

WIND POLLINATION AND REPRODUCTIVE ASSURANCE IN *LINANTHUS PARVIFLORUS* (POLEMONIACEAE), A SELF-INCOMPATIBLE ANNUAL¹

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Wind pollination was experimentally demonstrated in *Linanthus parviflorus* (Polemoniaceae), a predominantly bee-fly-pollinated, self-incompatible annual. Seed set in plants enclosed in mesh tents that excluded pollinators but allowed airborne pollen flow provided evidence for wind pollination, and the extent of seed set due to wind pollination was compared to that in open-pollinated controls and pollen-supplemented treatments. Additional controls were included to test for possible confounding effects of the mesh tent. Mean seed number in open-pollinated plants was 72.8–81.1% of that in pollen-supplemented plants, while wind pollination alone produced 49.5–52.2%, a smaller but substantial proportion of seed set with pollen supplementation. Further evidence for wind pollination was found in a comparison of sites differing in the extent of wind exposure in two populations of *L. parviflorus*. Airborne pollen counts were higher in exposed sites than in protected sites, and the difference was marginally significant. Seed set was significantly pollen limited in protected sites, but not in exposed sites. Taken together, the data suggest that wind pollination provides some reproductive assurance in this obligately outcrossing species. Wind pollination is hypothesized to represent an alternative to selfing as an evolutionary solution to the problem of temporal or spatial variation in pollination visitation.

Key words: *Linanthus parviflorus*; Polemoniaceae; pollen limitation; reproductive assurance; self-incompatibility; wind pollination.

In an obligately animal-pollinated species, visitation of pollinators is essential for reproductive success. Pollinator visitation rates have been found to vary both in space and time (Horvitz and Schemske, 1990; Primack and Inouye, 1993; Parker, 1997), and low visitation can limit reproduction in animal-pollinated species, despite considerable investment in floral displays. In a recent survey of the literature, female reproductive success was found to be significantly pollen limited in at least some years or sites in 62% of the 268 species studied (Burd, 1994).

Though outcrossing predominates in the angiosperms, self-fertilization also is common and has evolved from outcrossing in numerous plant taxa (Stebbins, 1974). Selection for reproductive assurance is thought to be one of the major factors involved in the evolutionary transformation from animal pollination to self-fertilization (Stebbins, 1957). When pollinators are scarce or absent, plants that can self-fertilize autonomously (without the service of a pollinator) will produce more seeds than obligately outcrossing plants. If this increase in seed set outweighs any cost to offspring quality due to inbreeding depression, mutations that confer selfing will be favored. Selection for reproductive assurance is expected to be especially important for annual plant species that have only a single opportunity for reproduction (Stebbins, 1950; Baker, 1959). The observed association between selfing

and the annual life history may reflect the importance of reproductive assurance for annual species (Lloyd, 1980; Barrett and Eckert, 1990).

Self-fertilization is thought to represent an evolutionary solution to pollen limitation of reproduction in many animal-pollinated plant lineages (reviewed in Barrett, 1988; Wyatt, 1988). Wind pollination might be viewed as a potential alternative solution, providing reproductive assurance in the face of variation in pollinator abundance. A few flowering plant families are predominantly wind-pollinated (e.g., Poaceae, Cyperaceae, and Plantaginaceae) and are characterized by a suite of distinctive floral traits adapted for this mode of pollination (Whitehead, 1969; Faegri and van der Pijl, 1979), but in addition to the discrete wind and animal pollination syndromes, a number of studies suggest that some plant taxa may undergo a combination of wind and insect pollination (Pojar, 1973; Lock and Hall, 1982; Stelleman, 1984; Gomez and Zamora, 1996). An association between wind pollination and colonization of areas with low insect abundance has been noted in two primarily insect-pollinated plant taxa (Berry and Calvo, 1989; Gomez and Zamora, 1996) and supports the hypothesis that wind pollination may provide reproductive assurance. The frequency of these mixed pollination systems in the angiosperms and the contribution of wind pollination to fitness in these species remains largely unknown.

Here I present evidence for wind pollination in *Linanthus parviflorus* (Polemoniaceae), a predominantly insect-pollinated, self-incompatible annual and suggest that wind pollination provides reproductive assurance in this species. The observation of extremely low pollinator visitation in some years in populations of *L. parviflorus* (Goodwillie, 1997b) suggests that pollen limitation is expected to be a critical problem for this obligately out-

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crossing species. Phylogenetic analysis of ITS (internal transcribed spacer) rDNA sequence data indicates that selfing has evolved several times in related *Linanthus* species (Goodwillie, in press), and selection for reproductive assurance is likely to have been an important factor in those evolutionary transformations. Because wind pollination in *L. parviflorus* can provide seed set in the absence of pollinators, it may represent an alternative means of reproductive assurance and allow for the persistence of *L. parviflorus* populations when pollinators are scarce.

This study provides several lines of evidence for wind pollination and its contribution to reproduction in *L. parviflorus*. The extent of wind pollination was experimentally tested in field manipulations replicated at two sites, and airborne *L. parviflorus* pollen was shown to be present in populations of the species. In addition, upper and lower plots in two populations of *L. parviflorus*, representing contrasting degrees of wind exposure, were compared to explore the relationship between the extent of pollen limitation and the abundance of airborne pollen, providing supporting evidence that wind pollination confers reproductive assurance.

MATERIALS AND METHODS

The species—*Linanthus parviflorus* (Benth.) Greene occurs abundantly in grasslands and open woodland areas throughout California (Hickman, 1993). Plants are 10–20 cm tall, with a terminal headlike inflorescence. The pink or white five-petaled flowers have a slender corolla tube, ~2.5 cm in length. Flowers are visited nearly exclusively by long-tongued bees (e.g., *Bombylius* spp.) that hover and probe the long corolla tube for nectar (Grant and Grant, 1965). Populations typically are large, with densities as high as 2400 plants/m². The flowering period extends from early April to early June, and seeds mature within 30 d. Self-fertilization is prevented in *L. parviflorus* by a highly effective sporophytic self-incompatibility system (Goodwillie, 1997a).

Study sites—All experiments and observations were carried out in the spring of 1996 in *L. parviflorus* populations at two study sites in Napa County, California, separated by ~6 km. The sites at Wantrup Reserve and Mast Hill are ecologically similar, characterized by grasslands and oak woodlands. An experiment investigating wind pollination was replicated at two plots within the Wantrup Reserve. Pollen limitation experiments were carried out in two plots at both the Wantrup Reserve and Mast Hill.

Airborne pollen counts—Pollen traps were used to test for the presence of airborne *L. parviflorus* pollen and to provide a relative measure of its density in all experimental plots. Glass microscope slides coated with vaseline and attached to wire stakes were placed in plots at flower level between 0900 and 1000 before flowers opened and collected between 1800 and 1900 after the flowers closed for the night. Pollen grains were stained with aniline blue in lactophenol (Kearns and Inouye, 1993), and counts were made of the number of grains deposited in the area of two coverslips (9.68 cm²) in a day. (For simplicity, this unit of measurement will be referred to as “pollen grains per slide”). Pollen was identified by reference to stained preparations of known *L. parviflorus* pollen. At each experimental plot, four pollen traps were set out on 4 d during peak flowering at randomly chosen locations within the population. Sampling days were consecutive when weather permitted, but on overcast or rainy days flowers remain closed and sampling was not possible. Because of slight differences in flowering phenology, sampling dates differed among plots.

For comparison, airborne pollen was quantified using the same meth-

ods and sampling protocol in two populations of a closely related species, *L. jepsonii*, adjacent to the study populations of *L. parviflorus* at Wantrup Reserve and Mast Hill. *L. jepsonii* has a similar morphology and flowering phenology and is visited by the same pollinators, but is self-compatible and partially selfing.

Wind pollination experiment—The capacity for wind pollination in *L. parviflorus* and its effectiveness relative to insect pollination were assessed by quantifying seed set in plants enclosed in mesh tents that exclude pollinators but allow passage of airborne pollen (see below) and comparing it to seed set in open-pollinated control plants. To test for possible confounding effects of the tent unrelated to pollination, seed set in two additional treatments, pollen supplementation and pollen supplementation with tent, were compared. Plants were subjected to these four treatments throughout the entire flowering period: (1) control (open pollination), (2) tent (wind pollination), (3) pollen supplementation, and (4) pollen supplementation with tent. A comparison of seed set in control and pollen-supplemented treatments provided an indication of the extent to which reproductive success was limited by pollen deposition in the population.

In tented treatments, entire plants were enclosed in a wire-framed tent stretched with 1-mm nylon mesh. In the pollen-supplemented treatments, pollen was applied to all open flowers by brushing the stigmas with the anthers of flowers from two donor plants. Pollen supplementation treatments were carried out on alternate days. Since flowers remain open for >2 d (unpublished data) this was sufficient to ensure that all flowers were treated. The experiment was replicated at two plots at the Wantrup Reserve, separated by ~500 m. At each plot, 30 groups of four adjacent plants of similar size (blocks) were marked at the start of the flowering season, and individual plants were randomly assigned to one of the four treatments. Fruits were collected as they matured, and total seeds were counted for each plant. The number of flowers produced by each plant was determined by counting calyces at the end of the season.

To test whether pollen flow was affected by the mesh tents, airborne pollen was quantified inside and outside tents. At a site adjacent to one of the experimental plots, eight pollen traps were set out on 6 d during peak flowering. On each day, four of the traps were randomly selected to be enclosed in pollinator exclusion tents. Traps were constructed and pollen grains were counted as described above.

Self-incompatibility—*Linanthus parviflorus* from populations in Napa County have been shown to be self-incompatible in greenhouse experiments (Goodwillie, 1997a). To determine whether *L. parviflorus* is fully self-incompatible under field conditions, pollen tube growth was compared in hand self- and outcross-pollinations on plants in the wind pollination experimental plots. Pollen tube assays are thought to be the best indication of self-incompatibility, since variation in seed set can be attributed to a number of other factors such as inbreeding depression or patterns of resource allocation (Weller, Donoghue, and Charlesworth, 1995). At each experimental plot, 30 randomly selected plants were enclosed in cardboard cylinders covered with tightly meshed fabric that excluded both wind- and insect-borne pollen but allowed light. On each plant, one flower was hand self-pollinated and one was hand cross-pollinated. Flowers were removed after they senesced, the style was fixed in 3:1 ethanol-acetic acid, and pollen tubes were inspected using the aniline blue staining method (Martin, 1959).

Pollen limitation in exposed and protected plots—Additional evidence for wind pollination in *L. parviflorus* and its role in reproductive assurance was obtained by exploring the relationship between pollen limitation and the amount of airborne pollen in plots that differ in wind exposure. If plants in exposed areas undergo more wind pollination, they are expected to show lower pollen limitation than areas protected from wind. Populations of *L. parviflorus* extending from windy hilltops

to lower, protected glades were studied at the Wantrup Reserve and Mast Hill.

To assess experimentally the magnitude of pollen limitation, 30–60 pairs of adjacent plants of similar size were marked in each of two plots at both sites, one in an upper, exposed area and one in a lower, more protected area. The exposed and protected plots at each site were separated by <15 m. One plant in each pair was randomly assigned to a pollen supplementation treatment, and the other served as an unmanipulated control. On pollen-supplemented plants, all flowers were hand-pollinated, as described above for the wind pollination experiment. Fruits of both hand-pollinated and control plants were collected when mature, and seeds and flowers were counted for each plant. Airborne pollen loads were quantified in the exposed and protected plots at both sites using the pollen trap technique and sampling protocol described above.

Data analysis—All statistical analyses were performed on SPSS version 7.5 (SPSS, 1997). The most relevant variable for comparison of treatments in both the wind pollination and pollen limitation experiments was the total number of seeds per plant, because this is a direct measure of total female fitness. However, a composite variable, seeds per flower, controls for variation in flower number and therefore may be more useful for detecting differences among treatments. All analyses were carried out for both dependent variables.

Wind pollination experiment—To statistically test for the occurrence of wind pollination in experimental plants, one-tailed one-sample *t* tests were used to determine whether seeds per plant and seeds per flower in tented treatments were significantly greater than zero. To test for an overall effect of pollination treatments on seeds per plant and seeds per flower, two-way nested ANOVA was used with plot as a random factor, block nested within plot, and treatment as a fixed factor. Error terms for this and all other mixed-model ANOVAs in the study were calculated using a linear combination of variance components of the random effects and quadratic terms of the fixed effects (SPSS, 1997). Comparisons of treatment pairs relevant to individual experimental questions (see Experimental design) were tested for significance using Scheffé's post hoc tests. The same analysis of variance model was used to test for differences in flower number.

Two-way ANOVA, with tent as a fixed factor and sampling date as a random factor, was used to test for an effect of the tent on pollen flow. The quantity of airborne pollen (log transformed) at the two experimental plots was compared using nested one-way ANOVA, with plot as a random factor and sampling date nested within plot. ANOVA was used to compare airborne pollen in *L. jepsonii* and *L. parviflorus*, with species as a fixed effect, site as a random effect, and sampling date nested within the interaction.

Pollen limitation in exposed and protected plots—Two-way nested ANOVA with site as a random factor, exposure (exposed vs. protected) as a fixed factor, and sampling date nested within the site by exposure interaction was used to test for differences in the mean airborne pollen count (log transformed). To test for significant pollen limitation at each plot (exposed and protected plots at Wantrup Reserve and Mast Hill), seeds per plant and seeds per flower in control and pollen supplementation treatments were compared using paired *t* tests, and significance levels were adjusted to hold the experimentwise Type I error rate at 0.05 using the sequential Bonferroni method (Rice, 1989).

RESULTS

Wind pollination experiment—**Airborne pollen counts**—Pollen traps in the wind pollination experimental plots at Wantrup Reserve revealed the presence of a substantial amount of airborne pollen (plot 1: mean = 239.6 pollen grains per slide, plot 2: mean = 161.2 pollen

grains per slide). Pollen grains from other plant species were rarely found on the traps. Pollen counts varied significantly among sampling dates ($F = 2.427$, $df = 10/34$, $P = 0.026$), and there was a marginally significant difference between the two experimental populations in airborne pollen counts ($F = 5.052$, $df = 1/10.28$, $P = 0.048$). In the comparison of tented and untented pollen traps, the effect of the mesh tent on airborne pollen flow was not significant (tented: mean = 341.9 pollen grains per slide, untented: mean = 375.9 pollen grains per slide, $F = 0.116$, $df = 1/5.03$, $P = 0.747$) but there was significant variation among the sampling dates ($F = 10.910$, $df = 5/5$, $P = 0.010$). *Linanthus parviflorus* populations had significantly higher pollen counts than *L. jepsonii* populations ($F = 8.0800$, $df = 1/6$, $P = 0.048$), with the mean for *L. jepsonii* only 27.6 pollen grains per slide, ~10% of the overall mean for *L. parviflorus* populations.

Self-incompatibility—In self-pollinated flowers, pollen tubes were absent in all styles in plot 1 and in all but one style in plot 2, while dense pollen tube growth was observed in styles of all cross-pollinated flowers (plot 1: mean = 88.5 tubes, $N = 30$, plot 2: mean = 56.9 tubes, $N = 30$). These data indicate that self-fertilization in *L. parviflorus* at the experimental sites is prevented by a highly effective self-incompatibility system.

Wind pollination—The number of seeds per plant and seeds per flower in wind-pollinated treatments at each plot was significantly greater than zero (seeds per plant, plot 1: $t = 7.606$, $df = 29$, $P < 0.0001$, plot 2: $t = 6.535$, $df = 27$, $P < 0.0001$; seeds per flower, plot 1: $t = 9.679$, $df = 29$, $P < 0.0001$, plot 2: $t = 9.060$, $df = 27$, $P < 0.0001$). The number of flowers per plant differed significantly between experimental plots, but treatments were not significantly different for flower number (Table 1), suggesting that there was no bias in the assignment of plants to treatments. There was no significant interaction between plot and treatment effects for either seed set variable. Treatments had a highly significant effect on both the number of seeds per plant and seeds per flower. In post hoc tests, a comparison between pollen supplementation and pollen supplementation with tent treatments showed no significant difference in the number of seeds per flower or seeds per plant, although for both variables the means for tented plants was higher (Fig. 1). Pollen-supplemented plants produced significantly more seeds per flower than control plants, indicating pollen limitation of reproduction; the difference between these treatments in seeds per plant was marginally significant (Fig. 1). Wind-pollinated plants (tent treatment) produced significantly fewer seeds per flower than control plants, while the difference in seeds per plant was not significant (Fig. 1).

To determine the relative contribution of wind pollination to seed set requires a comparison of pollination treatments. The most direct indication of the potential magnitude of wind pollination is a comparison of tent (wind pollinated) and control (wind and insect pollinated) treatments, but this comparison is confounded by any effect that the tent itself may have on reproductive success. Although the post hoc comparison of pollen supplementation and pollen supplementation with tent treatments

TABLE 1. Analysis of variance of wind pollination experiment replicated at two plots.

Character	Effect	df	SS	F	P
Number of flowers	Treatment	3	21.03	1.348	0.406
	Plot	1	234.59	11.176	0.002
	Treatment × Plot	3	15.60	2.163	0.094
	Block (within Plot)	56	1022.23	7.593	0.000
	Error	165	396.69		
Number of seeds per plant	Treatment	3	6088.44	30.803	0.009
	Plot	1	2183.70	6.745	0.016
	Treatment × Plot	3	197.66	0.464	0.708
	Block (within Plot)	56	22446.34	2.823	0.000
	Error	165	23425.30		
Number of seeds per flower	Treatment	3	180.35	519.892	0.000
	Plot	1	3.17	2.076	0.185
	Treatment × plot	3	0.35	0.052	0.984
	Block (within Plot)	56	203.96	1.636	0.009
	Error	165	367.213		

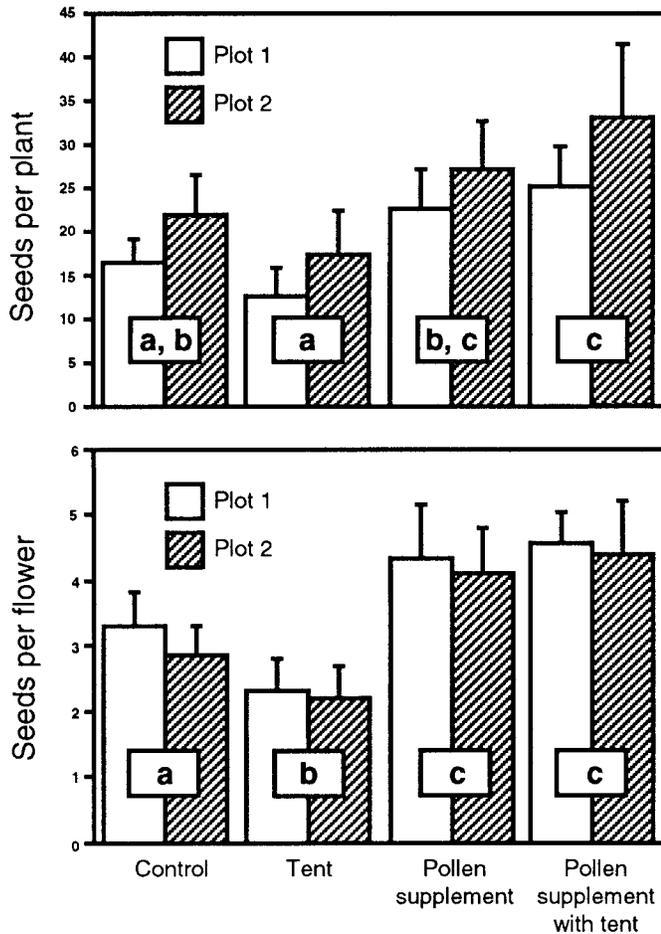


Fig. 1. Mean number of seeds per plant and seeds per flower for four pollination treatments at two sites. The overall effect of treatment is significant in two-way ANOVAs for both variables (Table 1). Treatment means marked with a common letter (a, b, c) are not significantly different in Scheffé's post hoc tests. Post hoc comparisons refer to overall treatment means across both sites; the treatment x site interaction was not significant (Table 1). Error bars indicate 2 SE.

indicated that the effect of tent was not significant (Fig. 1), higher means for the latter treatment at both sites suggest that a positive tent effect may exist but was not detected with the current sample size. An alternative method of comparison that factors out any possible tent effect is to compare two ratios: (1) the ratio of control to pollen supplementation treatments, indicating the proportion of full seed set (i.e., seed set with unlimited pollen) that was produced by insects and wind pollination together, and (2) the ratio of tent to pollen supplementation with tent treatments, indicating the proportion of full seed set that was produced by wind pollination alone. The values for the first ratio at plots 1 and 2 are 0.728 and 0.811 for seeds per plant and 0.749 and 0.687 for seeds per flower, respectively. Values for the second ratio at both plots, 0.495 and 0.522 for seeds per plant and 0.503 and 0.498 for seeds per flower, are lower as might be expected, but comparable in magnitude, suggesting that wind pollination has the potential to contribute substantially to seed set.

Pollen limitation at exposed and protected plots—Airborne pollen counts—Mean pollen counts ranged widely among the plots, from 161.2 to 497.9 pollen grains per slide (Fig. 2). Airborne pollen counts were not significantly different between Wantrup Reserve and Mast Hill, but the effect of wind exposure approached significance ($P = 0.106$) and there was significant variation among sampling dates (Table 2).

Pollen limitation—In the protected plots at both sites, the number of seeds per flower was pollen limited at experimentwise significance levels (Table 3). In addition, pollen limitation of seeds per plant was significant at the Mast Hill protected plot. In contrast, at exposed sites at both Mast Hill and Wantrup Reserve, no significant differences at either the individual test or tablewise level were found between control and treatment plants for either variable. The proportionate increase in seeds per plant due to pollen supplementation [(mean supplemented seeds/mean control seeds) - 1] ranged from 0.25 to 0.57 in protected plots, while pollen supplementation increased seeds per plant by <0.06 in exposed plots (Fig. 2).

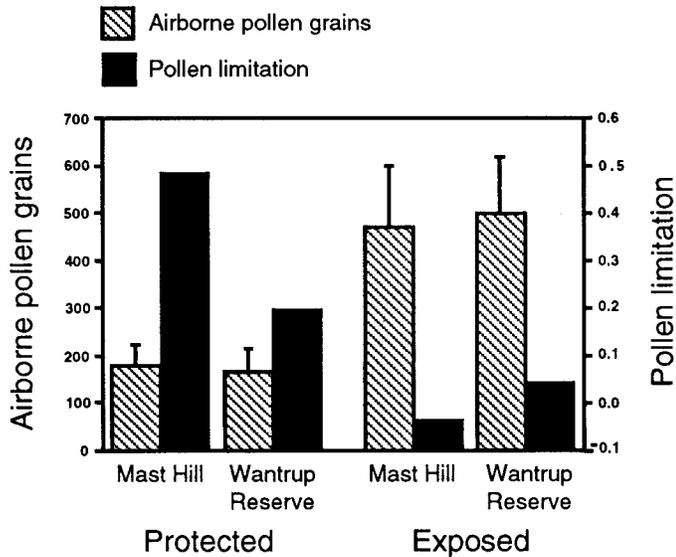


Fig. 2. Airborne pollen and pollen limitation in wind-exposed and protected plots. Pollen counts represent the mean number of grains per slide in a single day of sampling (see Materials and Methods). Error bars indicate 2 SE. Pollen limitation is represented by the proportionate increase in mean seed number resulting from pollen supplementation [(mean supplemented seeds/mean control seeds) - 1].

DISCUSSION

Wind pollination in *L. parviflorus*—The presence of airborne pollen in *L. parviflorus* populations and experimental evidence for seed production when insect pollinators are excluded combine to support the hypothesis that wind pollination can contribute to reproduction in *L. parviflorus*. The method used in this study for quantifying airborne pollen did not provide an estimate of actual stigmatic pollen loads resulting from wind pollination, but the large number of pollen grains captured each day was striking. By contrast, in populations of *L. jepsonii*, a closely related self-compatible species, airborne pollen counts were significantly lower, with mean airborne pollen only 10% of that in *L. parviflorus* populations.

At the experimental plots, insect and wind pollination together (control treatments) produced 70–80% of the number of seeds produced with pollen supplementation. Wind pollination alone produced substantial seed set, ~50% of seed set with pollen supplementation. Mean airborne pollen counts at the wind experiment plots were less than half of that at the exposed plot at Mast Hill in the pollen limitation study, suggesting that wind pollination at some sites may be considerably more extensive than the results for the wind pollination experiment indicate. The lack of pollen tube growth after hand self-pollination of flowers in wind exclusion tubes indicates that seed set in tented plants was not due to self-fertilization. Furthermore, negative results for self-pollinated flowers in the wind exclusion tubes eliminate an alternative explanation, that pollination in tented plants was effected by crawling insects.

Reproductive assurance—In the pollen limitation experiment, plants in upper, exposed plots where airborne pollen loads were high were not significantly pollen lim-

TABLE 2. Analysis of variance of airborne pollen counts at exposed and protected plots at two sites.

Effect	df	SS	F	P
Exposure	1	22.239	60.526	0.106
Site	1	0.002	0.000	0.987
Exposure × Site	1	0.394	0.118	0.737
Date (within Exposure × Site)	15	50.304	5.340	0.000
Error	57	35.794		

ited, while those in both of the lower, protected plots were found to be significantly pollen limited for seed number per flower. Though these data do not provide direct evidence of wind pollination, they support the experimental finding of wind pollination in *L. parviflorus*. The observed differences in the magnitude of pollen limitation could also be explained by differences in pollinator visitation rates at exposed and protected plots. Data are not available to fully eliminate this possibility, but exposed and protected plots at each site were part of the same continuous population, separated by <15 m, and pollinator visitation is unlikely to, and has not been observed to, differ at this scale. Furthermore, adjacent exposed and protected plots were not distinguished by any obvious ecological differences that might account for differences in pollen limitation.

The suggested relationship between airborne pollen and pollen limitation provides supporting evidence that wind pollination is occurring and leads to further conclusions as well. First, it suggests that wind pollination provides reproductive assurance at some sites, and second, it indicates that wind pollination may vary widely among *L. parviflorus* sites. Significant pollen limitation was found at both protected plots, and marginally significant pollen limitation was found in the wind pollination experimental plots, indicating that neither insect visitation nor wind pollination was sufficient to effect full seed set at these sites. The lack of pollen limitation and higher pollen counts at exposed plots suggest that wind pollination provides full reproductive assurance. Congruent with these findings is the fact that open, exposed habitats are more typical of *L. parviflorus*, at least in the study area; however, the demonstration of wind pollination at the experimental plots indicates that, even at sites with relatively lower airborne pollen counts, wind pollination can contribute substantially to reproduction in the absence of pollinators.

Wind pollination may provide partial or full reproductive assurance in years with low pollinator abundance. In five years of study of *L. parviflorus* populations in Napa County, I observed great variation in pollinator visitation rate. In 1995, a year with extensive flooding in the early spring, bee flies were virtually absent. If crashes in pollinator populations are frequent, selection for reproductive assurance is likely to be strong and may have played a role in the evolutionary transition from obligate, relatively specialized insect pollination to self-fertilization that has occurred repeatedly in related species. Selfing has evolved from self-incompatibility at least three times within this section of *Linanthus* (Goodwillie, in press), and the capacity for autonomous self-fertilization ensures that reproduction will occur even when pollinators are

TABLE 3. Mean seed set and results of paired *t* tests for pollen limitation in exposed and protected plots at two sites. *N* = the number of pairs of plants.

Variable	Plot	<i>N</i>	Control	Pollen supp.	<i>t</i>	<i>P</i>
Seeds per plant	Mast Hill, exposed	59	32.48	34.31	-0.609	0.545
	Wantrup Reserve, exposed	29	35.48	35.90	-0.123	0.903
	Mast Hill, protected	29	34.21	53.59	-4.053	<0.001 ^a
	Wantrup Reserve, protected	57	51.60	64.25	-2.165	0.035
Seeds per flower	Mast Hill, exposed	59	4.13	4.44	-0.976	0.333
	Wantrup Reserve, exposed	29	4.00	4.59	-1.470	0.153
	Mast Hill, protected	29	2.97	4.70	-4.773	0.001 ^a
	Wantrup Reserve, protected	57	4.04	5.19	-3.315	0.002 ^a

^a *P* values are significant after levels were adjusted for multiple comparisons.

absent or scarce. Evidence presented here suggests that wind pollination serves a similar function in *L. parviflorus*. Wind pollination was found to provide substantial reproductive assurance and may allow for the persistence of populations of a self-incompatible annual under conditions of low pollinator visitation. Of the self-incompatible species in *Linanthus* section *Leptosiphon*, *L. parviflorus* is the most widespread and common, occurring in large populations throughout much of California (Hickman, 1993). Furthermore, wind pollination in *L. parviflorus* may be hypothesized to contribute to the maintenance of self-incompatibility in the species; if wind pollination provides seed set in the absence of pollinators, it may reduce the selective advantage of selfing genotypes if they arise under pollinator-limited conditions.

Wind pollination and floral characters—If wind pollination contributes to the reproductive success of *L. parviflorus*, can we identify traits that represent adaptations for wind pollination? Many features of *L. parviflorus* flowers, like those of other species in the section, are clearly adapted for pollination by insects: showy corolla lobes, nectar-guide markings, and a long, nectar-containing corolla tube that receives the bee's proboscis. However, a distinctive feature of *L. parviflorus* flowers, reflexed corolla lobes, may be associated with wind pollination. In other species in the section, the corolla lobes are held at right angles to the corolla tube and are cupped slightly upward (e.g., *L. bicolor*, *L. jepsonii*, *L. acicularis*), or form a trumpet-like arrangement, with the lobes at a greater than 90° angle from the tube (e.g., *L. montanus*, *L. androsaceus*, *L. nudatus*). *Linanthus parviflorus* is unique in that the corolla lobes are often reflexed, bent back to form an acute angle to the corolla tube. The reflexing increases with flower age, in some plants to an angle of <15°. An intriguing hypothesis is that reflexed corolla lobe position in *L. parviflorus* represents an adaptation that promotes wind pollination by removing structures that impede air flow and exposing the stigma and anthers to airborne pollen. The highly reduced corollas that characterize typical wind-pollinated taxa are thought to serve the same function (Whitehead, 1969). Along with increased corolla lobe reflexing, stigma lobes elongate throughout the life of a flower of *L. parviflorus*, approximately doubling in length in 3 d in an unfertilized flower. Upon fertilization, stigma elongation stops and flower senescence occurs, usually within a day (personal observation). This phenomenon occurs to some degree in related self-compatible species, but is greatly exaggerated

in *L. parviflorus*. Stigma elongation may be viewed as a possible adaptation to wind pollination as well, in that continual elongation of an unpollinated stigma may increase its chances for intercepting airborne pollen. Long, plumose stigmas are typical of many exclusively wind-pollinated groups, such as the grasses (Whitehead, 1969; Faegri and van der Pijl, 1979).

The Polemoniaceae are well known for extraordinary diversity of pollination syndromes and mating systems (Grant and Grant, 1965), and this family has played an important part in the study of plant mating system evolution. Floral adaptations associated with animal pollination and autogamy have been studied in several species in the Polemoniaceae (Levin, 1972; Schoen, 1982; Galen and Stanton, 1989; Campbell et al., 1991). This study provides the first report of wind pollination in this family. Whether the phenomenon is unique to *L. parviflorus* or common to other species or genera in the Polemoniaceae is unknown. Indeed, the frequency of wind pollination in primarily insect-pollinated taxa has yet to be determined for higher plants. Evidence for mixed insect and wind pollination has been shown for a variety of plant taxa. In *Urginea maritima* (Liliaceae), wind-, insect- and self-pollination all occur (Dafni and Dukas, 1986). This Mediterranean species produces nectar-bearing flowers during late summer, a season that is characterized by low insect abundance. Wind pollination is thought to result primarily in self-fertilization, which cannot occur in this species without a pollen vector and has been hypothesized to represent a means for reproductive assurance in an environment harsh to insects. Wind pollination has been demonstrated in populations of *Hormathophylla spinosa* (Cruciferae), a primarily ant-pollinated self-incompatible species and is thought to allow colonization and persistence of populations in high montane environments with low insect abundance (Gomez and Zamora, 1996). Selection for reproductive assurance is hypothesized to have played a role in the evolution of wind pollination in high elevation species of *Espeletia* (Asteraceae), a genus of primarily insect-pollinated, exclusively self-incompatible species (Berry and Calvo, 1989). Contributions of insect pollination to primarily wind-pollinated species have also been reported (Clifford, 1962; Pojar, 1973; Adams, Perkins, and Estes, 1981; Lock and Hall, 1982; Stelleman, 1984). *Plantago lanceolata* (Plantaginaceae), typically regarded as a wind-pollinated species, has been shown to be partially insect pollinated at some sites (Clifford, 1962; Stelleman, 1984), with populations at those sites characterized by adhesive pollen hypothesized to facili-

tate transfer by insects (Stelleman, 1984). Similarly, a grass species, *Paspalum dilatatum*, with conspicuous purple anthers and stigmas and relatively large, adhesive pollen grains, was shown to be pollinated by halictid bees in addition to wind (Adams, Perkins, and Estes, 1981). Interestingly, in several of these studies (Pojar, 1973; Lock and Hall, 1982; Stelleman, 1984), the evolution of traits promoting insect pollination was attributed to selection for reproductive assurance in environments in which air movement of pollen is limited, such as in the forest understory or wet environments.

Mixed wind and insect pollination has received relatively little empirical study, and the small number of existing reports may not reflect its frequency among angiosperm taxa. Wind pollination has undoubtedly remained unnoticed in many insect-pollinated taxa. The recognition of wind pollination as a selective factor may contribute to our understanding of the evolution of floral traits in some plant species. Alternatively, wind pollination in some insect-pollinated taxa may represent an ecological consequence of harsh abiotic conditions, rather than an adaptation (Gomez and Zamora, 1996). But whether or not selection for this mode of pollination leads to adaptive character evolution, even a moderate degree of wind pollination may be important in the evolutionary trajectory of plant species and may have implications for the evolution of plant mating systems.

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