

Unilateral incompatibility and the effects of interspecific pollination for *Erythronium americanum* and *Erythronium albidum* (Liliaceae)

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To determine whether interspecific pollen transfer could reduce seed production by two sympatric lilies, *Erythronium albidum* and *Erythronium americanum*, we hand-pollinated flowers with mixtures of conspecific and heterospecific pollen. These species exhibited typical unilateral interspecific incompatibility, i.e., pollen tubes from the self-infertile species (*E. americanum*) grew apparently unimpeded in styles of the self-fertile species (*E. albidum*), whereas the reverse cross resulted in an incompatibility reaction. Because of this asymmetrical relation and faster growth by heterospecific pollen tubes in *E. albidum* stigmas than by conspecific tubes, pollination with abundant heterospecific pollen reduced fruit and seed production by *E. albidum*, but not by *E. americanum*, as long as the stigma also received some conspecific pollen. Unilateral incompatibility could benefit self-infertile species in reproductive interactions with closely related self-fertile species; however, this benefit remains to be demonstrated for naturally pollinated plants.

Key words: *Erythronium albidum*, *Erythronium americanum*, interspecific pollen transfer, pollination, unilateral incompatibility.

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Afin de déterminer si le transfert interspécifique de pollens peut réduire la production de graines chez deux liliacées sympatriques, l'*Erythronium albidum* et l'*Erythronium americanum*, les auteurs ont pollinisé des fleurs manuellement avec du pollen conspécifique et hétérospécifique. Ces espèces montrent une incompatibilité interspécifique unilatérale, i.e., les tubes polliniques de l'espèce auto-incompatible (*E. americanum*) semblent se développer sans difficulté dans les styles de l'espèce auto-compatible (*E. albidum*), alors que le croisement inverse se traduit par une réaction d'incompatibilité. À cause de cette relation asymétrique et d'une croissance plus rapide des tubes polliniques hétérospécifiques sur les stigmates de l'*E. albidum* comparativement aux tubes conspécifiques, une pollinisation abondante avec du pollen hétérospécifique réduit la production de fruits et de graines par l'*E. albidum*, mais non par l'*E. americanum*, aussi longtemps que les stigmates reçoivent également un minimum de pollen conspécifique. L'incompatibilité unilatérale pourrait être utile aux espèces auto-incompatibles en reproduction interactive avec des espèces voisines auto-compatibles; ce bénéfice reste à démontrer chez les plantes naturellement pollinisées.

Mots clés : *Erythronium albidum*, *Erythronium americanum*, pollinisation interspécifique, pollinisation, incompatibilité unilatérale.

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Introduction

Pollinators move between plant species with differing frequency, depending on circumstances, so that flowers often receive some heterospecific pollen (e.g., Waser 1978; Schemske 1981; Kephart 1983; Armbruster and Herzig 1984; Campbell and Motten 1985; Feinsinger et al. 1986; Kwak and Jennersten 1986; Galen and Newport 1988). This interspecific pollen transfer may initiate various processes that interfere with normal pollen germination, pollen tube growth, and (or) fertilization by conspecific pollen. Indeed, ecologists studying plant reproduction have proposed a diversity of interference mechanisms (reviewed by Rathcke 1983; Waser 1983), although demonstrations of ecologically important mechanisms are few and often species-specific (e.g., Thomson et al. 1981; Waser and Fugate 1986; cf. Galen and Gregory 1989).

In their efforts to produce hybrid varieties, plant breeders frequently cross-pollinate plants of different species. Their extensive experience with diverse taxa has revealed several

generalizations concerning the outcome of interspecific pollination. First, closely related species should be particularly susceptible to detrimental effects of interspecific pollination because their stigmas often provide a more suitable environment for pollen tube growth than do the stigmas of unrelated species (Martin 1970). Second, the success of crosses between self-compatible species and self-incompatible species typically depend on which species serves as the maternal parent. In particular, pollen tubes from self-incompatible species can often grow successfully in the pistils of self-compatible species, but the reverse cross produces an incompatibility reaction, an outcome known as unilateral interspecific incompatibility (Harrison and Darby 1955; Lewis and Crowe 1958; Nettancourt 1977).

Unilateral incompatibility and its potential ecological significance have apparently not been examined for species that naturally occupy the same habitat and flower concurrently, although Levin (1971) discussed its potential role in hybridization. We therefore examined the reproductive interactions between two sympatric lilies, *Erythronium albidum* Nutt. and *Erythronium americanum* Ker. Both species are perennial,

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spring-ephemeral herbs of deciduous forests in eastern North America, which flower before the forest canopy closes (Muller 1978; Schemske et al. 1978). These species differ in their response to self-pollination. Harder et al. (1985) found that only 33% of self-pollinated *E. americanum* flowers set fruit compared with 75% fruit set for outcrossed flowers (also see Bernhardt 1977). In contrast, Banks (1980) found that approximately 50% of *E. albidum* flowers produced fruit regardless of whether they received self or outcross pollen (also see Schemske et al. 1978). Because the mechanism controlling the outcome of self-pollination for *E. americanum* is unknown, we will refer to this species as self-infertile rather than self-incompatible. These tetraploid lilies differ in chromosome morphology and number (*E. americanum*, $2n = 48$, Parks and Hardin 1963; *E. albidum*, $2n = 44$, Robertson 1966), and they have not been reported to hybridize.

In this paper we consider the effects of hand-pollinations with mixtures of both species' pollen on pollen tube growth, and fruit and seed production. The observed responses indicate the potential for reproductive interference between these lilies through interspecific pollen transfer. In a separate paper we will examine the natural frequency of interspecific pollen transfer and its realized impact on the reproductive success of these plants.

Methods

We studied *E. americanum* and *E. albidum* during late April 1986 and 1989 in a sugar maple (*Acer saccharum* Marsh.) woodlot on the north shore of Fanshawe Lake (43°04'N, 81°11'W), near London, Ont. At this site the two species grow intermixed and flower concurrently. The flowers of these species (one flower per ramet) differ primarily in color (*E. americanum*, yellow; *E. albidum*, white); otherwise they are of similar size and morphology. Of particular relevance to this study, flowers of these species have equally long styles (*E. americanum*, mean (\pm SE) = 11.2 ± 0.18 mm; *E. albidum*, 11.5 ± 0.20 mm; $n = 30$ for each species, $t = 1.43$, $P > 0.1$) and produce equivalent numbers of ovules (*E. americanum*, mean (\pm SE) = 37.7 ± 1.1 ; *E. albidum*, 34.7 ± 1.6 ; $n = 30$ for each species, $t = 1.50$, $P > 0.1$). The same pollinators (primarily andrenid bees, especially *Andrena carlini* Cockerell) visit both species, and approximately 10% of pollinator flights between flowers involve a switch from one species to the other (L. D. Harder, M. B. Cruzan, and J. D. Thomson, unpublished data).

We used a series of hand-pollination treatments to determine the effects of mixed pollination on pollen tube growth, and fruit and seed production. Flowers were randomly assigned to pollination treatments, emasculated as buds (except for flowers receiving self-pollen), tagged, and then bagged until anthesis. We prepared pollen mixtures by placing different numbers of undehisced *E. americanum* and *E. albidum* anthers in vials, waiting for them to dehisce (< 1 h) and then shaking the vials to ensure thorough mixing. To assure a diversity of pollen donors, the anthers used in preparing these mixtures were collected from different ramets scattered over a large area (> 1800 m²) adjacent to the study site. Each recipient flower was liberally pollinated by dusting the stigma with a piece of tissue paper (≈ 0.5 cm²) that had been dipped in one pollen mixture, and the flower was rebagged. Five days later we unbagged the flowers. We collected all fruit approximately 5 weeks after pollination and counted the numbers of undeveloped ovules and developed seeds (fruit set requires development of some seeds).

The 1986 pollination experiment included the following four treatments: (i) 30 *E. americanum* anthers, (ii) 20 *E. americanum* anthers with 10 *E. albidum* anthers, (iii) 10 *E. americanum* anthers with 20 *E. albidum* anthers, and (iv) 30 *E. albidum* anthers. We later determined that *E. americanum* anthers produced three times more pollen than *E. albidum* anthers (L. D. Harder, M. B. Cruzan, and J. D.

Thomson, unpublished data), so that the proportions of the two pollens in the four pollination treatments were (i) 0% *E. albidum*, (ii) 14% *E. albidum*, (iii) 40% *E. albidum*, and (iv) 100% *E. albidum*. The plants used in this experiment were scattered throughout the study site. When we removed the bags from these flowers, we also collected their styles, which were then preserved in 70% ethanol until pollen tubes could be counted (see below).

In 1989 we conducted an additional pollination experiment that examined the relative effects of pollination with self-pollen, outcross pollen, or equal proportions of *E. albidum* and *E. americanum* pollen. We selected 10 clusters of ramets for each species that were scattered around the study site. From each cluster we randomly assigned one plant to each of the three pollination treatments. At each of three of these clusters we also caged two plants of both species during their flowering period to evaluate whether they could set seed by autogamous self-pollination. Fruit and seed set following outcross pollination did not differ significantly between 1986 and 1989 for either species ($P > 0.1$ in all cases). We have consequently pooled the results for the 2 years.

During 1989 we also conducted an experiment to determine the relative vigor of pollen tubes from self, outcross and heterospecific pollen. An *Erythronium* pistil has a three-lobed stigma and a hollow style. Each stigma lobe is associated with one canal of the Y-shaped stylar lumen. Pollen tubes originating in a particular stigmatic lobe grow together down their canal, separate from the tubes from other lobes (Cruzan 1990). This allowed us to place pollen from different sources on separate stigma lobes so that the growth rates of pollen from alternate treatments could be compared in the same style.

The plants used in this experiment were collected just before anthesis and their scapes were placed in water-filled vials in the laboratory. As these flowers opened (which occurred normally), we collected the undehisced anthers and allowed them to dehisce. We also clipped off one of the three stigma lobes to provide a reference point. The two remaining lobes were liberally pollinated with one of three types of pollen: self, conspecific outcross, or heterospecific. Each flower received two different treatments, which were applied randomly to the remaining stigma lobes. The styles of these flowers were collected 24 h later and preserved in 70% ethanol until the pollen tubes were counted. Pollen tube growth may not be complete after 24 h, so that differences in numbers of pollen tubes at style bases probably represent differences in growth rates rather than the actual numbers of tubes that would eventually traverse the style.

For both the 1986 field experiment and the 1989 laboratory experiment we counted the pollen tubes in the stigma and at the base of the style. The styles were split longitudinally and spread open to expose the pollen tubes. These were then stained with 1 drop of acidified aniline blue (0.1% in H₂O) and 1 drop of acetocarmine (40% acetic acid saturated with carmine) for 10 min. Pollen tubes were counted within 1 mm of the tip of the stigma lobes and within 1 mm of the base of the style under a dissecting microscope at 50 \times .

Results

Breeding systems

As previously reported (Bernhardt 1977; Schemske et al. 1978; Banks 1980; Harder et al. 1985), *E. albidum* and *E. americanum* responded differently to self-pollination. *Erythronium albidum* flowers were equally likely to set fruit following selfing or outcrossing (Table 1; one-tailed Fisher's exact test, $P = 0.142$), although a significantly smaller proportion of the ovules of selfed flowers produced seeds (Table 1; Wilcoxon's two-sample test, $X^2 = 10.96$, 1 df, $P < 0.001$). In contrast, none of the nine selfed *E. americanum* ramets located 5 weeks later had produced fruit, whereas 41.2% of the 17 outcrossed flowers set fruit (Table 1; one-tailed Fisher's exact test, $P = 0.043$). These results confirm that, at this site, *E. americanum* is self-infertile, whereas *E. albidum* is self-fertile but susceptible to reduced seed set on selfing. Overall,

TABLE 1. Percent fruit set and percent seed set for flowers that set fruit by selfed and outcrossed *E. albidum* and *E. americanum* flowers

Pollen source	<i>E. albidum</i>		<i>E. americanum</i>	
	%	<i>n</i>	%	<i>n</i>
Fruit set				
Self	70.0	10	0.0	9
Outcross	93.8	16	41.2	17
Median (\pm quartile) seed set				
Self	6.7 (6.1–10.3)	6		
Outcross	26.7 (17.4–29.6)	15	16.7 (14.6–22.7)	7

NOTE: Data for outcrossed flowers are pooled results from 1986 and 1989. *n*, sample size.

TABLE 2. Number of pollen tubes penetrating the stigma and at the style base following self-, outcross-, and heterospecific pollination

Pollen source	<i>E. albidum</i>		<i>E. americanum</i>	
	Mean \pm SE	<i>n</i>	Mean \pm SE	<i>n</i>
Penetrating stigma				
Self	53.0 \pm 7.19a	6	70.0 \pm 6.13a	8
Outcross	42.3 \pm 2.92a	6	54.9 \pm 5.57a	8
Heterospecific	47.6 \pm 7.33a	6	28.4 \pm 7.21b	8
Base of style*				
Self	2.8a (1.28–4.82)	6	4.5a (2.18–7.64)	8
Outcross	6.2a (3.42–9.73)	7	26.1b (21.3–31.35)	8
Heterospecific	19.6b (15.90–23.66)	7	0.7a (0.10–1.57)	8

NOTE: Values followed by different letters differ significantly at $P < 0.05$ based on results of Ryan's multiple comparisons between treatments for a given species.

*Statistics based on square root transformed data, hence asymmetrical standard errors.

selfing depressed fecundity for *E. albidum* by 81% relative to outcrossing (calculated as $1 - (f_s s_s / f_o s_o)$, where f_s and f_o are the proportional fruit set for selfed and outcrossed flowers, respectively, and s_s and s_o are the proportion of ovules setting seeds in fructiferous flowers for selfed and outcrossed flowers; see Schemske 1983).

None of the caged plants set fruit. Although few plants were involved ($n = 6$ for both species), these results agree with Banks' (1980) observations for *E. albidum* and suggest that even though this species is self-fertile, it does not set fruit through autogamous self-pollination.

Pollen tube growth after self-, outcross-, and heterospecific pollination

This experiment considered the number of pollen tubes in a stylar canal in relation to the type of pollen applied to a stigma lobe and the pollen type applied to the other lobe pollinated on the same stigma. Based on two-factor analyses of variance, neither the source of the competing pollen nor its interaction with the treatment received by the focal stigma lobe explained a significant proportion of the variation in the number of pollen tubes ($P > 0.05$ in all cases) for either species. We therefore present the results only as they relate to the source of the pollen received by a stigma lobe.

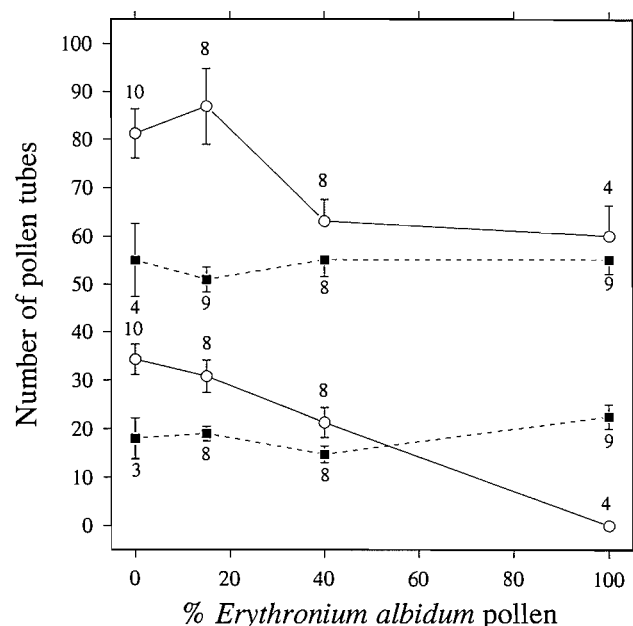


FIG. 1. Mean (\pm SE) number of pollen tubes penetrating the stigma and growing to the base of the style following hand-pollination with mixtures of *E. americanum* and *E. albidum* pollen. Solid lines connect mean responses for *E. americanum*; broken lines connect mean responses for *E. albidum*. The upper responses for each species relate the number of pollen tubes in the stigma and the lower responses indicate the mean number of tubes reaching the base of the style. Numbers adjacent to each standard error bar are sample sizes.

On average, fewer conspecific pollen tubes reached the bases of *E. albidum* styles after 24 h than *E. americanum* pollen tubes, regardless of their genetic relationship to the recipient plant. Pollination treatment did not affect the number of pollen tubes penetrating stigmas (Table 2; $F_{2,14} = 0.39$, $P > 0.5$); however, more heterospecific tubes reached the base of the style after 24 h than either self or outcross tubes ($F_{2,14} = 6.61$, $P < 0.01$). Pollen tubes failed to reach the base of the style for one self- and two outcross-pollinations.

In contrast, heterospecific pollen grew poorly in *E. americanum* styles. This poorer growth was evident within the stigma, as significantly fewer heterospecific pollen tubes penetrated *E. americanum* stigmas than self or outcross tubes (Table 2; $F_{2,18} = 13.24$, $P < 0.001$). Significantly more outcross pollen tubes reached the base of the style than either self or heterospecific tubes ($F_{2,18} = 7.28$, $P < 0.005$). Pollen tubes failed to reach the base of the style following most (6 out of 8) heterospecific pollinations and two self-pollinations.

Reproductive success following pollinations with interspecific pollen mixtures

Pollen grains germinated and began growing pollen tubes in all styles of both species, regardless of pollination treatment (0, 14, 40, or 100% *E. albidum* pollen; Fig. 1). Equivalent numbers of pollen tubes penetrated *E. albidum* stigmas for all four pollen mixtures ($F_{3,24} = 0.33$, $P > 0.75$). In contrast, more grains germinated on *E. americanum* stigmas following pollination with 0 or 14% *E. albidum* pollen than following pollination with 40 or 100% *E. albidum* pollen ($F_{3,26} = 4.63$, $P < 0.025$; Fig. 1).

Some pollen tubes grew the full length of the style for all treatments on both species, except for the four *E. americanum*

TABLE 3. Percent fruit and seed set by *E. americanum* and *E. albidum* following pollination with different percentages of heterospecific pollen (0, 14, 40, 50, or 100%)

	0		14		40		50		100	
	Median	<i>n</i>	Median	<i>n</i>	Median	<i>n</i>	Median	<i>n</i>	Media	<i>n</i>
<i>E. albidum</i>										
Fruit set	0.0	4	55.6	9	100.0	8	77.8	9	93.8	16
Seed set			4.9	5	9.6	8	17.1	6	26.7	15
			(2.3–8.3)		(5.9–15.4)		(7.4–20.0)		(17.4–29.6)	
<i>E. americanum</i>										
Fruit set	41.2	17	37.5	8	12.5	8	42.9	7	0.0	4
Seed set	16.7	7	35.9	3	26.1	1	18.0	3		—
	(14.6–22.7)		(25.8–36.7)				(16.7–27.8)			

NOTE: Data for seed set include only plants that set fruit and represent the median (\pm one quartile) percentage of ovules producing seeds. *n*, sample size.

styles that received pure *E. albidum* pollen (Fig. 1). Pollen tubes in the latter styles exhibited typical incompatibility responses, i.e., they developed inflated tips and ceased growth in the upper style. The number of pollen tubes at the base of *E. americanum* styles declined as the proportion of *E. albidum* pollen applied to the stigma increased (excluding pure *E. albidum* pollen, $F_{2,23} = 4.17$, $P < 0.05$; test for linear trend, $t_{23} = 2.79$, $P < 0.025$). In contrast, composition of the pollen mixture did not significantly affect the number of pollen tubes growing to the base of *E. albidum* styles ($F_{3,24} = 2.30$, $P > 0.1$). The number of pollen grains germinating was not included as a covariate in these analyses because it did not significantly affect the number of tubes reaching the base of the style (*E. americanum*, $F_{1,22} = 0.13$, $P > 0.7$; *E. albidum*, $F_{1,23} = 0.67$, $P > 0.25$).

During the 2 years of this study, we measured fruit and seed set for plants of both species that received one of five pollen mixtures, i.e., 0, 14, 40, 50, or 100% *E. albidum* pollen. Neither species set fruit following purely interspecific pollination, and responses to the other treatments differed for the two species (Table 3). Overall, 35.0% (14 out of 40) of *E. americanum* flowers and 85.4% (35 out of 41) of *E. albidum* flowers that received some conspecific pollen produced fruit. For *E. americanum*, pollination treatment did not significantly affect fruit set (Fisher's exact test, $P = 0.413$ with pure heterospecific treatment and $P = 0.538$ without pure heterospecific treatment). In contrast, pollination treatment did significantly affect fruit set by *E. albidum* (Fisher's exact test, $P = 0.0013$ with pure heterospecific treatment and $P = 0.05$ without pure heterospecific treatment); the likelihood of successful fruit set increased with the proportion of *E. albidum* pollen applied to the stigma (Table 3).

These hand-pollinations also revealed a difference between species in the effect of pollination treatment on the percentage of ovules producing seeds (Table 3). Pollination treatment did not significantly influence percent seed set for *E. americanum* (Kruskal–Wallis test, $H = 5.70$, 3 df, $P > 0.1$), although the relatively poor fruit set by this species reduced sample sizes. For *E. albidum*, percent seed set increased essentially linearly as the proportion of *E. albidum* pollen applied to stigmas increased ($H = 15.36$, 3 df, $P < 0.005$; test for linear trend, $X^2 = 14.46$, 1 df, $P < 0.001$; Marascuilo and McSweeney 1977).

Discussion

Erythronium albidum and *E. americanum* exhibited characteristic unilateral interspecific incompatibility (Harrison and

Darby 1955; Lewis and Crowe 1958; Nettancourt 1977). Pollen from the self-fertile species (*E. albidum*) experienced an incompatibility reaction in styles of the self-infertile species (*E. americanum*). In contrast, heterospecific pollen tubes not only developed successfully in *E. albidum* styles but also were apparently competitively superior to outcross, conspecific pollen tubes (Table 2). Note that these species have styles of equivalent length (see Methods), so that the absence of *E. albidum* pollen tubes at the bases of *E. americanum* styles could not have arisen from incongruity between style length and maximum pollen tube length, such as observed by Williams and Rouse (1990) for interspecific crosses of *Rhododendron*.

As a result of unilateral incompatibility, *E. americanum* pollen tubes growing in conspecific styles were probably little affected by the presence of *E. albidum* pollen when a stigma received pollen from both species. Most *E. americanum* ovules were therefore freely available for conspecific fertilization, and fruit and seed production by this species were not significantly affected by pollination with *E. albidum* pollen if conspecific pollen was also available (Table 3). In contrast, the presence of *E. americanum* pollen on *E. albidum* stigmas significantly reduced *E. albidum* fruit and seed set, either through interference between pollen tubes of the two species or through preemption of *E. albidum* ovules by *E. americanum* pollen tubes. These results resemble those of Kho and Baër (1971) who examined interspecific crosses in tulips (*Tulipa*), a member of the same lily subfamily as *Erythronium*. They found that heterospecific pollen tubes that entered the ovary started to coil after entering the embryo sac and failed to effect fertilization (also see Williams et al. 1982). Such behavior would probably disable the affected ovule from fertilization by a later arriving conspecific pollen tube.

These results foster an expectation of an asymmetric relation between plants that experience unilateral incompatibility, i.e., the self-fertile species is likely to suffer reduced fruit and seed production from abundant interspecific pollen transfer, whereas the self-infertile species should be exempt from such effects. Such a mechanism could be accompanied by several ecological and evolutionary consequences. In particular, unilateral incompatibility could affect the relative abundance of related species in a given community, with self-infertile species reproductively dominating self-fertile species. As a result, unilateral incompatibility may represent a cost of self-fertility. Such a cost could affect the optimal level of outcrossing in a mixed mating system or even promote complete dependence on selfing, depending on inbreeding constraints.

If unilateral incompatibility is as common as plant breeding

studies indicate (reviewed by Nettancourt 1977), why has its ecological significance not been recognized previously? Two explanations are possible: either appropriate data have not been collected during ecological studies, and (or) some feature of natural plant reproduction counteracts the effects of unilateral incompatibility. Although the number of ecological studies of the effects of interspecific pollen transfer is growing, few studies have examined species with the two attributes necessary for unilateral incompatibility. First, unilateral incompatibility should be more prevalent among related species because they are more likely to provide suitable conditions for the germination and growth of heterospecific pollen tubes (Martin 1970), so that this mechanism of reproductive interference should not be relevant to studies of interspecific pollination among unrelated species (e.g., Waser 1978; Campbell and Motten 1985; Kwak and Jennersten 1986; Galen and Gregory 1989). Second, unilateral incompatibility requires species with contrasting breeding systems, but studies of related species generally involve plants with the same breeding system (e.g., Schemske 1981; Armbruster and Herzig 1984; Randall and Hilu 1990). Ecological studies of interspecific pollination between congeneric or confamilial species with contrasting breeding systems seem to be rare.

Support for a feature of natural plant reproduction counteracting the effects of unilateral incompatibility is provided by Stucky's (1985) results for *Ipomoea hederacea*, a selfing species, and *Ipomoea purpurea*, an outcrossing species, grown in a common garden. Stucky (1985) pollinated bagged, emasculated, and intact flowers of both species with various pollen mixtures. For emasculated flowers, high proportions of heterospecific pollen (i.e., $\geq 50\%$) reduced seed production by *I. hederacea* but not *I. purpurea* (as long as some conspecific pollen was present), suggesting unilateral incompatibility. In contrast, intact flowers did not differ in seed production, regardless of the proportion of heterospecific pollen (except *I. purpurea* that received only *I. hederacea* pollen). This difference in response presumably resulted because the stigmas of intact flowers received self-pollen in addition to the experimental pollen mixture. As a result, stigmas of the self-compatible species probably received a smaller proportion of heterospecific pollen, thereby reducing the probability of ovule preemption.

In conclusion, results from diverse species (reviewed by Nettancourt 1977; see Williams et al. 1982; Stucky 1985; this study) suggest that unilateral incompatibility can bias the effects of interspecific pollen transfer in favor of the self-infertile species. Whether this particular mechanism plays a significant ecological role in determining a plant's seed production remains to be demonstrated.

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