

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*), rates of autonomous selfing were higher on Kent Island than on the mainland. Stamen-excision experiments in *I. versicolor* demonstrated that fruit set required pollen transfer in the absence of pollinators (*i.e.*, agamospermy did not occur). In hand-pollination experiments, five of six species (83%) (*R. canadense*, *L. groenlandicum*, *Smilacina trifolia*, *S. stellata* and *I. versicolor*) 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 before the last glacial period, a time when all plants and animals would have been eliminated from the island. Cooled by the Labrador Current, the island has relatively low species richness and a flora and fauna that are boreal in character, despite the island's temperate latitude and low elevation (less than 50 m at the highest point) (McCain *et al.*, 1973; McCain, 1975; McIlraith, 1986). For example, Swainson's Thrushes (*Catharus ustulatus*) and Blackpoll Warblers (*Dendroica striata*), denizens of montane and boreal forests in eastern North America, breed at sea level in Kent Island's dense, stunted spruce-fir forests, as do several carabid beetle species that are boreal specialists (Apigian and Wheelwright, 2000).

Study species.—Of the 260 species of vascular plants recorded on Kent Island, about 140 species have floral morphologies suggestive of pollination by insects (white, yellow, pink or blue petals or sepals; nectar production; stamens not excessively exerted). We selected 18 of the most abundant and conspicuous species that appeared to be insect-pollinated and that

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

TABLE 1.—Mean % fruit set (fruits per flower \times 100) among insect-pollinated plant species on Kent Island, New Brunswick. Except where noted otherwise, pollinator exclusion experiments were conducted in 1993. Control = unmanipulated open-pollinated flowers. Ratio of Autogamous to Natural Fruit Set = (fruit set for flowers from which pollinators were excluded)/(fruit set for open-pollinated flowers). Differences in fruit set between pollinator-exclusion treatment and control tested by Mann-Whitney U tests (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$)

Species	Control			Pollinators excluded			Ratio of autogamous to natural fruit set	P
	Fruit Set (%)	No. flowers	No. plants	Fruit set (%)	No. flowers	No. plants		
LILIACEAE								
<i>Maianthemum canadense</i>								
(1993)	0.0	416	19	0.0	472	20	—	1.00
(2001)	1.5	160	160	—	—	—	—	—
<i>Smilacina stellata</i>								
(1993)	38.5	181	17	0.0	213	19	0	***
(2001)	31.8	80	80	—	—	—	—	—
<i>Smilacina trifolia</i>								
(1993)	91.9	176	19	75.1	160	18	0.82	**
(2001)	18.1	120	120	—	—	—	—	—
IRIDACEAE								
<i>Sisyrinchium montanum</i>	84.0	71	20	87.2	72	20	1.04	0.75
<i>Iris versicolor</i>								
(1993)	70.4	41	41	36.8	35	35	0.52	0.08
(1998)	45.8	59	59	14.7	34	34	0.32	**
(2003)	72.7	33	33	—	—	—	—	—
SCROPHULARIACEAE								
<i>Rhinanthus crista-galli</i>	78.8	132	16	99.2	111	20	1.26	***
OXALIDACEAE								
<i>Oxalis montana</i>	15.0	20	20	0.0	20	20	0	0.10
ROSACEAE								
<i>Rubus hispidus</i>	74.0	155	17	72.1	172	18	0.97	0.50
ERICACEAE								
<i>Kalmia angustifolia</i>								
(1993)	88.3	85	5	65.0	104	5	0.74	0.07
(1994)	95.6	888	20	88.9	965	19	0.93	**
<i>Rhododendron canadense</i>								
(1993)	79.1	21	21	53.8	18	18	0.68	***
(1994)	86.7	240	53	49.0	86	19	0.57	***
<i>Ledum groenlandicum</i>								
(1993)	82.9	19	19	51.2	20	20	0.44	***
(1994)	83.2	554	23	50.9	482	20	0.61	***
<i>Vaccinium angustifolium</i>	53.9	166	17	23.4	130	19	0.43	**

TABLE 1.—Continued

Species	Control			Pollinators excluded			Ratio of autogamous to natural fruit set	P
	Fruit Set (%)	No. flowers	No. plants	Fruit set (%)	No. flowers	No. plants		
PRIMULACEAE								
<i>Trientalis borealis</i>	88.2	20	20	0.0	20	20	0	***
RANUNCULACEAE								
<i>Ranunculus acris</i>	91.2	81	16	78.5	106	18	0.86	0.13
CORNACEAE								
<i>Cornus canadensis</i>	14.6	545	14	0.0	571	19	0	***
ASTERACEAE								
<i>Chrysanthemum leucanthemum</i>	100.0 (0)	10	10	100.0 (0)	10	10	1.00	1.00
ONAGRACEAE								
<i>Epilobium angustifolium</i>	97.2	102	10	91.3	127	10	0.94	0.06
ORCHIDACEAE								
<i>Habenaria obtusata</i>	24.5	96	13	—	—	—	—	—

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 flowers were neither hand-pollinated nor bagged at any stage. In *Ledum groenlandicum*, we included a fifth treatment, the "augmented" treatment, to test for pollinator limitation. On these inflorescences, flowers were allowed to be open-pollinated and were never bagged, but they were hand-outcrossed in addition.

Hand-pollinations were performed on the first day during which most or all flowers in an inflorescence were open and their stigmas mature, as evidenced by their moist surfaces and distinct coloration. Flowers that had already opened and were no longer receptive were removed from experimental inflorescences. Following hand-pollination, flowers were immediately enclosed again (except in the augmented treatment) until we removed the bags in late July. In late September we determined % fruit set, and weighed and measured the length of mature fruits to 0.1 mm, averaging them within individual plants for each treatment. For a random subsample of fruits from 20 inflorescences (representing each of

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, or number of seeds per fruit.

Inbreeding depression in the Liliaceae.—We conducted hand-pollination experiments on three species in the family Liliaceae, *Smilacina trifolia* (three-leaved Solomon's seal), *S. stellata* (star-flowered Solomon's seal) and *Maianthemum canadense* (Canada mayflower) in 1995 and 2001. Replicate clones (genets) located 20–500 m apart were selected for each species (*S. trifolia*: N = 3 clones; *S. stellata*: N = 2; *M. canadense*: N = 4). Although the discreet boundaries, distinctive leaf and flower morphologies and uniform phenologies of sample plants allowed us to distinguish between clones, we did not test for genetic differences between plants and, therefore, could not rule out the possibility that a single "clone" was actually made up of several genotypes. Within clones, each of 20–35 plants (ramets) was randomly assigned to one of four treatments. In the "hand-selfed" treatment, plants were hand-pollinated using pollen from another plant from the same clone. In the "hand-outcrossed" treatment plants were hand-pollinated using pollen from a different clone located at least 50 m away. We performed hand-pollinations within one day of flowers opening, as soon as the stigma appeared moist. There were two controls. The first control was designed to evaluate the effect of physically contacting stigmas in the hand-pollination treatments; control plants were brushed lightly by a leaf but otherwise were allowed to be open-pollinated and were not hand-pollinated. In the other control, plants were allowed to be open-pollinated and were neither hand-pollinated nor were their stigmas contacted. Because none of these treatments involved bagging flowers to exclude pollinators, all flowers were likely to have received additional pollen from insect visitors, so the hand-pollination treatments in the Liliaceae represented pollen augmentations. Percent fruit set was determined at the end of July.

Because of Kent Island's remoteness and because this and other projects required us to remain on the island in June and July, we did not perform parallel experiments on mainland populations of Liliaceae or other plant species (with the exception of island-mainland comparisons of *Iris versicolor*; see below). Nonetheless, we compared the floral morphology of *S. trifolia* and *S. stellata* on Kent Island with that of populations on the much larger and less isolated island of Grand Manan. Flowers were collected from 5–7 clones from each species on each island. The length of petals and anthers was measured under a 40-power Leitz dissecting microscope to test the prediction that plants with larger pollinator faunas (e.g., on Grand Manan Island) would invest more in showy petals, whereas plants in more isolated habitats (e.g., Kent Island), where they may have to depend more on wind or autogamy for fertilization, would have smaller petals and more exerted anthers (i.e., greater anther:petal length ratios) (Holsinger, 1996; Inoue *et al.*, 1996).

Inbreeding depression, pollinator limitation and autogamy vs. agamospermy in Iris versicolor.—We estimated the effect of different levels of inbreeding in *Iris versicolor* (blueflag iris) with a series of hand-pollination experiments in 1996, 1998 and 2003. In the 1996 experiments, during the bud stage a single flower on each of 120 plants was bagged with bridal veil and assigned randomly to one of four treatments. On the day that the stigmatic surface became exposed, we removed all stamens and hand-pollinated the flower by brushing the stigma

with freshly dehisced stamens (*see* Back *et al.*, 1996 and Zink and Wheelwright, 1997 for descriptions of flower development in *I. versicolor*). Stamens used in the hand-pollinations were collected from one of four sources, depending upon treatment: from the same flower ("hand-selfed within ramets"), from a different plant but the same clone ("hand-selfed within clones"), from a different clone on Kent Island ("hand-outcrossed between clones") or from a different population on a neighboring island (Hay, Sheep or Grand Manan Island) ("hand-outcrossed between populations"). Immediately after hand-pollinations, the flowers were bagged again until the stigmas were no longer receptive. During the summer, herbivores reduced sample sizes from the initial 30 to 19–28 flowers per treatment. In early September, we determined fruit set and measured the length of fruit capsules.

We repeated hand-pollination experiments in 1998 in the Kent Island population and in a mainland population in Blacks Harbour, New Brunswick (45°05'N, 66°80'W). In the Kent Island population, we applied four pollination treatments: control (open-pollinated), hand-selfed within clones, hand-outcrossed between clones within the Kent Island population and hand-outcrossed between populations, using pollen from a mainland population (N = 28–59). In the Blacks Harbour population, we applied two treatments: hand-selfed within clones and hand-outcrossed between clones within the population (N = 31).

To examine the hypothesis that pollinator scarcity on islands has selected for a greater ability to reproduce via autogamy, we compared rates of autonomous selfing between the Kent Island and Blacks Harbour population. For 50 plants in each population, bags were placed around a metal cage (held up by a stake) which enclosed one flower per plant and prevented any contact between floral parts and the bag. Herbivory reduced sample sizes to 34 on Kent Island and 47 in Blacks Harbour. In the Kent Island population, we also assessed the extent to which reproduction was pollinator-limited by excising the anthers in (emasculating) one flower per plant for 47 plants and comparing fruit and seed set to flowers that were hand-outcrossed (*see* above).

Previous pollinator-exclusion experiments had demonstrated high levels of fruit set in *Iris versicolor* and other species (Table 1), so in 2003 we performed an experiment to distinguish between autogamy (requiring pollen transfer within flowers) and agamospermy (production of mature seeds without pollination) (*see* Amsellem *et al.*, 2001). We selected two *I. versicolor* clones separated by 1 km. Within each clone, one flower on each of 20 plants was enclosed in a soil-sample bag at the bud stage. The bags were tied at the base with wire around a strip of cotton to prevent small insects from crawling up the stem. Each flower was randomly assigned to one of four treatments: "anther excision" (emasculating), "stigma excision," "pollinator-exclusion" (flowers bagged but no manipulation of anthers or stigmas) and control (no pollinator-exclusion or excision of anthers or stamens). As soon as the petals of a given flower began to project from the bud (but before the flower had opened), bags were briefly removed and flowers were manually opened to allow excision of the anthers or stigmas using dissecting scissors (*cf.*, Rathcke, 2003). The bags were replaced in the "anther excision" treatment but not in the "stigma excision" treatment. Fly larvae destroyed 13% of *I. versicolor* flowers early in the experiment, so final sample sizes were less than 40 per treatment.

Pollinator-limitation in Habenaria obtusata.—In our final set of experiments (1993, 1996, 2002), we tested whether fruit set in *Habenaria obtusata* (one-leaf rein-orchid, Orchidaceae) was limited by the availability or behavior of pollinators on Kent Island. *H. obtusata* was chosen as a study species because its floral morphology indicated a much more specialized pollination biology than other insect-pollinated species on the island (Thien, 1969). Over a 2 wk period during July, all the flowers on 20 randomly selected plants (N = 160 flowers) from two different sites were hand-pollinated two days after opening, using as a pollen

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 pollinators had been excluded were significantly lower than from control, hand-outcrossed or hand-selfed flowers (Fig. 1, Table 2). The mean length of fruits from pollinator-exclusion flowers was also significantly less than that of hand-selfed and hand-outcrossed flowers, although not that of control flowers (Table 2).

Hand-pollination experiments demonstrated that *Rhododendron canadense* was self-compatible and showed no evidence of inbreeding depression, at least as expressed during the early stages of fruit development. Hand-selfed flowers set fruit at the same rate, produced fruits of the same length, and developed as many seeds per fruit as hand-outcrossed flowers (Fig. 1, Table 2). Seed production per flower (mean number of seeds per fruit \times number of fruits per flower) averaged 177.1 for hand-selfed plants and 140.2 for

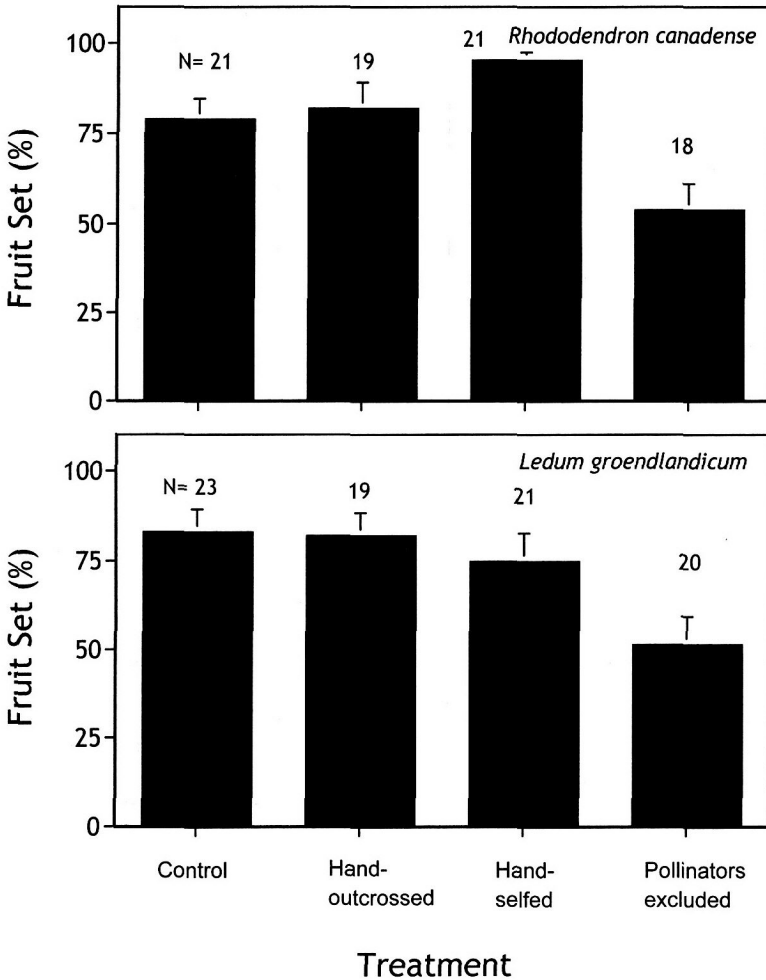


FIG. 1.—Mean % fruit set (mature fruits per flower) in *Rhododendron canadense* and *Ledum groenlandicum* (Ericaceae) inflorescences in which flowers were open-pollinated (control), hand-outcrossed or hand-selfed or had pollinators excluded. In the pollinator-exclusion and both hand-pollination treatments, flowers were bagged until stigmas were no longer receptive (see Methods). Sample sizes above bars refer to number of inflorescences per treatment; error bars represent ± 1 SE. Fisher PLSD tests showed significant differences in fruit set between the pollinator exclusion treatment and the other three treatments in both species, and between the hand-selfed treatment and control in *R. canadense*.

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,

TABLE 2.—Number of seeds per fruit and fruit length from *Rhododendron canadense* and *Ledum groenlandicum* flowers that were open-pollinated (control), hand-outcrossed, or hand-self-pollinated or from which pollinators were excluded. Data are presented as means (± 1 SD). For both species, the source of pollen in hand-pollination experiments (outcross vs. self) had no effect on the number of seeds per fruit or fruit length (no significant difference between treatments indicated by values sharing the same letter superscripts, based on Fisher PLSD tests) (see Fig. 1)

Treatment	Seeds (sd) per fruit	No. plants	Fruit (sd) length (mm)	No. plants
A. <i>Rhododendron canadense</i>				
Control	161.1 ^a (73.7)	18	10.6 ^{a,c} (1.5)	19
Hand-outcrossed	171.6 ^a (43.7)	18	11.0 ^a (1.3)	18
Hand-selfed	186.4 ^a (39.9)	20	11.4 ^a (1.2)	21
Pollinator-exclusion	114.8 ^b (71.4)	15	9.8 ^c (2.1)	15
B. <i>Ledum groenlandicum</i>				
Control	57.0 ^a (24.2)	20	4.6 ^a (0.5)	20
Hand-outcrossed	49.6 ^a (31.0)	18	4.5 ^a (0.6)	18
Hand-selfed	48.7 ^a (31.9)	19	4.3 ^a (0.6)	19
Pollinator-exclusion	22.2 ^b (15.0)	17	3.8 ^b (0.6)	17

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).

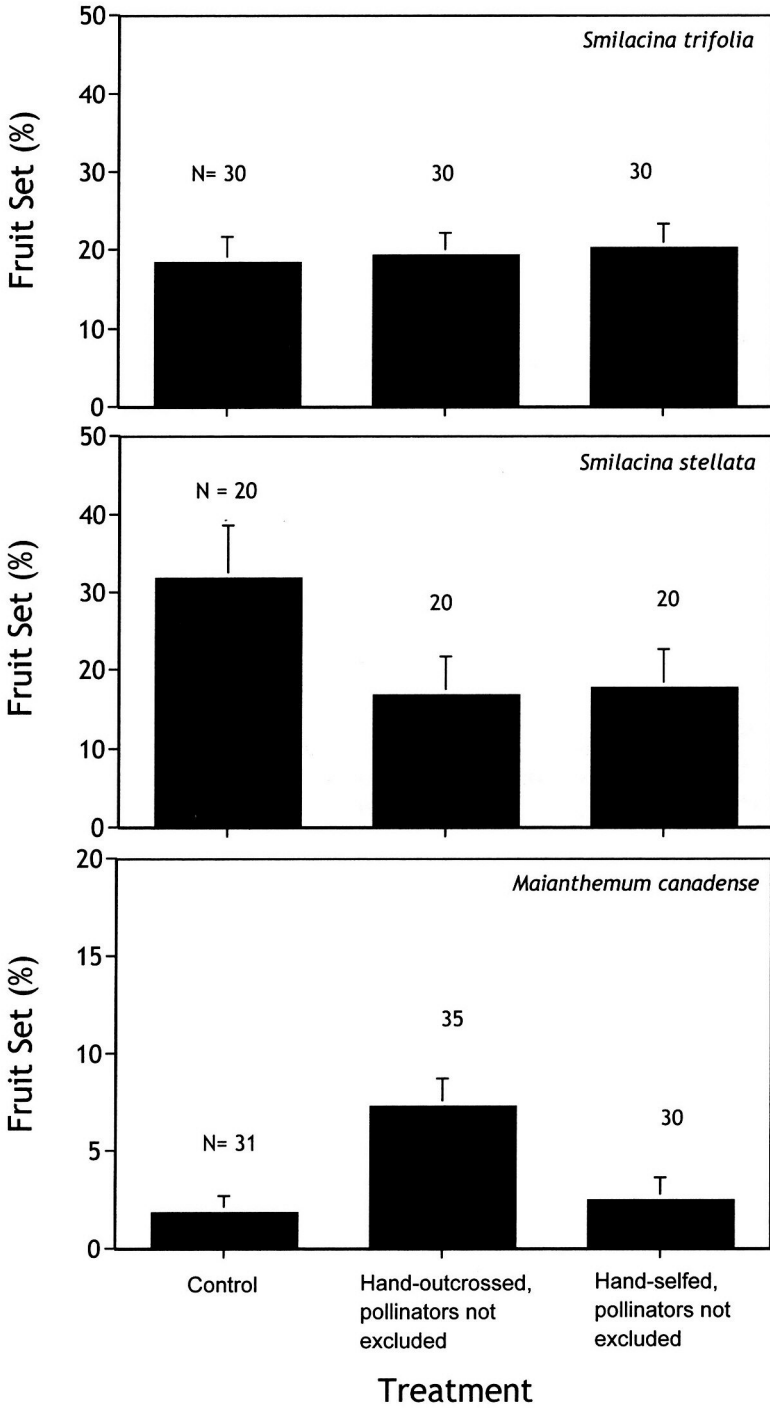
Ledum groenlandicum also showed no indication of inbreeding depression in terms of diminished fruit set or seed production. Hand-selfed flowers set fruit at a rate equivalent to that of hand-outcrossed flowers, and the fruits of both treatments were similar in length and number of seeds per fruit (Fig. 1, Table 2). Mean seed production per flower was 36.5 for hand-selfed plants and 40.7 for hand-outcrossed plants. There were no apparent differences in terms of size, shape or color of the seeds of hand-selfed versus hand-outcrossed fruits.

There was some evidence of pollinator limitation in *Ledum groenlandicum*. Augmented flowers set significantly more fruits than control (open-pollinated) flowers ($98.3 \pm 4.1\%$ vs. $82.0 \pm 25.6\%$; Mann-Whitney U test: $P = 0.04$). Their mean fruit length (4.4 ± 0.8 mm) and number of seeds per fruit (58.5 ± 40.1 , $N = 6$) were similar to controls (Table 2, $P > 0.41$).

Inbreeding depression in the Liliaceae.—Two of the three species of Liliaceae showed no evidence of inbreeding depression in terms of fruit set. There was no difference in fruit set between control (open-pollinated), hand-outcrossed and hand-selfed plants of *Smilacina trifolia* or *S. stellata* in experiments conducted in 2001 (Fig. 2; one-way ANOVAs: $P > 0.11$).

FIG. 2.—Mean % fruit set (mature fruits per flower) in *Smilacina trifolia*, *S. stellata* and *Maianthemum canadense* (Liliaceae) inflorescences in which flowers were open-pollinated (control), hand-outcrossed, or hand-selfed. None of the plants were bagged so the hand-pollination treatments represented pollen augmentation (see Methods). Sample sizes above bars refer to number of inflorescences per treatment; error bars represent ± 1 SE. Note differences in y-axis scale. Fruit set was independent of treatment in *Smilacina trifolia* and *S. stellata* but higher in hand-outcrossed inflorescences in *M. canadense*

→



In *S. trifolia*, fruit set was generally low and never exceeded 26% in any treatment or site [however, note high fruit set of open-pollinated *S. trifolia* flowers in 1993 (Table 1)]. In *S. stellata*, fruit set was intermediate in all treatments in one site (33–58%) but low in the other site (0–6%). Two-way ANOVAs showed significant site effects in both species ($P < 0.006$), no effect of treatment in *S. trifolia* ($P = 0.90$), but a significant overall effect of treatment in *S. stellata* ($P = 0.002$), although there was no difference in fruit set between hand-outcrossed and hand-selfed treatments (Fisher PLSD test: $P < 0.05$). Similar rates of fruit set between contact controls and open-pollinated controls (Fisher PLSD tests: $P > 0.35$) suggested that physically manipulating the stigma in the hand-pollination treatments had no negative effect.

Fruit set was especially low in the third species of Liliaceae, *Maianthemum canadense* (Fig. 2). Unlike the other two species of Liliaceae, in all four sites hand-outcrossed plants had higher fruit set than either hand-selfed or control plants (two-way ANOVA: site: $P = 0.001$; treatment: $P = 0.009$), suggesting that the species is prone to inbreeding depression and pollinator-limited on Kent Island.

Neither the length of anthers or petals nor anther:petal ratios differed between *Smilacina trifolia* populations on Kent Island and Grand Manan Island (Mann-Whitney U tests: $P > 0.24$), contrary to prediction. In *S. stellata*, the mean length of both anthers and petals was greater on Kent Island than on Grand Manan ($P < 0.007$), but there was no difference in anther:petal ratios between sites ($P = 0.14$).

Inbreeding depression, pollinator limitation and autogamy vs. agamospermy in Iris versicolor.—Hand-pollination experiments in 1996 revealed no inbreeding depression in *Iris versicolor*, at least as expressed by reduced fruit set, fruit size, or number of seeds per fruit. The probability of fruit set was independent of the source of pollen, whether from the same ramet, a different ramet but same clone, a different clone but same population, or a different population (Fig. 3; χ^2 test: $P = 0.57$). Likewise, there was no effect of pollen source on the size of mature fruits (length or width), the number of mature seeds per fruit, or the number of aborted seeds (ANOVAs: $P > 0.45$). Hand-pollination experiments in 1998 also found no evidence of inbreeding depression on Kent Island or in the mainland population: hand-selfed and hand-outcrossed flowers had an equal probability of setting fruits (χ^2 tests: Kent Island: $P = 0.72$; Blacks Harbour: $P = 0.90$) and did not differ significantly in seed set (t-tests: Kent Island: $P = 0.78$; Blacks Harbour: $P = 0.59$). There was a tendency for flowers that received pollen from mainland plants to produce more fruits and seeds than plants outcrossed to other clones within the Kent Island population, but differences were not statistically significant (% fruit set: χ^2 test: $P = 0.15$; number of seeds per fruit: t-test: $P = 0.07$). Of the flowers that produced fruits, however, we found significantly greater seed set in the island-mainland crosses than in the crosses between clones on Kent Island (t-test: $P = 0.006$).

Rates of autonomous selfing were significantly higher on Kent Island than in the mainland population (island vs. mainland % fruit set: 14.7% vs. 2.1%; χ^2 test: $P = 0.03$). We also found evidence for pollinator limitation of reproduction in Kent Island irises, suggesting that autonomous self-pollination may assure reproduction when pollinators are scarce. Compared to emasculated plants, hand-outcrossed plants had a significantly higher likelihood of setting fruits and produced more seeds per fruit (hand-outcrossed vs. emasculated % fruit set: 70.7% vs. 42.6%; χ^2 test: $P = 0.008$).

The capacity of most of the species we studied to set fruit even when insect pollinators were excluded (see *Pollinator-exclusion experiments and autogamy*, above) raised the question of whether seeds would develop without pollination via agamospermy, or whether pollen had to be transferred to stigmas by some means (e.g., wind or direct physical contact between the

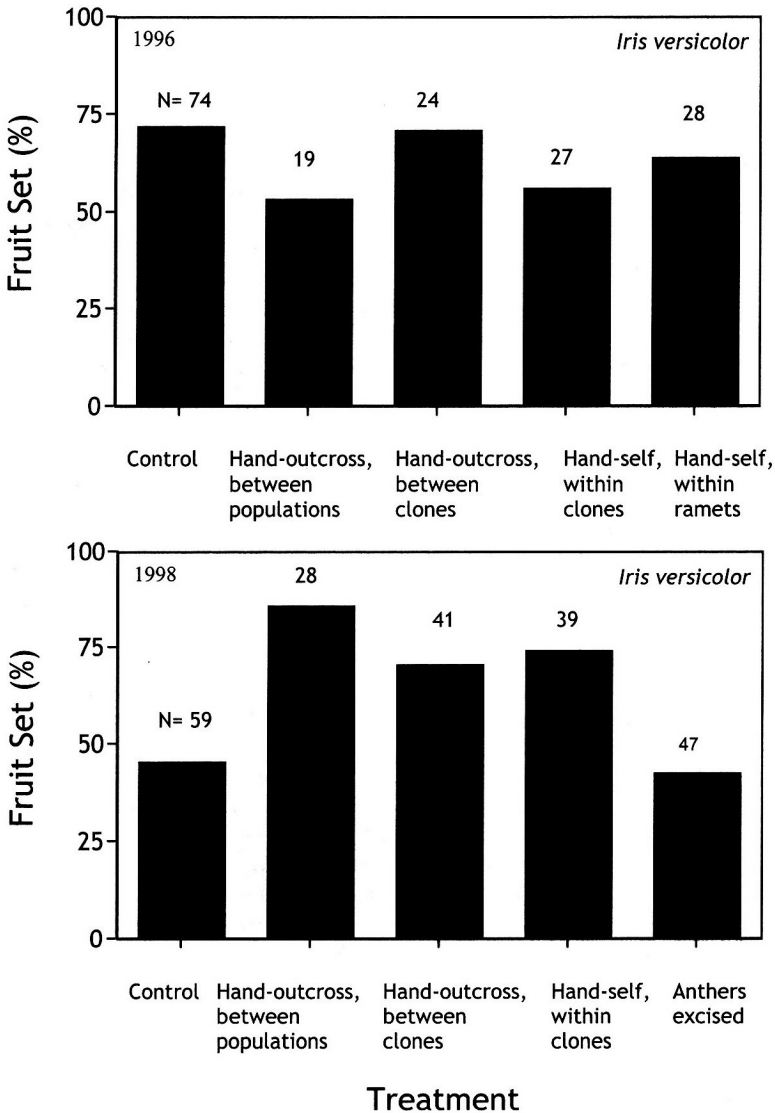


FIG. 3.—Mean % fruit set (mature fruits per flower) in *Iris versicolor* flowers on Kent Island that were open-pollinated (control) or hand-pollinated with pollen from different sources representing increasing degrees of inbreeding (outcrossing: between populations or clones; selfing: within clones or ramets; see Methods). In the experiments involving hand-outcrossing between populations, the pollen sources were flowers from nearby islands in the Grand Manan Archipelago in 1996, and flowers from the mainland (Blacks Harbour, New Brunswick) in 1998. Individual flowers were bagged in all treatments except controls and the anther-excision treatment. Sample sizes above bars refer to number of flowers per treatment; error bars represent ± 1 SE. Fruit set was independent of treatment in both years

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 effectiveness of pollen transfer by different species. Although their relative importance as pollinators is unknown, the general diversity of flower-visiting insects on Kent Island is better understood based on systematic collecting efforts during several summers (more than 1000 specimens identified to genus or species in the reference collection at the Bowdoin Scientific Station). The flower-visiting Diptera and butterflies are particularly well-sampled on Kent Island. As an indication of the reduced insect species richness on islands, only 25 species of flower flies (Syrphidae) are known to occur on Kent Island, compared to nearly 10 times that number on Mt. Desert Island, a much larger island located adjacent to the mainland in Maine at about the same latitude (C. Thompson, pers. comm.). Altogether, only about 60 species of flies have been identified so far on Kent Island. With just 20 butterfly species recorded on Kent Island [Maddox and Cannell (1982), pers. obs.], the island has fewer than 20% of the butterfly species that occur in Maine.

Although each of the 17 plant species we examined for autonomous self-pollination differed slightly in the relative abundance of the insect species they attracted, there was

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.*, 2001; Leimu, 2004). In particular they help to fill a gap in our understanding of the pollination biology of plants on boreal coastal islands. We found pollinator limitation to be common among the species of insect-pollinated plants studied on Kent Island (*cf.*, Burd, 1994; Rathcke, 2003). In five of the species in which we experimentally increased pollen loads (*Ledum groenlandicum*, *Rhododendron canadense*, *Maianthemum canadense*, *Iris versicolor* and *Habenaria obtusata*), fruit set was increased compared to open-pollinated controls, although we failed to find pollinator limitation in two other species (*Smilacina stellata* and *S. trifolia*) (Table 3) or in *Iris versicolor* in one set of experiments (1996; Fig. 3). We did not determine whether pollinator limitation on Kent Island results from a depauperate insect fauna (e.g., an island biogeographic effect), their inefficiency as pollinators, or some aspect of the island's physical environment (e.g., cool temperatures and frequent fog, which could influence the behavior of potential pollinators or explain the scarcity of such taxa as bees). We were also unable to assess pollinator limitation over the course of a plant's entire lifetime. Some species, particularly slow-growing perennials with underground storage organs such as the orchid *H. obtusata*, may appear pollinator-limited in one reproductive season while effectively being resource-limited over the course of several seasons (Ackerman and Montalvo, 1990) (S. Sipes, pers. comm.).

More than two-thirds of early-flowering, insect-pollinated plant species on Kent Island were not only self-compatible but were capable of setting fruit in the absence of flower visitors (although in two species, *Rhododendron canadense* and *Ledum groenlandicum*, autonomous self-fertilization resulted in fewer seeds per fruit compared to open-pollinated flowers). In the few mainland populations of the same and closely related species that have been studied, autogamy seems to be less common than on Kent Island. In our studies, autonomous self-pollination was much higher in *Iris versicolor* on Kent Island (15–37%) compared to a mainland population observed simultaneously (2%). Rates of autonomous selfing were also low in two populations in Ontario (7%) even though the mainland populations were self-compatible (Kron *et al.*, 1993; Back *et al.*, 1996).

Species in the family Ericaceae provide an additional example of island-mainland differences. In Virginia, *Kalmia angustifolia* was found to be autogamous, but fruit set was only one-sixth as high as on Kent Island [Nagy *et al.*, 1999; a separate study, however, found levels of autogamy in Virginia *K. angustifolia* similar to those on Kent Island (Rathcke,

TABLE 3.—Summary of experimental results on Kent Island insect-pollinated plants. Dashes indicate no information

Species	Natural fruit set	Pollinator limitation?	Autogamy?	Inbreeding depression?	Observations
<i>Chrysanthemum leucanthemum</i>	high	—	yes	—	introduced species
<i>Cornus canadensis</i>	low	—	no	—	flowers only in full sun
<i>Epilobium angustifolium</i>	high	—	yes	—	
<i>Habenaria obtusata</i>	low	yes	—		apparently pollinated by mosquitoes and geometrid moths; 2/3 pollinia removed at night
<i>Iris versicolor</i>	high	yes	yes	no	changes in stigma position to facilitate self-pollination when pollinators excluded; seed set requires pollen transfer (no agamospermy)
<i>Kalmia angustifolia</i>	high	—	yes	—	
<i>Ledum groenlandicum</i>	high	yes	yes	no	fewer seeds per fruit via autogamy
<i>Maianthemum canadense</i>	very low	yes	no	yes	flowers only in full sun; very low natural fruit set; reproduces mainly vegetatively
<i>Oxalis montana</i>	low	—	no	—	
<i>Ranunculus acris</i>	high	—	yes	—	
<i>Rhinanthus crista-galli</i>	high	—	yes	—	introduced species
<i>Rhododendron canadense</i>	high	yes	yes	no	fewer seeds per fruit via autogamy
<i>Rubus hispidus</i>	high	—	yes	—	
<i>Sisyrinchium montanum</i>	high	—	yes	—	
<i>Smilacina stellata</i>	intermediate	no	no	no	longer petals, anthers on Kent Island than Grand Manan
<i>Smilacina trifolia</i>	intermediate, variable	no	yes	no	no difference in length of petals, anthers on Kent Island and Grand Manan
<i>Trientalis borealis</i>	high	—	no	—	
<i>Vaccinium angustifolium</i>	intermediate	—	yes	—	

1988)]. A congener, *K. latifolia*, exhibited only 17% autogamous fruit set in Virginia and just 1% in Rhode Island (Rathcke, 1988; Rathcke and Real, 1993). In four other ericaceous species in Rhode Island, including *Rhododendron viscosum* and *Vaccinium cassinoides* (whose congeners on Kent Island exhibited 23–54% autogamous fruit set), pollinators were required for fruit set (Rathcke, 1988). In contrast, on Kent Island, the mean fruit set for four species of Ericaceae was 51% when pollinators were excluded. Without additional simultaneous experimental studies of island and mainland populations of the same species, controlling for differences in climate and physical environment, it is still an open question whether the pollination biology of plants on boreal islands is distinctive and favors self-

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 and pollinator-limited on the mainland (Worthen and Stiles, 1988; Ganger, 1997), apparently was not capable of autonomous self-pollination on Kent Island and did show evidence of inbreeding depression. Even with hand-outcrossing, however, fruit set never exceeded 12% in *M. canadense* at any site, and in control plants it averaged less than 2%, which raises the possibility of biparental inbreeding or extreme resource limitation in this species. Collectively, our results are consistent with the idea that inbreeding depression may be reduced in isolated habitats through founder effects or the purging of deleterious alleles (Lande and Schemske, 1985).

Islands differ from mainland habitats in numerous respects other than size and isolation, and it may be that differences in solar radiation, temperature, salt spray or other environmental factors may also shape plant breeding systems and the behavior of insect pollinators. Distinguishing between physiological responses (*e.g.*, low levels of insect pollination or more frequent autogamy due to cool temperatures) and evolutionary responses (*e.g.*, more frequent autogamy due to founder effects or local adaptation) will require common garden experiments. Ideally, plants of some of the species in this study could be grown from seeds collected on the mainland and on Kent Island, and their pollination biology investigated in both habitats. Another question that remains to be explored is whether the flora of small islands that are near the mainland is distinctive. Specifically, are the flowers of such islands more likely to be small, unspecialized or long-lived in order to take advantage of visits by generalist pollinators? Do they invest less in reproductive structures that are designed to attract pollinators (*e.g.*, nectar, petals) or do they have lower pollen-ovule ratios (Carlquist, 1974; Spears, 1987; Inoue *et al.*, 1996; Schueller, 2004)? Preliminary comparisons of the length of anthers and petals between *Smilacina stellata* and *S. trifolia* populations on Kent Island and the larger and less isolated island of Grand Manan lend little support so far for morphological divergence in response

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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