

Pollinator Limitation, Autogamy and Minimal Inbreeding Depression in Insect-pollinated Plants on a Boreal Island

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ABSTRACT.—We studied the pollination biology of 18 common insect-pollinated plant species on Kent Island, a boreal island in the Bay of Fundy, New Brunswick, Canada. Under natural conditions, fruit set was relatively high in most of the species studied [although it was very low (<1%) in *Maianthemum canadense*]. Hand-pollination increased fruit set in five of seven species examined (71%), indicating that fruit set may commonly be limited by the availability or behavior of pollinators on Kent Island. Twelve of 17 species examined (71%) were capable of substantial autonomous self-pollination (autogamy in the absence of pollinators), although fruit set averaged higher in open-pollinated flowers (65.7%) than in flowers from which insects were experimentally excluded (49.6%). The number of seeds per fruit was also less in autonomously self-pollinated flowers in two species (*Rhododendron canadense* and *Ledum groenlandicum*). In at least one species (*Iris versicolor*), we showed no evidence of inbreeding depression in terms of percent fruit set, fruit size, or number of seeds per fruit.

Overall, our results demonstrate that for many insect-pollinated plant species on Kent Island, pollinators are likely to be limiting, autogamy is common and inbreeding depression is negligible. Although pollinator limitation and autogamy regularly occur in mainland habitats as well, a review of the literature suggests that they may be more common on islands such as Kent Island. If such island-mainland differences are general, they may arise because genotypes and species capable of self-fertilization are more likely than obligate outcrossers to colonize and become established in isolated habitats.

INTRODUCTION

Islands tend to have fewer species and smaller populations of pollinators than mainland habitats, and flower visitors on islands often have generalized diets or reduced activity due to harsh weather (Hagerup, 1951; Carlquist, 1974; Linhart and Feinsinger, 1980; Barrett, 1996). As a result, fruit set of obligately outcrossing plant species on islands may be limited by the scarcity of flower visitors or by their ineffectiveness as pollinators (Baker, 1955; Barrett, 1996).

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Island plants also face the risk of inbreeding because of the high degree of relatedness among plants in populations founded by few individuals and the small size of island populations. In populations that have previously experienced extensive selfing, however, inbreeding depression may be reduced because most deleterious alleles will already have been purged by natural selection (Charlesworth and Charlesworth, 1987; Johnston and Schoen, 1996; Husband and Schemske, 1996). In the absence of inbreeding depression, pollinator scarcity or inefficiency on islands could actually favor self-compatibility and self-fertilization. In fact, even with substantial inbreeding depression selfing may be favored over outcrossing if the reproductive assurance value of selfing is large enough (Levin, 1972; Schemske and Lande, 1985; Lloyd, 1992; Whisler and Snow, 1992; Schoen et al., 1996). Autonomous self-pollination [autogamy that does not require pollen transfer by insects or other animal vectors: Lloyd and Schoen (1992)] may be an extreme adaptation to provide reproductive assurance in pollinator-poor habitats. Self-compatibility and autogamy are hypothesized to be particularly advantageous during the initial stages of colonization when population sizes are small and the probability of pollen transfer between plants is low (Baker, 1955; Stebbins, 1957; Cox, 1989; Holsinger, 1996; Barrett, 1996; Moeller and Geber, 2005).

Although the distinctive pollination biology of the floras of large isolated archipelagos—Hawaii, New Zealand, the Galápagos—has been well-documented, less is known about reproduction in individual plant species with both mainland and island populations (Barrett, 1996). Investigations of inshore islands provide a unique opportunity to understand ecological and evolutionary processes that influence island colonization and its potential long-term consequences for plant breeding systems. To increase our understanding of pollination biology of inshore islands (Inoue et al., 1996; Larson and Barrett, 1998; Bernardello et al., 2001; Mavraganis et al., 2001; Leimu, 2004), especially boreal islands, which have hardly been examined, we studied a simple plant community on a small island in the Bay of Fundy in eastern Canada. We surveyed the most common early-flowering, insect-pollinated species for autonomous self-pollination by excluding pollinators. In addition, we conducted hand-pollination experiments from 1993 through 2003 to assess pollinator limitation and inbreeding depression in a variety of species in this island plant community.

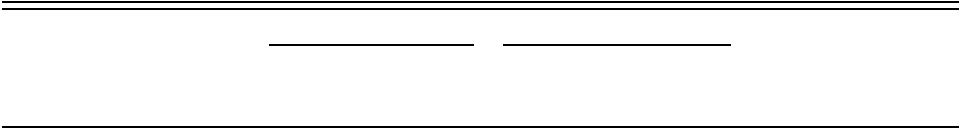
METHODS

Study site.—Our study site was Kent Island, New Brunswick, an 80-ha island located 9 km south of Grand Manan Island (44°35'N, 66°45'W). Kent Island, the outermost vegetated island in the Grand Manan Archipelago, is isolated by more than 25 km from the coasts of Maine, New Brunswick and Nova Scotia. It has not been connected to the mainland since well

flowered on the island during the early summer (late May through early July). Our sample of insect-pollinated species did not include species represented by just a few individuals, species that did not begin to flower until late summer, or, with two exceptions, introduced species (introduced species make up about 25% of the flora, predominantly grasses and herbs). Collectively, the study species represented 12 plant families (Table 1). Of special interest were species in the Ericaceae, Iridaceae and Liliaceae because of their abundance on the island, their easily manipulated floral displays, the presence of several species per family and the existence of comparative studies of mainland populations of the same or closely related species. Our experiments and observations on the pollination biology of Kent Island plants were carried out during June and July of various summers from 1993 through 2003, as described below. Nonparametric statistical tests (χ^2 , Mann-Whitney U, Wilcoxon Sign Rank) were performed on categorical data and data for which the assumptions of parametric analyses (e.g., normal distributions) were not justified. For those tests and for t-tests and ANOVAs, we used StatView on the Macintosh (SAS Institute Inc., 1999).

Pollinator-exclusion experiments and autogamy.—We tested for autonomous self-pollination in 17 species by performing pollinator-exclusion experiments. (Hereafter, in the context of pollinator-exclusion experiments, we use the terms “autonomous self-pollination” and “autogamy” interchangeably.) For each species studied in 1993, we selected plants from at least two different sites located 0.1–1.0 km apart, depending upon the species. Within each site, 10 individual plants per species served as open-pollinated controls. Ten other plants were randomly chosen for the “pollinator-exclusion” treatment. Pollinators were excluded from flowers by placing bags made of bridal veil over inflorescences during the bud stage. Plants were censused daily and bags were removed after the stigmas were clearly no longer receptive (usually 7–10 d after the petals had withered and the stigma had become discolored and dry). At the end of July we determined “% fruit set” as the number of well-developed fruits (with normal-looking seeds) divided by the number of flowers at the beginning of the experiment ($\times 100$). In the case of plant species that produced a single flower (e.g., *Trientalis borealis*) or species in which we typically observed just a single flower per plant (e.g., *Iris versicolor*), fruit set for a given plant was scored as either 0% or 100% and averaged across individuals. Significant site effects appeared in only three of the 17 species (*Sisyrinchium montanum*, *Rhinanthus crista-galli* and *Ranunculus acris*), so within species we combined samples from different sites for analysis. The total sample size in 1993 for the two treatments and two sites was 40 plants per species (see Table 1). Snowshoe hares (*Lepus americanus*), muskrats (*Ondatra zibethica*), gulls (*Larus* spp.) and other herbivores destroyed some of our experimental plants, so final sample sizes varied slightly between treatments and species. Replicate tests for autogamy were performed on a subset of the 17 species during the summers of 1994, 1998, 2001 and 2003, using similar methods but larger samples, as indicated in Table 1.

Autogamy, inbreeding depression and pollinator limitation in the Ericaceae.—To determine whether fruit and seed set on Kent Island was limited by pollinators, and whether self-pollination resulted in lower reproductive success than outcrossing (inbreeding depression), we performed hand-pollination in addition to pollinator-exclusion experiments on two species in the family Ericaceae, *Rhododendron canadense* (rhodora) and *Ledum groenlandicum* (Labrador-tea) in 1993 and 1994. We attempted to ensure that experimental plants (*R. canadense*: N = 21 plants; *L. groenlandicum*: N = 23) represented different genets by choosing individuals separated by at least 4 m. Four inflorescences per plant were randomly selected and (except for control inflorescences) covered during the bud stage with Hubco Sentry soil-sample bags (Hutchinson Bag Corporation) which were permeable to air, moisture and reduced sunlight, but which prevented pollinators from visiting the



inflorescences. On average, experimental inflorescences had 5.1 (± 1.1) flowers in *R. canadense* and 24.1 (± 8.0) flowers in *L. groenlandicum* and did not differ between treatments (ANOVAs: $P > 0.20$; except as noted otherwise, all descriptive statistics are given as mean ± 1 SD).

On every plant each inflorescence was randomly assigned to one of four treatments. In the “hand-selfed” treatment, the stigma of every flower in the inflorescence was lightly contacted 3–5 times with two mature and recently dehisced anthers from a flower from the same plant. In the “hand-outcrossed” treatment, pollen was transferred to stigmas in the same way except that we used anthers from two other conspecific plants, both of which were located at least 4 m away. (Because we did not remove anthers from experimental flowers, we could not rule out the possibility that hand-outcrossed flowers also received pollen from the same flower.) In the “pollinator-exclusion” treatment, insects were excluded by bags until stigmas were no longer receptive, as described in the previous section (Pollinator-exclusion experiments and autogamy), and flowers were not hand-pollinated. A fourth inflorescence on each plant was used as a control to assess the effectiveness of natural pollination; these

the treatments), we counted the minute seeds. Significant regressions of dry mass of individual fruits on the number of seeds per fruit allowed us to estimate the number of seeds per fruit for the rest of the sample (least square regression: *Rhododendron canadense*: $r^2 = 0.62$; *Ledum groenlandicum*: $r^2 = 0.60$; $P < 0.01$). We observed no differences between treatments in terms of seed size or appearance. Note that in these and other species in this study we did not test for aspects of inbreeding depression expressed later in life, such as reduced germination rate, seedling growth rate or adult survival and fecundity. Thus, in this paper we use the term “inbreeding depression” to refer to reduced fruit set, fruit size, orpa361 .sion:

source pollinia from two different plants located at least 5 m away. Pollinia were removed by touching a pin to the viscous disk at their base and pollen was immediately deposited directly on the stigmatic surfaces of receptive flowers using the point of the pin. Twenty other plants served as open-pollinated controls. Plants were censused at sunrise and sunset during the flowering period to document natural rates and timing of pollinium-removal by insects. In late September we collected and measured seed capsules. Damage by herbivores reduced the final sample size to 8 hand-pollinated plants and 13 controls. The surviving hand-pollinated and control plants were similar in terms of the number of flowers per plant (8.0 ± 2.3 flowers vs. 7.1 ± 2.7 flowers), plant height (21.3 ± 4.2 cm vs. 19.5 ± 4.8 cm), leaf length (7.5 ± 1.3 cm vs. 6.6 ± 2.0 cm) and leaf width (2.8 ± 0.7 cm vs. 2.6 ± 0.8 cm) (Mann-Whitney U tests: $P > 0.29$).

Insect observations.—Between 1993 and 2003, we opportunistically collected insects visiting the flowers of each plant species and made incidental observations of their behavior at various times of day (morning, midday, afternoon) throughout the season. In the case of *Habenaria obtusata*, we also conducted censuses at sunrise and sunset and sampled nocturnal insects for pollinia using blacklight traps. Specimens were sent to the U.S. Systematic Entomology Laboratory for identification.

RESULTS

Pollinator-exclusion experiments and autogamy.—Most of the common early flowering insect-pollinated plant species on Kent Island set at least some fruits without insects visiting their flowers (Table 1). Of the 17 species surveyed, 12 (71%) were capable of autonomous self-pollination. In *Maianthemum canadense*, one of the species that set no fruits when pollinators were excluded, open-pollinated plants also set no fruits in 1993 (which was typical of most years, based on casual observations between 1987 and 2004) and very few fruits in 2001. Across all 17 species, mean fruit set via autogamy was on average 25% less than fruit set for open-pollinated plants (49.6% vs. 65.7%, averaging % fruit set across years within species studied in more than one year; Wilcoxon Sign Rank test: $P = 0.02$). In 11 species autogamous fruit set was substantial, at least 50% as high as fruit set resulting from natural pollination. In eight species it was at least 80% as high, and in three species autogamous fruit set equaled or exceeded fruit set in open-pollinated controls. Nonetheless, in eight of the 17 species (47%), control plants set significantly more fruits than plants from which pollinators had been excluded (Table 1; Mann Whitney-U tests: $P < 0.05$).

Autogamy, inbreeding depression and pollinator limitation in the Ericaceae.—Percent fruit set in *Rhododendron canadense* depended upon whether pollinators were excluded and whether flowers were hand-pollinated (Fig. 1; ANOVA on arcsin-transformed % fruit set: $P = 0.0001$). In two different years *R. canadense* showed high levels of autonomous self-pollination (Table 1), but rates of fruit set and the number of seeds per fruit from flowers from which

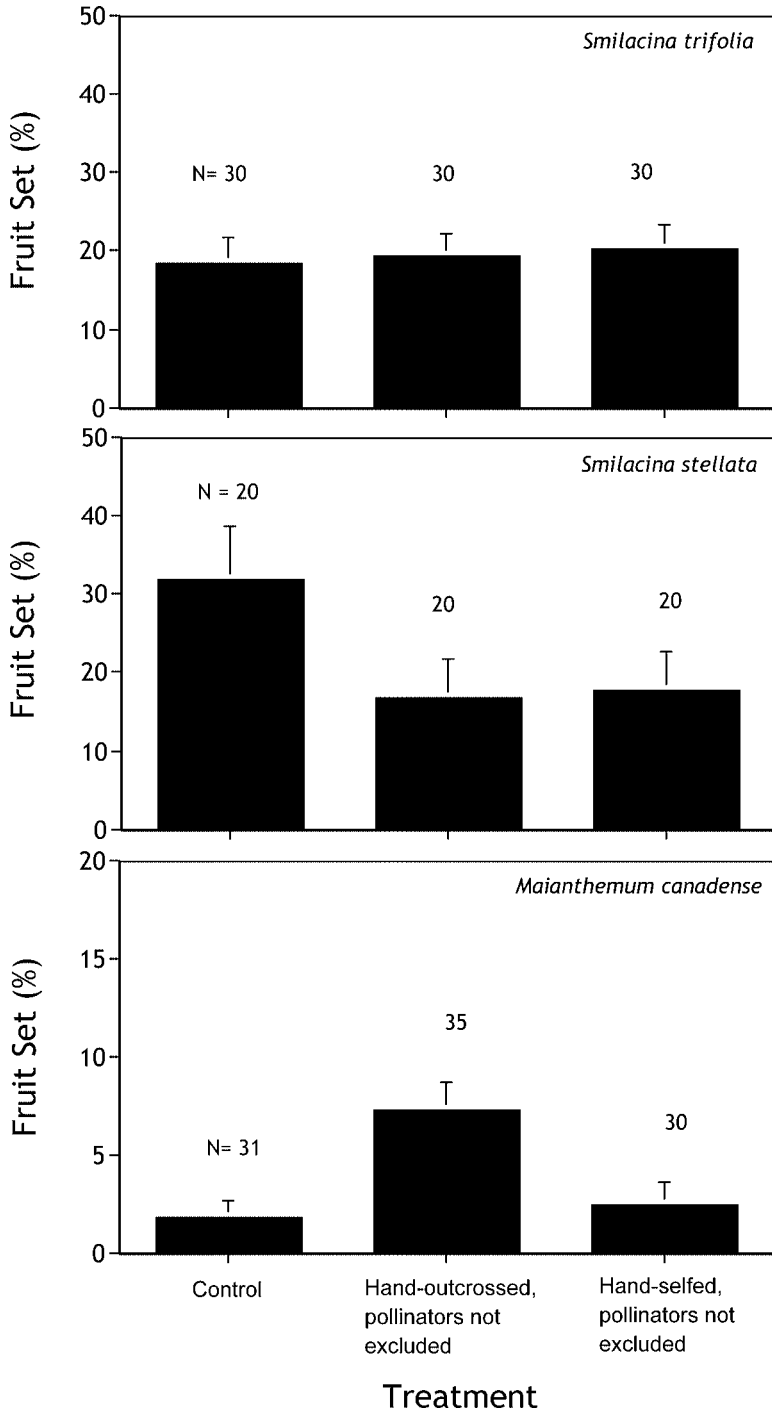
hand-outcrossed plants. Seeds of hand-selfed and hand-outcrossed flowers were indistinguishable in size, shape and color.

The fact that hand-selfed flowers tended to have higher fruit set, longer fruits and more seeds per fruit than control (open-pollinated) flowers suggests that *Rhododendron canadense* is pollinator-limited on Kent Island (Fig. 1, Table 2; Mann-Whitney U test comparing hand-selfed and control treatments: fruit set: $P = 0.003$; fruit length: $P = 0.04$; number of seeds per fruit: $P = 0.06$). Hand-outcrossed flowers had about the same fruit set, fruit length,

and number of seeds per fruit as control flowers (Fig. 1, Table 2; Mann-Whitney U test: $P > 0.26$).

As in *Rhododendron canadense*, fruit set in *Ledum groenlandicum* depended strongly upon treatment (Fig. 1; ANOVA on arcsin-transformed % fruit set: $P = 0.006$). *Ledum groenlandicum* showed high levels of autonomous self-pollination in two different years (Table 1), but fruit set was significantly lower, fruit length shorter, and seeds per fruit fewer compared to control, hand-selfed or hand-outcrossed flowers (Fig. 1, Table 2).

Ledum groenlandicum also showed no indication of inbreeding depression in terms of diminished fruit set or seed production. Hand-selfed flowers set fruit at a rate equivalent to that of hand-outcrossed flowers, and the fruits of both treatments were similar in length and



In *S. trifolia*

stigma and stamens: Kron et al., 1993, Zink and Wheelwright, 1997). In 2003 we attempted to distinguish between these two possibilities in *Iris versicolor*. By the end of July, fruits began to develop from 65% of bagged *I. versicolor* flowers from which anthers had been removed (N = 31) and 67% of flowers from which stigmas had been removed (N = 33), compared to 77% of control flowers (N = 66) (χ^2 test: P = 0.33). By mid-September, however, all of the flowers from which anthers had been removed, and all but one of the flowers from which stigmas had been removed, had aborted their fruits, whereas 46% of control plants had mature fruits (P < 0.0001). The soil-sample bags we used to exclude pollinators throughout the flowering period appeared to have had a strongly negative effect on fruit development in this species, so we cannot be certain that removing anthers and excluding pollinators prevents fruit set or that agamospermy does not occur in emasculated *I. versicolor* flowers (cf., Young and Young, 1992). However, when we restricted our analysis to flowers that had not been bagged after opening, control (open-pollinated) plants produced significantly more fruits than flowers from which stigmas had been removed (P < 0.0001). Thus, pollen transfer (even if only within a flower) appears to be necessary for substantial fruit set in *I. versicolor*.

Pollinator-limitation in *Habenaria obtusata*.—Hand-pollination experiments demonstrated pollinator limitation in *Habenaria obtusata*. Hand-outcrossed plants had a mean fruit set of 68.4% compared to 24.5% for control (open-pollinated) plants (Mann-Whitney U test: P < 0.01). With millions of tiny seeds per fruit, it was impractical to count seeds, so we used fruit length as a proxy for seed set, after verifying that swollen ovaries contained normal-looking seeds (cf., Sipes and Tepedino, 1995). Fruits of hand-outcrossed flowers were similar in length to those of control flowers but significantly larger in diameter (P = 0.11 and 0.03, respectively). On average, 40.3% of the flowers on a plant had at least one pollinium removed, and 14.7% had both pollinia removed, but pollinia were observed deposited on fewer than 1% of open-pollinated flowers (N = 156 flowers from 21 plants). There was no correlation between the number of pollinia removed per inflorescence and subsequent fruit set, suggesting that male and female components of reproductive success were relatively independent (least square regression: $r^2 = 0.01$, P > 0.50).

Observations of insects.—The most frequently observed flower-visiting insects on Kent Island were, in general order of abundance, flies in the families Syrphidae [genera: *Toxomerus*, *Eupeode*, *Meliscaeva*, *Sericomyia*, *Helophilus* and *Eristalis* (6 species in the latter genus)], Calliphoridae (*Calliphora*, *Cynomya* and *Lucilia*), Stratiomyidae, Muscidae (*Fannia*), Lauxaniidae, Ephydriidae, and Coelopidae, as well as bees [*Bombus* (3 species)] and butterflies (*Pieris*, *Vanessa*, *Speyeria*). We did not quantify insect visit rates or test for the

a high degree of overlap in flower visitors among plant species. Many of the same species of syrphid flies, for example, could be found at a variety of unrelated plant species, indicating the general nature of their diets. Closely related plants (e.g., *Smilacina stellata* and *S. trifolia*, or *Sisyrinchium* and *Iris*) differed in key aspects of their pollination biology such as whether or not they were capable of autogamy (Table 1), but they shared many of the same insect visitors. *Habenaria obtusata* had perhaps the most specialized pollination biology. Only small spiders were seen at flowers during numerous nocturnal and diurnal censuses, but pollinia were found on three species of geometrid moths and one mosquito species (*Aedes vexans*) (see Thien, 1969). Pollinia were twice as likely to be removed at night as during the day, based on dawn and dusk flower censuses in 2001 and 2002 (Wilcoxon Sign Rank test: $P < 0.001$).

Our experimental results on pollinator limitation, autogamy, and inbreeding depression in insect-pollinated plants on Kent Island are summarized in Table 3.

DISCUSSION

The results of this study add to the growing literature on the reproductive biology of plant populations on small islands (Inoue et al., 1996; Larson and Barrett, 1998; Bernardello et al., 2001; Mavraganis et al.

fertilization as reproductive assurance (Baker, 1955). Nonetheless, this brief literature survey suggests that the frequency of autonomous self-fertilization on Kent Island may be higher than in plant species generally and provides additional support for the generalization that the pollination biology of island and mainland plants differs (Allard, 1975; Rathcke, 1988; Barrett, 1996).

We failed to find evidence of inbreeding depression in two species of Ericaceae (*Rhododendron canadense* and *Ledum groenlandicum*) or in two species of Liliaceae (*Smilacina trifolia* and *S. stellata*) [at least through the early stages of development: see Husband and Schemske (1996)], a result that contrasts with some studies of related mainland species (Jaynes, 1968; Rathcke and Real, 1993). Nor was there any indication of inbreeding depression in *Iris versicolor* on Kent Island (or in a mainland population): fruit set and fruit size did not depend on whether pollen came from the same flower, the same clone, the same population, or different populations. Outbreeding depression due to founder effects or local adaptation might have been expected to occur on Kent Island, as it does in certain island plant species even over small spatial scales (Quilichini et al., 2001). Nonetheless, we found no evidence of reduced fruit set in *I. versicolor* in crosses between islands or crosses between Kent Island and the mainland. In fact, we found some evidence that crosses between island and mainland populations resulted in higher seed set than outcrossing within the Kent Island population, which suggests that outcrossed seeds in Kent Island irises may result from matings between close relatives (biparental inbreeding; Uyeonoyama, 1986). These results support the idea that island populations may be founded by few colonists that are able to persist through reproduction by autonomous selfing and/or clonal growth. A third species of Liliaceae (*Maianthemum canadense*), known to be self-incompatible

to different pollinator assemblages, but the comparative study of island and mainland pollination biology is rich with possibilities.

If plants on Kent Island and other isolated habitats differ from mainland populations in terms of self-compatibility, autogamy and inbreeding depression, there are two likely explanations. One explanation is that island plants have evolved in situ in response to strong directional selection driven by a scarcity of effective pollinators or potential mates (Moeller and Geber, 2005). Given the recency of glaciation in northeastern North America and a high frequency of population turnovers on islands, plant species currently on Kent Island may not have been there long enough to have evolved selfing from outcrossing. A more plausible explanation is that genotypes and species capable of self-fertilization have a higher probability of becoming established in isolated habitats like Kent Island than obligate outcrossers. For colonization events involving a single seed, vegetative reproduction or self-fertilization would be a prerequisite for establishment. In this light, isolation would act as a selective filter, with autogamy and self-compatibility being important screens (Baker, 1955; Schueller, 2004).

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