for any measure of reproductive success. Two out of three populations of *Linanthus jepsonii*, the partially outcrossing but self-compatible species, were not pollen limited. In contrast, the self-incompatible species *Linanthus parviflorus* was found to be pollen limited in some or all measures

Table 2

ANOVA for Pollen Limitation of Seed Number (Ratio of Open-Pollinated to Pollen-Supplemented Plant in Each Pair)				
Effect	df	MS	F	P
Species	2	7.959	3.489	0.258
Year	1	11.512	17.655	0.051
Population (species)	6	3.764	1.790	0.248
Species \times year	2	0.651	0.313	0.742
Year \times population (species)	6	2.103	2.703	0.013
Error	1087	845.580		

Note. Ratios were log transformed. See text for explanation of error term calculations.

Table 3

ANOVA of Pollen Limitation for Seed Number (Ratio of Open-Pollinated to Pollen-Supplemented Plants in Each Pair) for Each Species

Species/effect	df	MS	F	P
<i>Linanthus bicolor</i> :				
Year	1	4.638	2.792	0.236
Population	2	0.331	0.198	0.834
Year × population	2	1.667	2.081	0.126
Error	332	265.907		
<i>Linanthus jepsonii</i> :				
Year	1	1.000	2.466	0.257
Population	2	5.649	13.925	0.067
Year × population	2	0.406	0.704	0.495
Error	317	182.731		
<i>Linanthus parviflorus</i> :				
Year	1	8.148	1.954	0.296
Population	2	5.313	1.254	0.444
Year × population	2	4.237	4.675	0.010
Error	438	396.942		

Note. Ratios were log transformed. See text for explanation of error term calculations.

of reproductive success in two of the three populations in both years, and mean seed production in one population more than doubled when pollen was supplemented. In summary, the two self-compatible species appear to experience lower pollen limitation, overall, than the self-incompatible species. It seems unlikely that this pattern can be explained by differences among the species in pollinator abundance; populations of the three species occurred in close proximity, and their flowering phenologies were similar (fig. 1). Instead, it appears that pollen limitation is low in most populations of *L. jepsonii* and *L. bicolor* because autogamous self-fertilization is providing reproductive assurance.

The results reported here for *Linanthus* are congruent with the pattern that emerges from surveys of selfing and outcrossing species. Self-incompatible species were found to have significantly greater pollen limitation than self-compatible species in a survey of studies for 46 self-incompatible and 117 self-compatible species (Burd 1994). Similarly, Larson and Barrett (2000) found that the mean pollen limitation in 102 self-compatible species surveyed was approximately half the magnitude of that observed in 66 self-incompatible species. Moreover, this difference was significant even when phylogenetic relationships were taken into account. In a study of breeding systems and pollination in 34 tree species in a tropical forest community, self-incompatible species were often extremely pollen limited, whereas hand-pollination of self-compatible species increased seed set by a much smaller magnitude (Bawa 1974). In a test of the reproductive assurance hypothesis, pin and thrum outcrossing morphs in populations of *Primula vulgaris* were often found to be pollen limited, and seed set in open-pollinated pin and thrum flowers was significantly lower than that of self-fertile homostylous morphs in some sites (Piper et al. 1986). This result was interpreted as evidence that selection for reproductive assurance may have played a major role in the evolution of self-fertilization in *P. vulgaris*.

Temporal and Spatial Variation in Pollen Limitation

Although the summary of population-level tests indicates that *L. parviflorus*, the obligately outcrossing species, is often pollen limited and the self-compatible species are generally not, the difference among species in pollen limitation was not significant in the full ANOVA (table 2). Perhaps this result is not surprising given the magnitude of temporal and spatial variation in pollen limitation found within each species (fig. 3); the ranges of population means for the three species overlap to a considerable extent. Furthermore, the whole-model and species-level ANOVAs provide evidence for between-year and among-population variation in pollen limitation as well as variation among populations in temporal patterns (year × population interactions). The pattern seen here of variation in pollen limitation among species, between years, and among populations is complex and may reflect a number of factors.

Temporal or spatial variation in pollen limitation has been demonstrated in a number of studies (e.g., Johnston 1991; Dieringer 1992; Karoly 1992; Murphy and Vasseur 1995; Dudash and Fenster 1997; Jacquemart 1997; Parker 1997; Baker et al. 2000) and usually has been attributed to differences in pollinator abundance. Pollinator assemblages and visitation rates have been shown to vary in time and space (Horvitz and Schemske 1990; Primack and Inouye 1993; Utelli and Roy 2000), and this factor is undoubtedly a major determinant of variation in pollen limitation in many cases. Yet the relationship between visitation and the magnitude of pollen limitation has been addressed empirically in relatively few studies (Parker 1997; Larson and Barrett 1999; O'Neil 1999) and may not always account for observed differences in pollen limitation. An alternative (or complementary) explanation is that, even when the amount of pollen delivered is constant, variation in resource availability may cause variation in pollen limitation because resources determine the potential for reproduction when pollen is not limiting. In addition, variable pollen limitation may result from a combination of variation in pollen delivery and resource availability. Figure 4 presents hypothetical data in which each of these three ecological circumstances results in the same pattern of variation in pollen limitation.

Although interest has focused on resource versus pollen limitation of reproduction and how these two factors may interact (Haig and Westoby 1988; Zimmerman and Pyke 1988; Campbell and Halama 1993), the contribution of resource availability to variation in pollen limitation and the potential implications of this for mating system evolution have received

Table 4

ANOVA of Seed Number in Pollen-Supplemented Plants (Log Transformed)

Effect	df	MS	F	P
Species	2	43.623	14.850	0.107
Year	1	41.791	13.729	0.065
Population (species)	6	1.878	0.938	0.530
Species × year	2	3.060	1.547	0.286
Year × population (species)	6	2.002	3.238	0.004
Error	1096	677.658		

Note. See text for explanation of error term calculations.

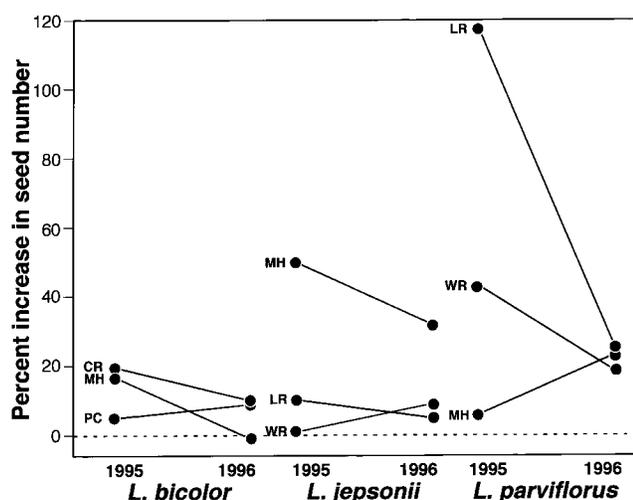


Fig. 3 Percentage increase in mean seed number in pollen-supplemented plants relative to open-pollinated plants. Dashed line at zero indicates no difference between treatment means. Solid lines connect means for each population in 1995 and 1996. CR = Cedar Roughs, LR = Litto Ranch, MH = Mast Hill, PC = Pope Creek, WR = Wantrup Reserve.

little consideration. In one of the few studies to mention this source of variation, Karoly (1992) suggested that observed differences in the magnitude of pollen limitation in two populations of *Lupinus nanus* may be caused by differences in resource levels. A related idea—that the extent of seed set is mediated by the availability of pollinators only under certain temperature and wind conditions—was demonstrated in a study of *Ranunculus acris* (Totland and Eide 1999). A definitive study of the factors underlying variation in pollen limitation in *Linanthus* would require an experimental investigation of resource limitation and observational data on visitation. A number of lines of evidence in this study, however, yield some insight into this question.

Overall, pollen limitation was significantly higher in 1995 (table 2). The increase in seed number with pollen supplementation, averaged across all species and populations, was 22% in 1995, compared with 16% in 1996. As noted above, this difference could result from variation in pollen delivery, resource availability, or both. Information about visitation is limited to anecdotal observations but indicates that pollinator abundance was unusually low in 1995, perhaps because of severe early-spring flooding. In that season, I encountered only two to three bees in a typical day of pollination treatments and censuses on *L. parviflorus* and *L. jepsonii*. In contrast, throughout the 1996 season and in the two previous field seasons, several pollinator visits were often observed in a single hour in the field. Higher flowering densities in 1995 (fig. 1) might have magnified the effect of low pollinator abundance by further reducing the probability that a flower was visited, as has been proposed for other species (Fritz and Nilsson 1994).

Despite the suggestion of lower average flower visitation in 1995, it appears likely that variation in resource availability made an equal or larger contribution to between-year differ-

ences in pollen limitation. The hypothesis that higher resource availability contributed to higher pollen limitation in 1995 is supported by the fact that the mean seed set in pollen-supplemented plants across all species was significantly higher in 1995 (59.9) than in 1996 (42.7; table 4). Water availability is likely to set a critical limit to both the magnitude and timing of reproduction in all three species. Flowering begins as the early-spring rains end, and flower and fruit production must be completed before the soils dry out in early summer. Total precipitation from March to June was substantially higher in 1995 than in 1996 (21.6 in vs. 10.1 in) and continued later into the season (National Climatic Data Center). Note that the flowering period in 1995 extended several weeks beyond that in 1996 (fig. 1).

On inspection of figure 2, the only population for which increased pollen delivery appears to be the cause of a reduction in pollen limitation in 1996 is *L. parviflorus* at Litto Ranch; open-pollinated seed production increased dramatically in 1996, whereas seed set in pollen-supplemented plants was relatively constant in the 2 yr. However, higher resource availability, not lower pollen delivery, appears to have resulted in greater pollen limitation in 1995 for *L. parviflorus* at Wantrup Reserve; although seed set in open-pollinated plants was nearly equal in the 2 yr, seed production in pollen-supplemented plants was higher in 1996 (fig. 2). An effect of resource availability on pollen limitation is also indicated for *L. jepsonii* at Mast Hill; greater pollen limitation was observed in 1995, despite higher open-pollinated seed set, because pollen-supplemented seed set in 1995 exceeded that in 1996 by an even larger magnitude (fig. 2).

Similar to the between-year trends, multiple factors may have contributed to the spatial variation in pollen limitation that was observed for *L. parviflorus* and *L. jepsonii*. In *L. parviflorus*, pollen limitation was highly significant for several

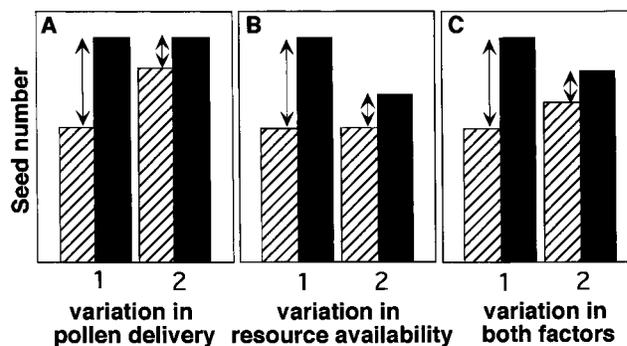


Fig. 4 A graphical representation of factors that may contribute to spatial or temporal variation in pollen limitation. Hypothetical seed number data for pollen-supplemented plants (filled bars) and open-pollinated plants (hatched bars) are shown. Replicates 1 and 2 may represent either different years or different sites. A, Resource availability (as indicated by seed number in pollen-supplemented plants) remains constant from 1 to 2, but pollen delivery (as indicated by seed number in open-pollinated plants) is higher in 2, resulting in reduced pollen limitation. B, Pollen delivery remains constant, but resource availability is lower in 2, resulting in reduced pollen limitation. C, Both higher pollen delivery and lower resources in 2 contribute to reduced pollen limitation.

measures of reproductive success in the Wantrup Reserve and Litto Ranch populations in 1995, whereas the difference between treatments was not significant at the Mast Hill population in that year (table 1; figs. 2, 3). However, of the three *L. parviflorus* populations, Mast Hill had the highest seed set in pollen-supplemented plants in 1995, which indicates that, in this case, variation in resources cannot account for the observed variation in pollen limitation. All populations of *L. parviflorus* have been found to be strongly self-incompatible (Goodwillie 1997; C. Goodwillie, unpublished data), so occasional selfing can be ruled out as an explanation for low pollen limitation at Mast Hill. Spatial variation in pollen limitation could result from variation among sites in pollinator visitation. Although this hypothesis cannot be rejected without formal data on visitation, no such differences between sites were noted in anecdotal observations, and bee fly visitation rates appeared to be universally low at all sites in 1995.

An alternative cause of variation in pollen delivery seems more likely: that wind pollination of *L. parviflorus* is more extensive at Mast Hill than at the other sites. This hypothesis is indicated by a comparison of habitats at the three sites. At Mast Hill, most of the experimental plants were found on the crest of an open, windy hillside, whereas the other two populations occur in more protected oak woodlands. Moreover, in the previous study (Goodwillie 1999b), differences in airborne pollen counts in exposed and protected sites approached significance, with the highest pollen counts found at an exposed site adjacent to the Mast Hill population used in the study reported here. Thus, extensive wind pollination at the Mast Hill population of *L. parviflorus* could account for low pollen limitation, even in a year with apparently low pollinator abundance. In addition, temporal variation in wind pollination may explain why pollen limitation at Mast Hill was greater in 1996, despite the suggestion of higher pollen visitation in that year. The flowering phenology data shown for Mast Hill indicate a brief, sharp peak in flowering (fig. 1); weather conditions on those few days could greatly affect the contribution of wind pollination to reproduction in a given year. In summary, variation in wind pollination may be an important contributor to spatial and temporal variation in pollen limitation in *L. parviflorus*.

Linanthus jepsonii populations also varied in the magnitude of pollen limitation (fig. 3). No significant pollen limitation was detected in two populations, as is expected if delayed selfing confers reproductive assurance. At Mast Hill, however, pollen supplementation resulted in significant increases in seed number of 50% in 1995 and of 31% in 1996. As with *L. parviflorus*, anecdotal observations did not detect spatial variation in pollinator visitation, but this hypothesis for variable pollen limitation in *L. jepsonii* cannot be rejected completely without more data. A number of other potential explanations seem unlikely. Resource availability, as indicated by the magnitude of pollen-supplemented seed set, was comparable in the three populations (fig. 2), so that factor appears not to have contributed to the observed variation. Morphological differences among *L. jepsonii* populations might affect the potential for autogamous selfing and therefore the extent of pollen limitation. However, stigma-anther separation, the floral trait most likely to affect the autogamy rate, was not significantly greater at Mast Hill (C. Goodwillie, unpublished data), and

autogamy rates for *L. jepsonii* plants did not differ among the three populations (Goodwillie 2000). Furthermore, inbreeding depression at the seed set stage was found to be uniformly low in all three *L. jepsonii* populations (Goodwillie 2000) and, thus, is unlikely to be the cause of among-population variation in open-pollinated seed set.

Yet another explanation for greater pollen limitation in *L. jepsonii* at Mast Hill is indicated by the high frequency of partial or complete male sterility in that population. Obvious male steriles lacking normal anthers were frequently encountered in the Mast Hill population, and in an inbreeding depression experiment with plants from the three populations, 38% of selfed progeny and 17% of outcrossed progeny from Mast Hill were either partially or completely male sterile, a level over three times as high as that in either of the other two *L. jepsonii* populations (Goodwillie 2000). In addition, plants with reduced pollen fertility were found to have significantly lower autogamous fruit production than plants with normal pollen. Thus, the greater pollen limitation in the Mast Hill population is most likely the result of a substantial proportion of plants that have reduced autogamy because of pollen sterility but that are capable of higher seed set when flowers are cross pollinated.

Mating System Evolution in *Linanthus*

In the self-incompatible species, pollen limitation was highly significant at some sites in some years. If populations of a self-incompatible ancestor of *L. jepsonii* and *L. bicolor* experienced episodes of low pollinator abundance, especially when accompanied by high resource availability, as appears to have occurred in 1995, pollen limitation would impose strong positive selection in favor of mutations that confer self-compatibility. The finding of substantial temporal and spatial variation in pollen limitation in *L. parviflorus* is congruent with current views of mating system evolution in the genus and in angiosperms in general. The angiosperms are characterized by an extraordinary diversity of mating systems. Phylogenetic studies of *Linanthus* (Goodwillie 1999a) and other families and genera (reviewed by Weller and Sakai 1999) have revealed similar patterns: transitions from outcrossing to selfing have occurred multiple times, yet other lineages remain outcrossing. Variation in pollen limitation may play a role in determining why one lineage maintains outcrossing mechanisms and another evolves toward self-fertilization.

Though pollen limitation was often significant in *L. parviflorus*, the magnitude was fairly low relative to the several-fold effects of pollen supplementation reported for other outcrossing species (reviewed by Burd 1994). Despite what appears to have been unusually low pollinator visitation in 1995, the maximum increase in seed set observed in this study was just over twofold. The evidence presented above points to a major contribution of wind pollination at the Mast Hill population of *L. parviflorus* in particular, but it is likely that wind pollination may occur to a lesser degree in all populations of this species and provides some reproductive assurance. This idea is congruent with the finding of substantially higher pollen limitation of seeds/fruit than of fruits/flower in *L. parviflorus* populations. The reverse situation, in which most or all of the pollen limitation occurs at the level of fruits/flower, is consid-

erably more common (Burd 1994), as perhaps might be expected if only some proportion of flowers are visited but a single pollinator visit is sufficient to effect high seed set in those flowers. With wind pollination, however, we might expect most flowers to intercept at least a small amount of airborne pollen, and therefore to produce a fruit, but additional pollen might be necessary for full seed set. Wind pollination is facilitated in *L. parviflorus* by its occupation of open areas, by high population densities, and perhaps by features of floral morphology that expose the stigma and anthers to wind (Goodwillie 1999a). Other self-incompatible species in the section, including the sister species to the lineage comprising *L. jepsonii* and *L. bicolor*, do not share these attributes and do not appear to be wind pollinated. It is likely that the self-incompatible ancestor of *L. jepsonii* and *L. bicolor* was not wind pollinated and therefore was more vulnerable to severe

pollen limitation than was *L. parviflorus*. Thus, selection for reproductive assurance in populations that were subject to low pollinator visitation, and perhaps particularly when accompanied by high resource availability, may have been primarily responsible for the loss of self-incompatibility in these species.

Acknowledgments

I thank D. Schemske for guidance in the design, execution, and analysis of this study; R. Olmstead and J. Stiller for valuable comments during manuscript preparation; J. Callizo and M. Groom for field assistance; and the Napa County Land Trust for providing accommodations at the field site. This research was supported by a grant from the Hardman Foundation and NSF grant DEB 94-11869 to D. Schemske and C. Goodwillie.

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