

## Contribution of Delayed Autonomous Selfing to Reproductive Success in Mountain Laurel, *Kalmia latifolia* (Ericaceae)

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**ABSTRACT.**—The release of anthers held under tension which catapult pollen onto the stigma near the end of floral life has been proposed as a mechanism for autonomous self-fertilization in normally outcrossing *Kalmia latifolia*. We examined the importance of this delayed autonomous self-pollination to fruit production and the efficiency of the pollen deposition mechanism under field conditions. By comparing intact open-pollinated flowers with emasculated flowers we found that autonomous self-pollination did not increase fruit production. To evaluate the efficiency of the catapult mechanism of selfing, we compared the relative fruit production of flowers that were hand-pollinated with self-pollen with those that self-pollinated spontaneously. Fruit set was higher in hand-selfed flowers than in the autonomously-selfed flowers, indicating that the natural mechanism of selfing is inefficient. Through the observation of flowers protected from pollinators we determined the natural pattern of floral phenology and anther release. The effect of timing of autonomous selfing on fruit production was assessed by manipulating the time of anther release. The timing of selfing is not related to fruit production and varies widely among flowers. Although autonomous selfing did not contribute to fruit production under natural conditions, it can assure reproduction in the absence of pollinators.

### INTRODUCTION

The incidence of self-pollination in primarily outcrossing plants has been documented in a variety of taxa (Levin, 1972; Schoen, 1982; Richards, 1986; Lloyd and Schoen, 1992; Klips and Snow, 1997). Autonomous selfing may be advantageous in outcrossing species if among-flower pollination is limited since it assures some reproduction (Wyatt, 1983). “Autonomous self-pollination” is defined as that which occurs spontaneously, without an external agent (Lloyd, 1979; Lloyd and Schoen, 1992). Lloyd (1979) proposed three modes of autonomous selfing within a flower: “prior,” “competing” and “delayed,” depending on the relative timing of selfing and outcrossing. Prior selfing occurs before there are opportunities for outcrossing; competing selfing involves competition for ovules between co-occurring self- and outcross-pollen. Both of these modes can lower the potential for outcrossing. Delayed self-fertilization occurs when the opportunity for outcrossing is past and is always considered advantageous (Lloyd, 1980). Although the potential negative consequences of selfing (*e.g.*, decrease in genetic variation, reduced seed production, reduced progeny vigor) have been observed in genera which normally outcross (Charlesworth and Charlesworth, 1987; Dole and Ritland, 1993; references therein), self pollen may contribute to a plant’s reproductive fitness by fertilizing ovules not fertilized by limited outcross pollen (Wyatt, 1983; Klips and Snow, 1997). For example, Solbrig and Rollins (1977) concluded

that self-fertilizing *Leavenworthia* flowers had a 3:2 reproductive advantage over flowers from populations that outcrossed but did not self.

The morphology of *Kalmia latifolia* flowers reflects specialization for bumblebee pollination. The anthers are inserted into invaginations in the corolla and held reflexed under tension until they are triggered by a visiting insect pollinator or released spontaneously toward the end of floral life (Rathcke and Real, 1993). Upon release, the anthers spring inward, throwing pollen onto the pollinator's body or, if no pollinator is present, onto the stigma resulting in self-pollination.

Although *Kalmia latifolia* is traditionally considered an outcrossing species, the frequency of delayed autonomous self-pollination ("selfing") has been shown to vary among populations. Flowers from populations in Rhode Island do not self, whereas 17% of flowers in Virginia populations spontaneously self-fertilize in the absence of pollinators (Rathcke and Real, 1993). At the two sites hand pollination with self-pollen resulted in similar levels of fruit production, suggesting that differences in fruit production are due to differences in the quantity of self-pollen reaching the stigma, not to differences in self-incompatibility or fruit abortion (Rathcke and Real, 1993).

Automatic self-pollination may be selected for environments where pollinator visitation is limited. Piper *et al.* (1986) suggested that inconsistent pollinator activity may result in a higher relative fertility for selfers as compared to outcrossers (*see also* Wyatt, 1983). Natural selection may therefore favor selfing over outcrossing under some conditions. For the Virginia population of *Kalmia latifolia*, competition for pollinators with the simultaneously flowering shrub *Vaccinium erythrocarpum* (Ericaceae) may limit pollinator visitation (Real and Rathcke, 1991; Rathcke and Real, 1993). *Kalmia latifolia* flowers are thought to depend exclusively on bumblebees for pollination, therefore limited pollinator visits may reduce reproductive success. Selfing, therefore, would assure *K. latifolia* reproduction.

Although reproductive assurance has been implicated as a primary factor leading to selection for delayed autonomous selfing, few studies have quantitatively measured its impact on plant fitness (Schoen and Lloyd, 1992). We tested the hypothesis raised by Rathcke and Real (1993) that selfing increases reproductive success in a Virginia population of *Kalmia latifolia*. We also investigated the proposed mechanism of autonomy. We addressed three questions: (1) Does delayed autonomous selfing increase fruit production? (2) Is the release of anthers an effective mechanism for self-pollination? and (3) Does the time of anther release affect fruit production?

#### METHODS

*Study species and sites.*—The two study populations of mountain laurel (*Kalmia latifolia* L., Ericaceae) are located on Salt Pond Mountain in the Southern Appalachian Mountains of Giles County, Virginia (37°22'N, 80°31'W). One population is located in the understory of a shaded deciduous forest ("Moonshine Dell," elevation 1162 m); the other is on a treeless and windswept rock outcropping ("Bald Knob," elevation 1330 m). The sites are separated by 1.6 km. Experiments were conducted just before and during peak flowering in June and July of 1996.

*Floral phenology.*—To determine the natural pattern of anther release, 5 flowers from single inflorescences on 18 plants at Moonshine Dell were labeled and observed daily for the number of stamens released of the 10 possible. Inflorescences were bagged with a fine-mesh netting to exclude pollinators. We also measured floral longevity, defined as the time between anthesis and when the corolla either fell off or became withered and brown.

*Contribution of selfing to fitness.*—The contribution of selfing to reproductive success was measured by comparing the fruit production of naturally pollinated, emasculated flowers

with intact flowers. In the emasculation treatment the anthers were removed from flowers when still in bud to ensure that fruit production would be the result of pollinator activity. To make sure emasculation did not negatively affect reproductive potential, 9 emasculated flowers were augmented with outcross pollen; 78% of these flowers produced fruit. This figure is comparable to the fruit set for cross pollinations of unmanipulated flowers in other studies (80%, Rathcke and Real, 1993) and is higher than emasculated flowers that were not augmented in this study. There was no fruit production on 5 bagged and emasculated flowers. Emasculations were performed on 10 flowers per plant, each on a different inflorescence. On the same inflorescences, 10 unmanipulated flowers at a similar phenological stage were labeled as controls. This experiment sampled 10 haphazardly chosen plants at Moonshine Dell and 12 at Bald Knob, a total of 220 pairs of flowers. Counting seeds was not practical due to their small size, so fruit production was measured and used as a fitness estimate (Rathcke and Real, 1993). The proportion of flowers that produced fruit per plant was analyzed, making the plant the experimental unit. Fruit production was arcsin transformed and analyzed using a two-factor fixed effects ANOVA with site and pollination treatment as main effects (GLM procedure, SAS 1988).

*Efficiency of selfing.*—The reproductive success of flowers supplemented with self-pollen was compared to that of autonomous self-pollinated flowers to determine the efficiency of selfing. In both treatments a fine-mesh netting was placed around one inflorescence per plant to exclude pollinators. In the self-pollen supplementation treatment 10, 3–4 d-old flowers within each inflorescence were hand pollinated with self-pollen. Pollen was collected from the anthers of several different flowers on the same plant and placed on a glass slide. The pollen was then transferred to a receptive stigma either directly from the slide or with the use of forceps. By providing the stigmas with pollen this treatment ensured that pollen was available for fertilization. The autonomous selfing treatment consisted of bagged unmanipulated inflorescences on the same plants as the supplementation treatment. Both treatments were applied to 18 haphazardly chosen plants at Moonshine Dell. For each treatment the proportion of flowers that set fruit on each plant was calculated and analyzed using a nonparametric Mann-Whitney test, due to the large number of zeros in the unmanipulated treatment.

To examine the differences in reproductive success of self- and outcross-pollen we compared the fruit production of flowers supplemented with self-pollen (treatment described above) with that of the intact flowers that were naturally pollinated in the first experiment (“contribution of autonomous selfing to fitness”) at Moonshine Dell. Differences in fruit set were analyzed using a two-tailed Student’s *t*-test; data did not require transformation.

*Timing of selfing.*—The effect of timing of selfing on fruit production was studied by experimentally manipulating the time of anther release and observing subsequent fruit production. Three inflorescences on each of the 18 haphazardly chosen plants at Moonshine Dell used above were bagged, and each inflorescence received one of three treatments. Two of the treatments involved imitating the proposed mechanism of selfing by hand-releasing the stamens at either 3 or 7 d after anthesis (just before and just after the modal day of anther release, *see* Results). Inflorescences of the third treatment were not manipulated but were observed for time of natural anther release. Ten flowers on each inflorescence were used in the experiment. In the manipulated treatments all 10 stamens were released at once. Proportion of fruit set was calculated for each treatment on each plant. Differences in fruit production among the treatments were analyzed using a nonparametric Kruskal-Wallis test due to the large number of flowers that did not produce fruits.

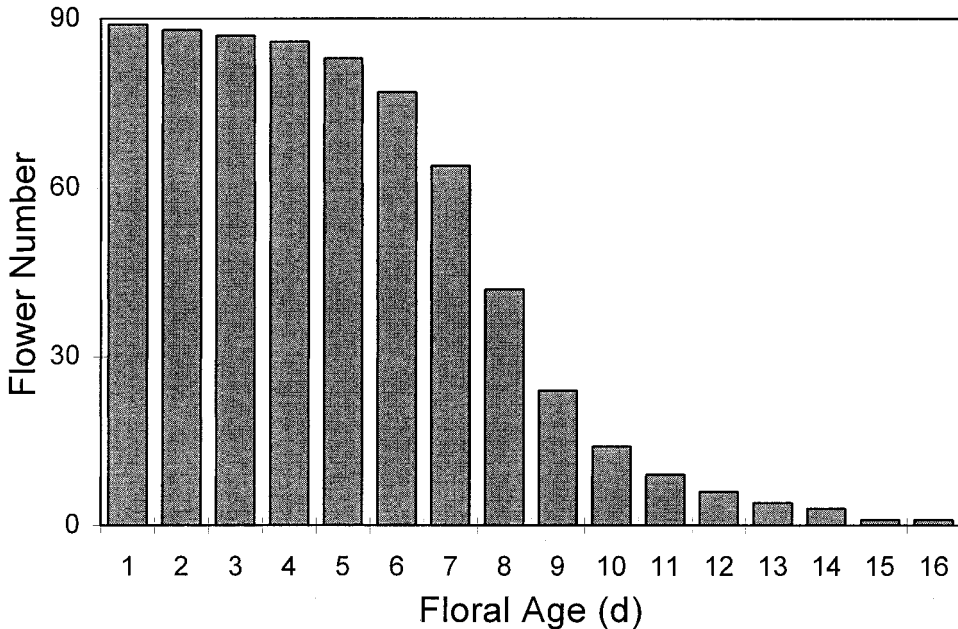


FIG. 1.—Number of flowers “alive” out of 90 vs. d after anthesis (floral age). All flowers were protected from pollinators and removed from the “alive” group when the corolla fell off or became withered and brown

#### RESULTS

*Floral phenology.*—Observations of bagged unmanipulated flowers provided an account of the floral life history in the absence of pollinators. Of the 90 flowers observed, 11 (12%) produced fruit. The floral life-span ranged from 2 to 16 d, with a mean of 7.6 d (Fig. 1). The fifth and sixth days of floral life represent days on which most flowers released their first anthers (17% and 15%, respectively; Fig. 2). By the sixth day of floral life, 67% of the flowers had released at least one anther. The total number of anthers released increased uniformly over time (Fig. 3). The mean number of anthers released was 4.5; however, 12% of flowers did not release any anthers. Of the 11 unmanipulated flowers that produced fruit, 18% did not release any anthers, 18% released all 10, and only 36% released more than 5 anthers throughout floral life (Table 2). Fruit production was not significantly associated with floral life span ( $t = 0.38$ ,  $P = 0.70$ ,  $df = 14$ ), total number of anthers released ( $t = 0.16$ ,  $P = 0.87$ ,  $df = 13$ ) or floral age at which the first anther was released ( $t = 0.016$ ,  $P = 0.98$ ,  $df = 10$ ). Although the corollas fell off at the end of floral life, the age at which this occurred was not consistent. The corolla fell off before the petals became brown and withered in 73% of the 11 unmanipulated flowers that produced fruits, in contrast with 42% of the 78 flowers that did not produce fruits.

*Contribution of selfing to fitness.*—Fruit production in *Kalmia latifolia* at both sites was unaffected by the emasculation treatment. The ANOVA revealed no significant differences in the proportion fruit production of the emasculated (mean  $\pm$  SE,  $0.62 \pm 0.05$ ) and control flowers ( $0.59 \pm 0.06$ ; Table 1). There was a trend for a greater proportion of flowers to

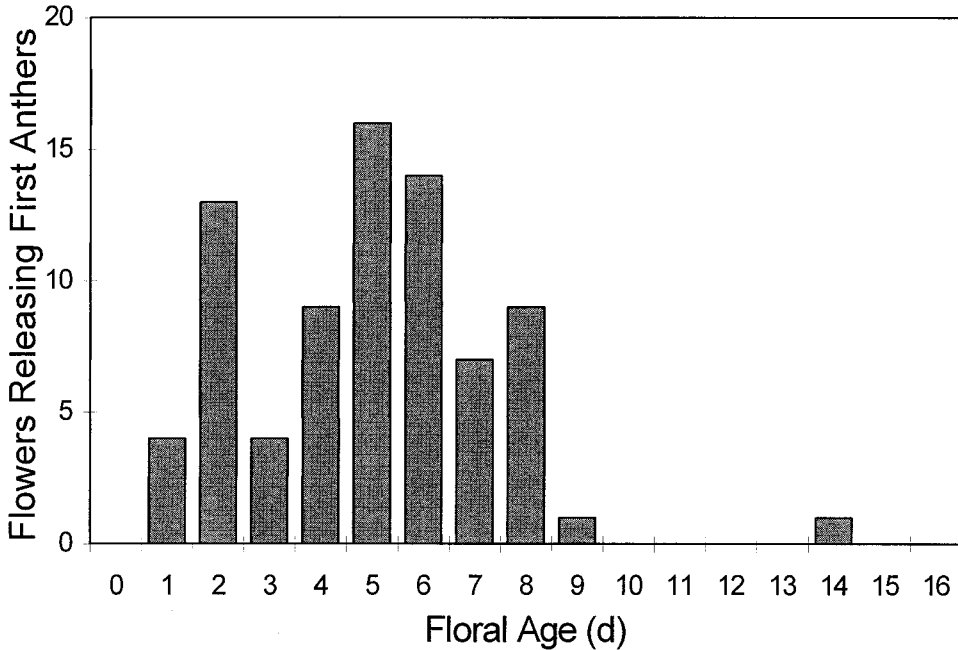


FIG. 2.—Number of flowers spontaneously releasing their first anther by floral age. Flowers were protected from pollinators

produce fruit at Bald Knob ( $0.67 \pm 0.05$ ) than Moonshine Dell ( $0.53 \pm 0.06$ ), possibly indicating higher pollinator activity.

*Efficiency of selfing.*—Fruit set was significantly greater in flowers supplemented with self-pollen (38%) than in those that only had access to their own pollen (11%;  $U = 229$ ,  $P < 0.05$ ). Inflorescences that were hand-pollinated with self-pollen had lower fruit production than naturally pollinated flowers (38% vs. 54%). This difference, however, is not significant ( $t = 1.11$ ,  $P = 0.28$ ,  $df = 20$ ).

*Timing of selfing.*—The floral age at which the anthers are released has little relationship to fruit production. The proportion of flowers producing fruit on control (Mean  $\pm$  SE,  $0.121 \pm 0.040$ ) and experimental inflorescences in which anthers were manually released at 3 d ( $0.062 \pm 0.024$ ) or 7 d ( $0.076 \pm 0.024$ ) after anthesis were not significantly different (Kruskal-Wallis  $H = 8.74$ ,  $P > 0.05$ ,  $df = 2$ ).

#### DISCUSSION

*Contribution of selfing to fitness.*—Autonomous self-pollination did not contribute significantly to fruit production of open pollinated flowers at either site. Klips and Snow (1997) observed a similar pattern in *Hibiscus*. Our results suggest that although there is the potential for selfing in this species, it does not contribute to fruit production under the conditions present in this study. There are two possible explanations. If flowers are pollinator limited, then autonomous selfing may not compensate for the inadequate pollination. Alternatively, pollinators may not be limiting fruit production. Rathcke and Real (1993) and Real and Rathcke (1991) concluded that the plants at Bald Knob were pollinator-limited and that bumblebee visits to *K. latifolia* flowers were relatively infrequent. In con-

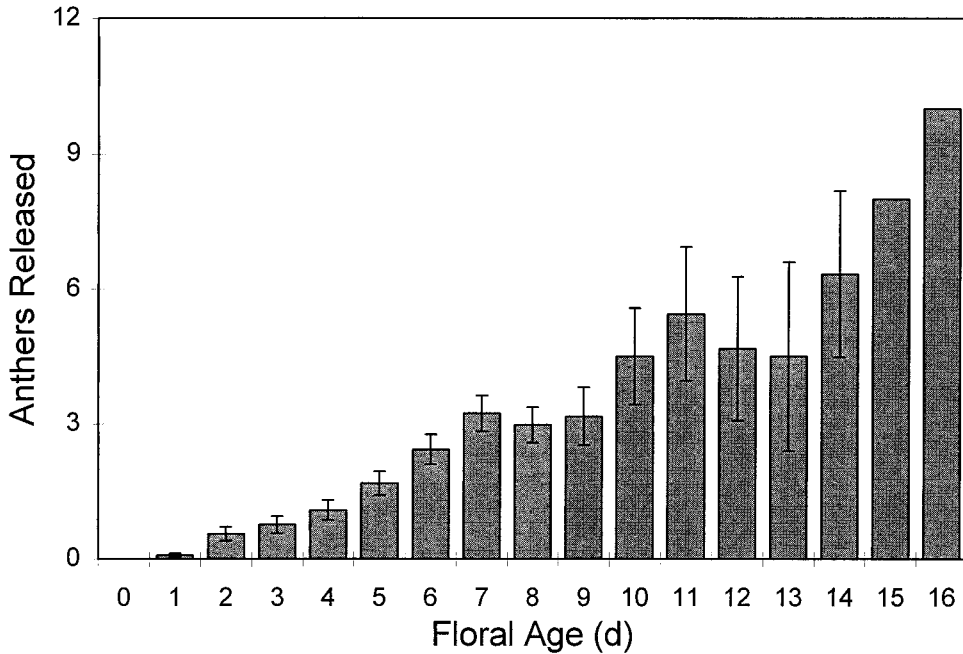


FIG. 3.—Mean ( $\pm$ SE) cumulative anthers released by floral age. Flowers were unmanipulated and protected from pollinators. Sample sizes for each floral age are the number of flowers in Fig. 1

trast, during this study bumblebees were numerous and often seen visiting flowers (LS pers. obs.). In addition, Rathcke and Real (1993) reported a mean fruit set for unmanipulated flowers that was considerably lower than that observed under our 1996 field conditions (37% vs. 63%). However, fruit production following self-pollination was also higher in the present study—suggesting that factors other than pollinator service differed between the years of the two studies.

*Efficiency of selfing.*—The significant increase in the fruit production of the hand-selfed flowers over the bagged, autonomously selfing flowers implies that the reproductive potential of selfing is limited more by pollen quantity than by the pollen quality. Klips and Snow (1997) made a similar conclusion based on their study of *Hibiscus*. If the selfing mechanism effectively provided the stigma with pollen, then fruit production of the two treatments

TABLE 1.—ANOVA on the proportion of fruit produced by emasculated and unmanipulated flowers at two sites. Data are arcsin transformed

Source	df	SS	F	P
Model	3	0.141	1.29	0.292
Site	1	0.377	3.51	0.068
Emasculation	1	0.014	0.13	0.719
Site $\times$ Emasculation	1	0.019	0.18	0.672
Error	40	4.296		

TABLE 2.—Individual anther release observations from bagged, unmanipulated flowers that produced fruit

Total number of anthers released	Floral life (d)	Floral age when first anthers were released (d)
0	6	—
0	9	—
1	10	8
2	8	7
3	7	6
3	7	7
4	7	2
7	8	4
8	4	2
10	9	5
10	6	2

should have been similar. Since the hand self-pollinations were conducted with relatively young pollen (1–2 d younger than the modal age of natural self pollinations) it is possible that they were done with pollen of higher than normal quality. However, while slightly early, the pollination timing was well within the age range of normal anther release. Our finding does not correspond with previous results that found *Kalmia latifolia* flowers augmented with self-pollen displayed fruit sets similar to those of autonomously selfed flowers (Rathcke and Real, 1993).

We were not able to detect effects of the timing of anther release, the number of anthers released or floral longevity on the efficiency of autonomous self-pollination. The average age at which anther release began was 4.7 d, with most flowers (67.4%) releasing anthers within 6 d of anthesis (Fig. 2). Based on these data, the manipulated anther release treatments were performed before and after the natural release time. The lack of difference in fruit production among flowers with different release schedules suggests that the floral age at which the anthers are released does not influence fruit production within the range tested. Furthermore, fruit set by bagged flowers showed that all ten anthers need not be released for fruit production to occur. Neither the number of anthers released, the final age of the flower nor the age at which the first anthers are released affects the likelihood of fruit production in flowers that self-pollinate.

These results indicate the rate of autonomous selfing is not a direct function of spontaneous anther release which does not appear to be a requirement for self-fertilization. Autonomous selfing can occur passively as the corolla is shed. In some other species, flowers that shed their corollas set more seed when pollinators are limiting than flowers that do not (Dole, 1990). The abscission of the corolla occurred in 73% of the control flowers that produced fruit, and only in 42% of those that did not. However, further investigation is necessary to fully understand the mechanism of delayed selfing in this species.

Even though autonomous self-pollination does not contribute substantially to fitness under the conditions experienced in this study, successful reproduction of 11% of flowers in the absence of pollinators suggests selfing may provide some reproductive assurance. In a study exploring the role of reproductive assurance in the evolution of self-fertilization, Piper *et al.* (1986) found temporal and spatial variation in the relative fertility of the different morphs in distylous *Primula vulgaris* that was partially due to pollinator availability. Our results which illustrate a limited potential for autonomous self-fertilization, in conjunction

with those from a year in which pollinators were limiting (Rathcke and Real, 1993), suggest that reproductive assurance by autonomous selfing may be important to this species, but that it is not likely to contribute substantially to fitness in years when pollinator service is plentiful.

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