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Facultative Self-pollination in Island Irises

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ABSTRACT.—Blue flag (*Iris versicolor*) is an insect-pollinated clonal plant found in wet habitats throughout northeastern North America. Our study of an isolated population on Kent Island, New Brunswick, showed that blue flag was self-compatible. Moreover, we found no evidence of inbreeding depression. Although individual flowers were protandrous, the timing of male and female phases and the precise behavior of flowers depended upon whether the flowers had been pollinated. Naturally pollinated or hand-pollinated flowers closed their stigmas shortly after pollination, making them no longer receptive. Flowers from which pollinators had been excluded prolonged the period during which stigmas were open and reflexed downwards, increasing the likelihood that they could come into contact with their own anthers or petals onto which pollen had fallen. In the absence of insects, plants growing in exposed habitats apparently took advantage of wind to achieve pollination. Hand-pollination experiments showed no evidence of inbreeding depression. Fruit set, capsule size and the number of seeds per capsule did not depend on whether pollen came from the same flower, the same clone, the same population or different populations. Facultative autonomous self-pollination may be advantageous whenever pollination and outcrossing are unlikely, as in inbred populations, in small or clonal populations, or in populations where insect pollinators are scarce, ineffective or constrained by harsh environmental conditions.

INTRODUCTION

Plants have evolved a variety of traits that have been interpreted as adaptations to avoid inbreeding, including separation of male and female flowers in space and time (dioecy, dichogamy), distinctive floral polymorphisms (*e.g.*, heterostyly), and various self-incompatibility mechanisms (Darwin, 1876; Bateman, 1952; Faegri and van der Pijl, 1979; Dickinson, 1994). Under certain situations, however, plants that are unable to be fertilized by pollen from the same flower, ramet, or clone may have lower reproductive success than plants that are capable of selfing. For example, if pollinators are scarce or inefficient, or if plant populations are small or consist mainly of related individuals, outcrossing may be less likely than inbreeding. Rare or clonal species commonly face such situations, as do plants that occur in physically stressful environments, in fragmented habitats, and on islands (Kevan, 1972; Linhart and Feinsinger, 1980; Handel, 1985; Jennersten, 1988; Menges, 1991; Rathcke and Jules, 1993). Asexual reproduction, self-compatibility, and autonomous self-pollination (autogamy, the ability to self-fertilize in the absence of pollinators) enable plants to reproduce when outcrossing is disadvantageous, difficult or impossible (Lloyd and Schoen, 1992).

One plant species that lends itself to investigations of self-pollination is blue flag, *Iris versicolor* (Iridaceae), an insect-pollinated herb which exhibits clonal growth and frequently occurs in isolated or physically stressful wet habitats throughout northeastern North America. Working at a site in Ontario, Kron *et al.* (1993) demonstrated that *I. versicolor* was completely self-compatible. Moreover, their study found no evidence of inbreeding depression. Insects commonly moved between flowers of the same clone, and even between stigmas (pollination units) of the same flower, causing high rates of selfing. Nonetheless, outcrossing in *I. versicolor* was shown to be promoted to some degree by protandry: individual flowers passed through a male stage during which the stigma was not yet receptive before passing

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through a female phase. Kron *et al.* (1993) suggested that the final stage, in which the stigmas curl down and sometimes contact the anthers, could lead to autonomous self-pollination.

In the present study our aim was to determine the extent of self-compatibility and inbreeding depression in an isolated population of *Iris versicolor* occurring on an oceanic island. We also measured the amount of time that individual flowers spent in different phases to test whether pollination influenced subsequent floral behavior and to investigate the mechanisms behind autonomous self-pollination.

METHODS

Our study was conducted in June and July 1994 and 1995, at the Bowdoin Scientific Station, located on Kent Island, in the Bay of Fundy, New Brunswick, Canada (44°35′N, 66°46′W). The 80-ha island is isolated from the mainland of Maine and Nova Scotia by 20 km and from the island of Grand Manan, the nearest large body of land, by 9 km.

Iris versicolor is common in moist open areas on Kent Island and most of the larger islands in the Grand Manan Archipelago. A native perennial, it reproduces both asexually, through vegetative or clonal growth, and sexually. Its large showy flowers are composed of three functional "pollination units" (Pande and Singh, 1979; Kron *et al.*, 1993). The central style is divided into three branches. On the underside of the distal end of each style branch is a stigma flap which opens, reflexes downward, and becomes receptive during the female phase. Tucked underneath each style branch is an anther extending up towards the base of the stigma flap. Each style branch lies above a large sepal which forms an incompletely enclosed tunnel through which insects must crawl in order to reach the nectaries at the base of the sepal. In the process, the anthers contact the insects' backs, dusting them with pollen. When the stigma flaps are open, they partially block the entrance and force insects to brush past them as they enter (*see* Kron *et al.*, 1993). In the mainland population that Kron *et al.* (1993) studied, bumblebees (*Bombus* spp.) were the principal pollinators. On Kent Island, however, bumblebees comprise fewer than 1% of the insect visitors to *I. versicolor*, which are predominantly visited (and presumably pollinated) by flies, particularly seaweed flies (Coelopidae).

We carried out four separate experiments on irises. In the first experiment we monitored the duration of different reproductive stages in 60 flowers, each from distinct clones. Flowers were considered to be from separate clones if clusters of ramets were isolated from each other by at least 5 m of other vegetation. In some cases flowers from different clones could also be recognized by their distinctive colors. Each morning (0700–0900 h) and afternoon (1600–1800 h), we examined flowers, recording when they first opened, when their anthers dehisced and when their petals withered. One-third of the flowers in the sample for the first experiment were not manipulated (control flowers; N = 20). One-third were "bagged": insects were excluded by enclosing the flowers in loose bags of white bridal veil held away from the flower by wire frames. The bags were sealed at the bottom with a twist-tie around the stalk and supported by metal surveyors' stakes. The remaining one-third of the flowers in the sample for the first experiment were hand-pollinated (*see* below). Sample sizes presented below vary at different floral stages because of damage to some of the flowers caused by rain and animals (mostly muskrats, *Ondatra zibetheca* and herring gulls, *Larus argentatus*).

To evaluate the influence of wind on pollination, we performed a second experiment on flowers in sites sheltered by shrubs and trees on at least three sides (protected sites) or in open fields (exposed sites). In both protected and exposed sites, 40 unopened flowers were chosen, each from a different clone. Half of the flowers at each type of site were randomly

designated as controls and were allowed to be visited by insects naturally (free pollination treatment). The other 20 flowers were bagged for the duration of the experiment (autonomous self-pollination treatment). We removed the bags once the flowers had obviously senesced and the stigmas were no longer receptive (within 10 days of opening, Kron *et al.*, 1993). Mature-sized fruits were collected at the end of July.

In the third experiment, a sample of 45 additional irises was established in an open field to investigate whether pollen deposited on the stigma influenced floral behavior, specifically the closing or reflexing downward (curling) of the stigma flap. All flowers were bagged in the bud stage. Fifteen flowers were hand-pollinated as soon as the stigma flap opened and immediately bagged again (hand-outcrossed treatment). We pollinated flowers by gently brushing the anther of a flower from a different clone against their stigmas. Another 15 flowers were bagged throughout the experiment but otherwise not manipulated in any way (autonomous self-pollination treatment). The remaining 15 flowers were bagged without being pollinated, but in addition they were emasculated by excising their anthers once the flower opened but before anthers had dehisced (anther removal treatment). To control for the effect of physical contact during hand-pollination, we brushed the stigmas of flowers in the autonomous self-pollination and anther removal treatments with a feather. We categorized the position of stigmas as closed, partially open (the stigma forming an angle of 20–80° with the style branch), completely open (80–140°), and reflexed (>140°). When reflexed, the stigmas sometimes directly contacted the anthers of the same flower. Flowers were observed each morning and afternoon as described above.

In our fourth experiment, in 1995 we selected 28 flowers from each of four clones (total N = 112 flowers). From each clone, an equal number of flowers were assigned to one of four treatments: self-pollination (using pollen from the flower itself); within-clone pollination (using pollen from three different flowers from the same clone); between-clone pollination (using pollen from flowers of three different clones on Kent Island); and between-population pollination (using pollen from flowers of clones from three neighboring islands, Sheep, Hay and Grand Manan islands). We determined fruit set, measured mature fruit capsule size, and counted the number of seeds per capsule for a subset of the plants in September 1995.

RESULTS

Experiment 1.—In flowers that were not experimentally manipulated (control flowers), anther dehiscence occurred an average of 0.6 days (*SD* = ±0.2, N = 14) after the flower began to open. Most pollen had fallen or been removed 1.0 days (±0.4) after opening. The stigma flap first opened and became receptive after 1.6 days (±0.5), approximately 1 day after anther dehiscence. In four of the control flowers, stigma flaps reflexed downward 2.0 days (±0.9, N = 4) after the flower opened. The mean longevity of control flowers (time between flower opening and senescence as indicated by withered petals) was 2.9 days (±0.2, N = 6). Bagging or hand-pollinating flowers did not appear to affect the timing of anther dehiscence, stigma flap opening, or senescence compared to control flowers (ANOVA: $P > 0.30$ for all comparisons). However, stigma flaps reflexed downward in 55% of the bagged flowers, in only 10% of the control flowers, and in 0% of the hand-pollinated flowers (chi-square test: $P < 0.01$).

Experiment 2.—When pollinators were excluded by bagging flowers in areas differing in their exposure to wind, 53% of bagged flowers showed autonomous self-pollination in exposed areas as compared to only 18% in protected areas (Table 1; chi-square test: P = 0.067). However, there was little or no effect of degree of exposure on fruit set in freely pollinated plants in the same experiment (Table 1; P = 0.81). There was no significant

TABLE 1.—Proportion of flowers that set fruit in different experiments. Although initial sample sizes were 20 in Experiment 2, by the time of fruit set they were reduced because of damage by herbivores. (Fruit set was not monitored in Experiment 1.)

Treatment	Fruit set (%)	N
Experiment 2		
Free pollination—exposed sites	70.6	17
Free pollination—protected sites	61.1	18
Autonomous self-pollination—exposed sites	52.6	19
Autonomous self-pollination—protected sites	17.7	17
Experiment 3		
Hand-outcrossed	73.3	15
Autonomous self-pollination	40.0	15
Anthers removed, flowers bagged	6.7	15

difference in fruit set between bagged and freely pollinated flowers in exposed sites ($P = 0.45$), but freely pollinated flowers had higher fruit set than bagged flowers in protected sites (Table 1; $P = 0.02$).

Experiment 3.—Most hand-outcrossed flowers (64%) closed their stigmas within 1.5 days after pollination, making them unreceptive to additional pollen. In contrast, fewer than 15% of stigmas closed in flowers that were not cross-pollinated (autonomous self-pollination treatment), and 0% closed in emasculated flowers (anther removal treatment; Fig. 1A). In hand outcrossed plants, only 11% of stigmas reflexed downwards to contact stamens, whereas stigmas reflexed in 63% of flowers that were not hand-pollinated and in 94% of emasculated flowers ($N = 45$ in each treatment; chi-square test: $P < 0.0001$; Fig. 1B). There was substantial variation among flowers within treatments and even among pollination units

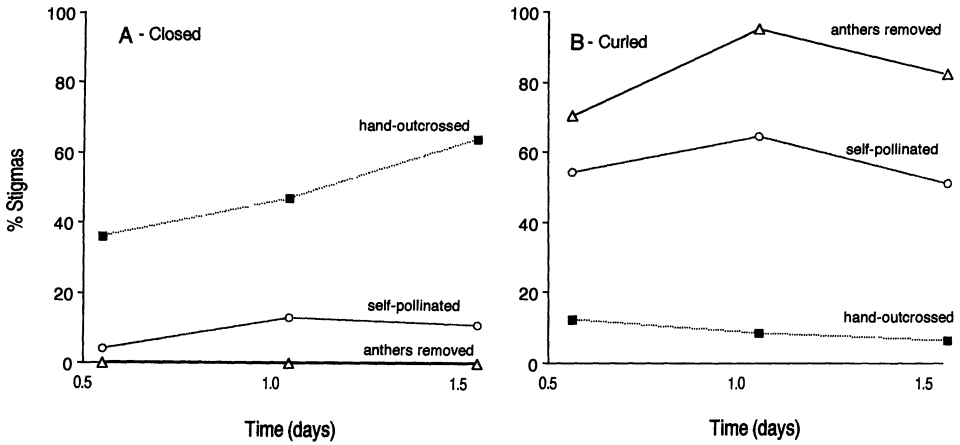

Fig. 1.—Proportion of flowers with stigma flaps (a) closed (unreceptive to pollen) or (b) with stigma flaps curled (reflexed downwards to receive pollen) as a function of time since hand-outcrossing or anther removal. Flowers were not manipulated in the self-pollination treatment. $N = 15$ flowers for each treatment

within flowers. A single flower sometimes had one stigma flap reflexed while the other two were closed.

Overall, more than 70% of hand-outcrossed flowers set fruit, compared to 40% for autonomous self-pollinated flowers. Only one emasculated flower (7%) set fruit. The effect of treatment on fruit set was statistically significant (chi-square test: $P < 0.001$). However, there was no significant effect of treatment on fruit size for those flowers that did set fruit (Mann-Whitney U test for differences in mass, length and width: $P > 0.42$ for all comparisons; $N = 11$ hand-outcrossed and six autonomous self-pollinated flowers).

Experiment 4.—In the final experiment, which involved hand-pollinating flowers using different sources of pollen, we found no indication of inbreeding depression (at least through the stage of fruit and seed set). Fruit set was equivalent regardless of whether pollen was from the same flower, the same clone, different clones, or different populations (Table 2; chi-square test: $P = 0.57$). If anything, there was a suggestion that pollen from more distant populations may have resulted in reduced fruit set (Table 2; *see* Waser and Price, 1989). Overall, fruit set in the 1995 hand-pollination experiments was 61% ($N = 98$ flowers), slightly lower than fruit set in the hand-outcrossed experiment the previous year (experiment 3). There was no significant effect of pollination treatment on capsule dimensions or the number of seeds per capsule (Table 2). On average only 51% of the seeds per capsule ($N = 38$) appeared fully developed, but neither the proportion nor the number of fully developed seeds per capsule varied with pollination treatment (ANOVA: $P = 0.65$).

DISCUSSION

The spatial separation of stigmas and anthers in *Iris versicolor* and the 1-day temporal separation between the initiation of male and female phases appear to have originated as adaptations to promote outbreeding. Nonetheless, on Kent Island the species is self-compatible and there is no evidence of inbreeding depression with regard to fruit set, fruit capsule size or seeds per capsule. In fact, flowers that have not received pollen from other sources have the ability to pollinate themselves facultatively by reflexing their stigmas downwards to contact their own anthers and petals. Autonomous self-pollination resulted in slightly lower rates of fruit set (40%) compared to hand-pollinated plants (73% in 1994, 61% in 1995) or freely pollinated controls (66%; Table 1). Plants growing in exposed habitats, however, apparently took advantage of the wind to elevate the probability of autonomous self-pollination. In the absence of insect pollinators, wind may increase pollination directly by carrying pollen through the air, or indirectly by forcing reflexed stigmas to brush

TABLE 2.—The effect of pollen source on proportion of flowers that set fruits, mean capsule dimensions, and number of seeds per capsule (±1 SD) in Experiment 4. Sample sizes for % fruit set were 28, 27, 24 and 19 for the four treatments, respectively; for capsule dimensions and seed number sample sizes were 6, 7, 14 and 6. There were no differences in fruit set (chi-square test: $P = 0.57$), capsule length, capsule diameter or seed number between treatments (ANOVA: $P > 0.30$)

	Pollen source			
	Same flower	Same clone	Different clone	Different population
% fruit set	64.3	55.7	70.8	52.6
Capsule length (cm)	2.8 (0.6)	3.3 (0.7)	3.3 (0.7)	3.1 (0.3)
Capsule diameter (cm)	1.0 (0.1)	1.1 (0.3)	1.0 (0.2)	1.0 (0.2)
Seeds/capsule	75.0 (11.8)	99.3 (20.7)	92.2 (18.6)	87.0 (21.6)

against petals of the same flower where pollen has settled, or by causing neighboring flowers to come into contact with each other.

One possible artifact introduced by our bagging experiments was the fact that once the anthers dehisced, pollen could not be removed by insects. Much of the pollen therefore remained in the anthers or fell on petals beneath the stigmas, where it may have been more likely to have ended up on the stigmas either through direct contact or wind transport. In other words, the rate of autonomous self-pollination could be somewhat lower under natural conditions if insects remove pollen from flowers but are inefficient in depositing it on stigmas. We should also point out that we measured inbreeding depression only in terms of fruit set, capsule size, and seeds per capsule. To establish definitively that self-pollination does not result in inbreeding depression, one should also determine characteristics of the progeny, such as germination rates, growth rate, adult fecundity, and so forth.

Self-pollination may be particularly advantageous whenever the probability of outcrossing is low or pollination is limited, as in inbred plant populations, in small or clonal populations, or in populations where insect pollinators are scarce, ineffective, or constrained by harsh environmental conditions (Whisler and Snow, 1992). The fact that Kron *et al.* (1993) found a high degree of autonomous self-pollination in a mainland iris population with large numbers of effective pollinators (bumblebees) tends to suggest that the conditions that characterize Kent Island's *Iris versicolor* populations (isolation, small population sizes, harsh environment) are not the only factors influencing the evolution of facultative self-pollination in this species. Clonal growth, for example (Handel, 1985), could also lead to high frequencies of self-pollination. Alternatively, populations of *I. versicolor* across northeastern North America may have experienced historical periods of intense inbreeding, perhaps during glaciation, which could have purged deleterious alleles, reduced inbreeding depression, and relaxed selection against self-pollination (*see* Lande and Schemske, 1985; Jarne and Charlesworth, 1993). In any event, autonomous self-pollination and the apparent absence of inbreeding depression (at least through the stages of fruit and seed set) may liberate *I. versicolor* from a dependence on insect pollen vectors. Because autonomous self-pollination is facultative, occurring only when a flower has not already received pollen and is nearing the end of its floral life, the option of outcrossing is still preserved in *I. versicolor.* Such reproductive flexibility may preadapt species like *I. versicolor* to colonize isolated habitats such as oceanic islands.

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