

COST OF REPRODUCTION IN THE PINK LADY'S SLIPPER ORCHID (*CYPRIPEDIUM ACAULE*, ORCHIDACEAE): AN ELEVEN-YEAR EXPERIMENTAL STUDY OF THREE POPULATIONS¹

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An 11-yr experimental study of the cost of reproduction in three wild populations of the perennial orchid *Cypripedium acaule* contrasted experimental plants that were repeatedly hand-pollinated and often made fruits with control plants that were not hand-pollinated and only rarely made fruits. Repeated flowering without subsequent fruit production resulted in no detectable reduction in either plant size or probability of flowering in subsequent years. A cost of fruit production was evident in experimental plants in all three populations in terms of a reduced probability of flowering and smaller leaf area in subsequent years, but was not evident in terms of mortality rate. Experimental effects of fruit production reached maximum values at 3–7 yr, depending on the population. The probability of remaining dormant below ground in a given year was strongly dependent on plant size in the previous year. Furthermore, the length of the dormancy period (one to several years) was a significant and inverse function of plant size just prior to dormancy. Sample sizes and the consequent ability to detect experimental effects declined over time as more plants died or stopped flowering. Four to seven years appears to be an optimal duration for studies of the cost of reproduction in perennial herbs similar to this species. Studies lasting less than 4 yr may be too brief to reveal experimental effects, whereas those lasting more than 7 yr may fail to reveal new insights.

Key words: cost of reproduction; *Cypripedium acaule*; life history; orchid; Orchidaceae; pollination; perennial herb; population biology.

Life history models of plants and animals assume that there is a trade-off between present reproduction and future growth, survival, and reproduction, which is usually referred to as the cost of reproduction (Stearns, 1989; Partridge and Harvey, 1988; Roff, 1992). Researchers have conducted experiments and detailed observations to support or refute these models. Experiments in which randomly selected individuals are manipulated to have higher or lower levels of reproduction than control individuals have proved to be a powerful tool for investigating the costs of reproduction (Resnick, 1992; Calvo, 1993). In general, experiments involving both plants and animals have provided evidence for the theory of a cost of reproduction (Zimmerman and Pyke, 1988; Snow and Whigham, 1989; Zimmerman and Aide, 1989; Ackerman and Montalvo, 1990; Primack and Hall, 1990; Primack, Miao, and Becker, 1994). However, a number of experiments with plants have either failed to demonstrate a cost of reproduction (Reekie and Bazzaz, 1987; Horvitz and Schemske, 1988; Calvo, 1990; Fox and Stevens, 1991; Ågren and Willson, 1994; Lehtilä and Syrjänen, 1995), or have found evidence only when plants are defoliated, which presumably results in a reduced energy budget. A difficulty with most of the experiments published to date

is that they typically only last one or two seasons. Such a brief time perspective may give an inadequate picture for long-lived species that may require several years to store up the energy required for reproduction (Clark and Clark, 1988). In such species, a cost of reproduction may only become evident after several years and several reproductive episodes. Conversely, initial evidence of a cost of reproduction may prove to be insignificant if the experiment is continued for many years.

In this paper, we report on the results of an 11-yr study of the cost of reproduction in three populations of the pink lady's slipper orchid (*Cypripedium acaule*), a long-lived perennial. To our knowledge, this is the longest-running study of the cost of reproduction, and as such can provide insight into population-level phenomena that is not possible in shorter studies. A cost of reproduction was evident in a 4-yr study of two of these populations (Primack and Hall, 1990). In extending the study through 11 yr, our first objective was to determine whether evidence of a cost of reproduction persists or even increases over several consecutive years of fruit production. Or alternatively, is there a limit to the number of consecutive years hand-pollinated plants will produce fruit? Further, is there a predictable recovery period exhibited by plants after successive years of fruit production? Our second objective was to estimate the time frame suitable for the study of reproductive costs in natural populations of this native perennial species based on our observations of the timing of the appearance and disappearance of experimental effects. Long-term population studies involving multiple parameters are expensive to conduct and often yield complicated data sets that are difficult to analyze. How many reproductive seasons are needed to investigate these phenomena in an efficient manner?

¹ Manuscript received 18 August 1997; revision accepted 30 May 1998.

Dozens of people assisted with the field work through the years, including undergraduate and graduate students from Boston University and volunteers from the Broadmoor Audubon Sanctuary. Permission to use the field sites came from Broadmoor Audubon Sanctuary, the Arnold Arboretum of Harvard University, and the Newton Conservation Commission. Marsha Pomeroy, among others, helped with data management. The manuscript benefited from the comments of Dennis Whigham, Lisa Delisio, Jennifer Smith, and Elizabeth Newell.

TABLE 1. Characteristics of three experimental *Cypripedium acaule* populations.

Character	Year	Case Estates		Hammond Woods		Broadmoor		
		Control	Experimental	Control	Experimental	Control	Experimental	
% fruit set ^a	1984	0 (64)	93.8 (64)***	1.5 (65)	93.8 (64)***			
	1985	5.3 (57)	95.6 (45)***	2.9 (34)	87.5 (32)***	1.3 (77)	91.1 (79)***	
	1986	2.1 (48)	65.5 (29)***	4.7 (64)	74.1 (58)***	1.5 (69)	81.3 (75)***	
	1987	0 (41)	95.2 (21)***	5.9 (68)	89.4 (47)***	2.7 (74)	79.2 (48)***	
	1988	5.6 (18)	88.9 (9)***	0 (52)	63.0 (27)***	25.0 (40)	76.9 (26)***	
	1989	0 (14)	100 (3)**	3.9 (51)	80.6 (36)***	1.5 (66)	44.2 (43)***	
	1990	25.0 (8)	60 (5)	9.8 (51)	86.7 (30)***	12.9 (62)	87.8 (41)***	
	1991	0 (1)	0 (1)	5.9 (51)	84.0 (25)***	8.9 (45)	92.9 (14)***	
	1992	—	—	1.9 (54)	80.0 (20)***	4.4 (45)	70.0 (20)***	
	1993	—	—	0 (47)	81.3 (32)***	8.0 (50)	73.1 (26)***	
	1994	—	—	3.0 (33)	50.0 (12)***	18.2 (11)	— (0)	
	Cumulative deaths ^b	1985	2	2	1	0		
		1986	6	9	1	0	3	0
		1987	9	16	0	0	6	6
1988		12	17	1	1	6	3	
1989		11	19	4	2	7	4	
1990		12	23*	6	8	11	8	
1991		23	31	8	8	12	7	
1992				11	10	16	13	
1993				11	15	19	12	
1994				20	14	20	18	

Note: Significant differences between control and experimental plants are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, $P < 0.001$).

^a Total number of flowers present in the population is shown in parentheses. Differences were tested using χ^2 .

^b Plants sometimes reappeared after not being present for one or more years. Consequently, the number of cumulative deaths can decline as well as increase.

MATERIALS AND METHODS

This paper describes an extension of a previously reported 4-yr study of the cost of reproduction in two populations of *C. acaule* (Primack and Hall, 1990). The original study involved two woodland populations of the species in eastern Massachusetts, Case Estates (CE), in a mature pine plantation in Weston, and Hammond Woods (HW), a deciduous, rocky woodland conservation area in Newton. Our earlier papers provide further information on site characteristics, natural history, and experimental design (Primack, Miao, and Becker, 1994; Primack, 1996). In 1984, 128 plants at CE and 129 plants at HW were selected that each had two leaves and a single flower; other plants in the population may not have been flowering or had more than two leaves and were not used in the study. Plants were measured for leaf area (leaf length and width), numbered, and mapped. Individuals were randomly assigned to be either hand-pollinated experimental plants, or unpollinated control plants. Pollen was taken from plants just outside of the research site. Hand-pollinated flowers had a high probability of developing into fruits, while unpollinated flowers had a very low probability of being pollinated by bees and developing into fruits. In all subsequent years, tagged plants were measured for their leaf area and number of flowers. The experimental treatment was repeated throughout the study period in that all plants that had initially been assigned to controls were unmanipulated and all experimental plants assigned to the hand-pollination treatment were pollinated in years when they flowered. Plants at HW were studied until 1994, for a total of 11 yr. Observations at CE ceased in 1991 after eight study seasons, because so few experimental and control plants were alive and in flower, apparently due to a disease that damaged the leaves several years in a row. Some plants in the study became dormant for one or more years; they could be recognized because they re-emerged as adults in subsequent years in exactly the same place where they grew before (see also Gill, 1996).

In 1985, a third woodland population, Broadmoor (BM) Audubon Sanctuary in Natick, Massachusetts, was selected for study. The BM population included 156 flowering plants of a wider variety of sizes than occurred at the other sites, from plants with two leaves to plants

with 16 leaves. As in other populations, individuals were randomly assigned to be hand-pollinated (experimental plants) or unpollinated (control plants). The experiment ran for ten field seasons, from 1985 to 1994, and is being reported on here for the first time.

Because a substantial number of plants died (or entered a dormant, belowground state) during the study, we analyzed the data both for the entire population of plants alive in any year as well as for only the plants that survived through the final year of the experiment. Because both analyses gave similar results, only results involving all plants are reported, unless otherwise stated. Statistical analyses are provided in the Results.

RESULTS

Fruit set—Percentage fruit set increased dramatically with hand-pollination (Table 1). Over the course of the study, mean fruit set for control plants was 2.8, 3.7, and 6.5% of total flower production at CE, HW, and BM, respectively, whereas mean fruit set for hand-pollinated plants was 88.1, 82.0, and 78.5% of total flower production, respectively. Over the 11-yr period at HW, all control plants combined produced only 21 fruits (less than one fruit per plant), whereas the experimental plants produced 314 fruits (roughly five fruits per plant).

Fruit set varied considerably among years, even though flowers on experimental plants always had a much higher probability of producing fruits relative to flowers on control plants (Table 1). At BM, for example, fruit set on control plants varied from a low of 1.3% in 1985 to a high of 25.0% in 1988; experimental plants varied from a low of 44.2% fruit set in 1989 to a high of 92.9% in 1991.

At all three sites, every experimental plant produced at least one fruit over the course of the study, whereas the majority of control plants failed to produce even one

TABLE 2. Number of plants classified by total flower and fruit production for each site and treatment (CE: 1984–1991; HW: 1984–1994; BM: 1985–1994). In general, most control plants produced no fruits, while each experimental plant produced ≥ 1 fruit. For each category of flower production, the mean initial size of the plants is given, with the standard deviation in parentheses.

No. of flowers produced	Control						Experimental								No. of plants
	Mean size in first year (SD)	Number of fruits produced				No. of plants	Mean size in first year (SD)	Number of fruits produced							
		0	1	2	3			1–2	3–4	5–6	7–8	9–10	11–12	≥ 13	
Case Estates															
1–2	142.1 (54.9)	13	1	0	—	14	152.4 (54.9)	31	—	—	—	—	—	—	31
3–4	162.5 (51.7)	29	2	0	0	31	191.9 (54.3)	7	21	—	—	—	—	—	28
5–6	197.1 (69.7)	10	3	0	0	13	178.0 (17.7)	0	3	2	—	—	—	—	5
7–8	223.0 (23.9)	3	0	0	0	3	—	—	—	—	—	—	—	—	—
9–10	297.9 (—)	1	0	0	0	1	—	—	—	—	—	—	—	—	—
11–12	267.9 (25.4)	1	1	0	0	2	—	—	—	—	—	—	—	—	—
Hammond Woods															
1–2	154.2 (—)	1	0	0	—	1	133.2 (—)	1	—	—	—	—	—	—	1
3–4	153.2 (37.7)	7	3	0	0	10	159.1 (49.0)	6	13	—	—	—	—	—	19
5–6	168.3 (35.5)	6	3	0	0	9	188.1 (52.7)	0	8	13	—	—	—	—	21
7–8	193.7 (63.8)	8	4	1	0	13	215.1 (68.4)	0	3	9	3	—	—	—	15
9–10	189.6 (56.8)	9	3	0	0	12	212.9 (69.5)	0	0	1	1	2	—	—	4
11–12	176.5 (78.2)	8	2	0	0	10	217.3 (19.7)	0	0	0	0	2	0	—	2
13–14	267.2 (16.4)	2	1	1	0	4	208.9 (54.6)	0	0	0	0	1	1	0	2
15–16	177.3 (53.4)	2	1	0	0	3	—	—	—	—	—	—	—	—	—
17–18	244.3 (—)	1	0	0	0	1	—	—	—	—	—	—	—	—	—
19–20	214.9 (109.2)	2	0	0	0	2	—	—	—	—	—	—	—	—	—
Broadmoor															
1–2	147.7 (40.7)	8	0	0	—	8	112.2 (50.5)	8	—	—	—	—	—	—	8
3–4	157.9 (68.1)	10	0	0	0	10	189.8 (54.2)	5	7	—	—	—	—	—	12
5–6	186.5 (75.4)	2	3	1	0	6	198.8 (70.6)	2	7	4	—	—	—	—	13
7–8	170.0 (81.0)	4	6	2	0	12	201.6 (39.2)	0	0	5	3	—	—	—	8
9–10	183.1 (69.1)	2	1	1	0	4	184.2 (34.7)	0	0	2	4	2	—	—	8
11–12	120.7 (—)	0	1	0	0	1	213.4 (79.0)	0	0	0	2	1	0	—	3
13–14	191.6 (—)	0	1	0	0	1	—	—	—	—	—	—	—	—	—
15–16	270.4 (25.9)	1	1	0	1	3	—	—	—	—	—	—	—	—	—
17–18	239.2 (8.8)	1	0	1	0	2	261.8 (2.7)	0	0	0	0	0	1	1	2
19–20	243.2 (—)	1	0	0	0	1	232.5 (—)	0	0	0	0	0	0	1	1
21–22	257.5 (26.1)	0	1	1	0	2	—	—	—	—	—	—	—	—	—
23–24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
25–26	179.0 (34.2)	1	1	1	1	4	269.4 (—)	0	0	0	0	0	0	1	1
27–28	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
≥ 30	255.9 (3.0)	2	0	0	0	2	—	—	—	—	—	—	—	—	—

fruit (Table 2). Some control plants at each site, however, produced one fruit during the study due to natural insect pollination, and a small number of control plants fruited two or three times. In contrast, the majority of experimental plants at HW and BM produced five or more fruits over the 11- and 10-yr study periods, respectively. At BM, three vigorous experimental plants produced 13, 15, and 17 fruits over 10 yr.

Flowering—In each population, the percentage of flowering individuals was highly variable among years. However, flower production was always strongly influenced by initial plant size (Table 2). Regardless of treatment, larger plants tended to produce more flowers over the course of the study (initial plant size \times total flower production: $R = 0.43, 0.27,$ and 0.63 (all $P < 0.001$), for CE, HW, and BM, respectively) and to flower in more years than smaller plants (initial plant size \times number of years in flower: $R = 0.39, 0.28,$ and 0.25 (all $P < 0.001$), for CE, HW, and BM, respectively).

The percentage of flowering individuals was significantly reduced by the hand-pollination treatment (Fig. 1).

At CE, experimental plants had a dramatically lower probability of flowering than control plants during 1986, 1987, and 1989, with the maximum effect occurring in 1987 after 3 yr of treatment. Over time, however, there was a substantial decline in the probability of flowering in all plants at CE, to the point where only two plants flowered in 1991. This general decline in flowering, which was apparently caused by disease, obscured any possible experimental effects in 1990 and 1991.

At HW, experimental plants began to exhibit significantly lower probabilities of flowering in comparison with control plants starting in 1987 and continuing through 1994 (excluding 1993). The lack of significance in 1993 was due to the strong flowering of experimental plants. In 1992 and 1994, twice as many control plants as experimental plants produced flowers (Fig. 1).

At BM, experimental plants had a significantly lower probability of flowering in comparison with control plants in 1987, but then no effect was evident from 1988 to 1990. Differences in the probability of flowering were again significant from 1991 to 1994. Although flowering was poor in both groups in 1994, still 19.4% of the con-

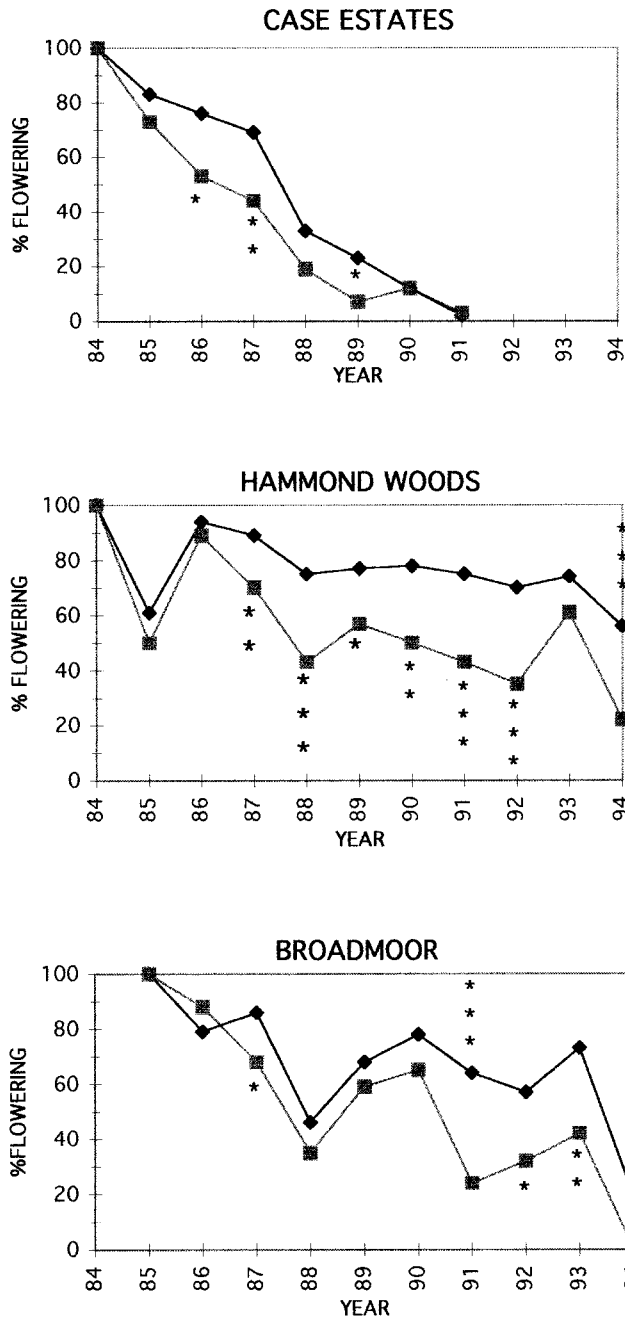


Fig. 1. Trends over time in percentage of plants in flower for control plants (diamonds) and experimental plants (squares). Differences between control and experimental plants are determined using χ^2 and indicated by stars (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). In Figs. 1–3, sample sizes for each site and year are given in Table 1, as the number of plants at start less the number of plants that died.

control plants flowered in contrast to no flowering by experimental plants (Fig. 1). The reason for this poor flowering is not known, as site conditions at BM did not change notably over the course of the study.

Regardless of population, for many experimental plants fruiting ability was restricted by the reduction in flower production that occurred over the course of the study. For example, at HW only eight experimental plants

TABLE 3. Repeated-measures ANOVA for the effects of treatment and years on leaf area. F statistics are given for analysis with treatment as the factor and years as the repeated measure.

Effects	Population		
	Case Estates	Hammond Woods	Broadmoor
Year	73.93***	7.34***	8.69***
Treatment	1.59†	7.62**	3.67†
Year \times Treatment	2.13*	7.92***	2.99**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † not significant.

had ≥ 9 flowers, and none produced > 14 flowers during the 11-yr period (Table 2). In contrast, 32 control plants produced ≥ 9 flowers, and six plants had > 14 flowers.

Plant size—The hand-pollination treatment caused a reduction in plant size in all three populations, though to different degrees, and at different times. The strength of the treatment effect varied among years at all sites, as indicated by the significant interaction terms in the repeated-measures analysis (Table 3). The significant interaction occurs because experimental and control plant groups had the same mean initial leaf area, but gradually diverged over time due to the experimental treatment. For each population, the decline in mean plant size due to hand-pollination was most rapid in the first 3–4 yr, followed by a longer period of gradual decline in plant size (Fig. 2). By the end of the study, experimental plants were on average 15 cm² (CE) to 77 cm² (HW) smaller than control plants. Differences between mean ultimate sizes of experimental and control plants were significant in two of the three populations.

At CE, the effects of the experimental treatment were most dramatic during 1985 and 1986, after which the general decrease in plant size due to disease weakens, but does not eliminate the experimental effect of hand-pollination (Fig. 2). In general, plants decreased in size over time at CE, but the rate of decrease was more rapid for experimental plants. In 1991, experimental plants were still significantly smaller than control plants.

At HW, mean plant size was highly variable over time. In general, control plants increased in size while experimental plants decreased in size, however slightly. Differences in mean plant size between the experimental and control groups reached significance in 1985 after just one treatment and continued to increase throughout the remainder of the study. From 1986 to 1994, experimental plants were significantly smaller ($P < 0.001$) than control plants, except for 1988 when a fire burned many plants before they could be measured. By 1993, experimental plants were less than two-thirds of the size of control plants (Fig. 3).

At BM, experimental effects on plant size became evident after 3 yr of treatment. From 1985 to 1988, plant size increased for control plants but remained stable for experimental plants, resulting in a divergence of mean plant size over time. Starting in 1990, the experimental plants declined in mean size while the control plants fluctuated in mean size. The experimental effect on plant size reached significance in 1988 and was significant in 1989 and 1991–1993. The experimental effect gradually weakened over time, however, in part due to a decline in sam-

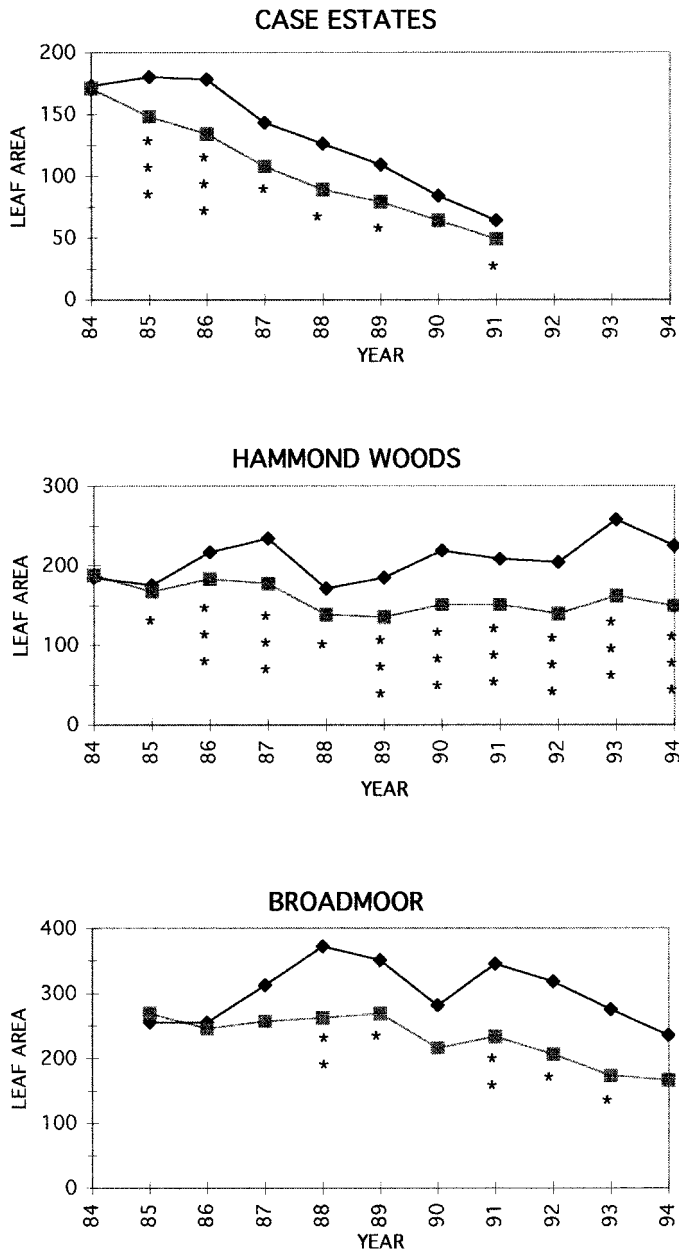


Fig. 2. Trends in mean leaf area over time for control plants (diamonds) and experimental plants (squares) at three experimental populations. Differences between control and experimental plants are indicated by stars (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Differences were tested using ANCOVA. As initial plant size strongly influenced plant size in subsequent years (Primack and Hall, 1990; see also this study), initial plant size was used as a covariate in each ANOVA.

ple size. By 1994 experimental plants and control plants were not significantly different in size.

Mortality—At HW and BM, the mortality rates for control plants were slightly higher than for experimental plants, whereas at CE the opposite was true (Table 1). Overall, there was no significant effect of experimental treatment on mortality rate. Here, reported mortality should be treated as apparent mortality only, as in many cases plants that were considered dead reappeared 1 or 2

yr later. Such a phenomenon could be due to plants remaining in a dormant stage, or to the aboveground parts being eaten or damaged so that plants have no apparent foliage that year and are not seen. At CE, mortality was probably due to a pathogen that attacked leaves. At all three sites, plants were sometimes killed by falling trees and branches or by burrowing rodents. Plants also died in groups, suggesting local outbreaks of disease.

Cost of flower production—It was shown in the previous study that production of flowers resulted in no significant and consistent cost to plants in the CE and HW populations in terms of plant size over a 4-yr period (Primack and Hall, 1990). Here we expanded the analysis to three populations over a period of 8–11 yr and similarly found no consistent cost of flower production manifest in year-to-year change in plant size. Of 26 analyses (ANOVAS: difference in leaf area from year_N to year_{N+1}) contrasting flowering and nonflowering plants, only six (two for each population) showed a significant effect of flowering on the maintenance of plant size. In fact, in all three populations ultimate plant size generally increased as a function of the mean number of years in flower, though this relationship was variable, particularly for BM.

Cost of fruit production—The effect of persistent annual fruit production measured in terms of differences in plant size in the year following reproduction was highly variable among populations (Table 4). At CE, a significant cost of fruiting in terms of leaf area was found after the first reproductive period. This effect continued through the fourth consecutive year of fruiting. At HW, significant costs of reproduction were evident after two and three consecutive years of fruiting, as reported previously (Primack and Hall, 1990). By the 4th yr, however, the effect of annual fruiting disappeared. Statistical analyses of the cost of fruit production in subsequent years (5–11) at HW were not possible due to insufficient numbers of plants in these highly reproductive classes. At BM, the effect of persistent annual fruiting on plant size in the year following fruiting was not evident, as significant effects were found in the second year only. Again, despite the occurrence of a few individuals with spectacular fruiting histories, statistical comparisons were restricted to plants with ≤ 5 fruits (over 5 yr) due to insufficient sample sizes of plants fruiting repeatedly.

The absolute cost of reproduction was calculated as loss of leaf area (in square centimetres) on a per fruit basis (Table 4: “Adjusted Differences”). Across populations, these estimates were fairly consistent, ranging in a loss of 15–30 cm² of leaf area for each fruit produced. At CE, the estimated per-fruit loss in plant size decreased gradually over the first 4 yr, perhaps because only the most vigorous plants were able to produce fruits in five consecutive years.

Patterns of dropout and recovery of experimental plants—In each population, very few experimental plants were present and flowering in every year of the study. Instead, the majority of experimental plants failed to appear above ground, or appeared but failed to flower during one or more period lasting one to several years each. During these periods, such plants were called “dropouts”

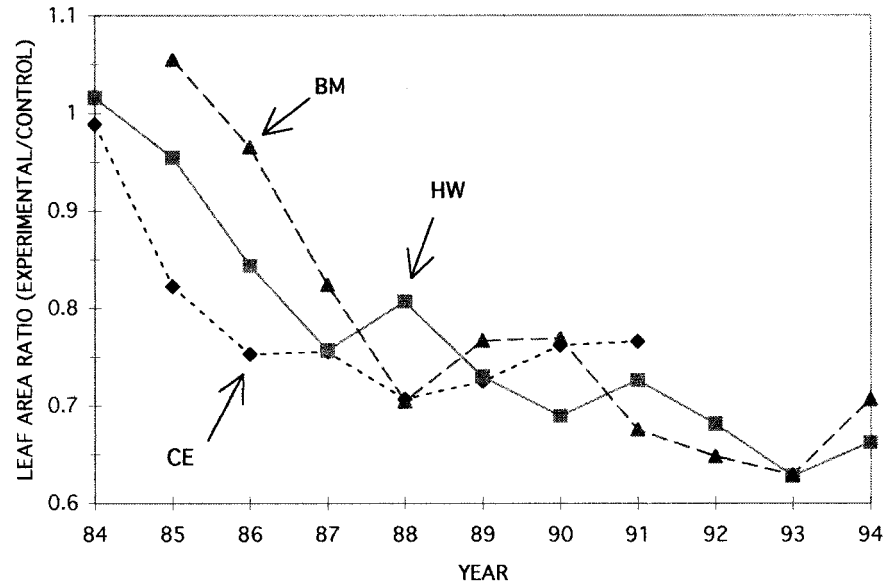


Fig. 3. Mean leaf area of experimental plants divided by mean leaf area of control plants. diamonds, Case Estates; squares, Hammond Woods; triangles, Broadmoor Sanctuary.

as they essentially dropped out of the hand-pollination experiment. We calculated the mean plant size in the year before dropping out of the experiment both for all dropout periods (i.e., when no flowers were produced), and for the subset of these dropout periods during which the plants failed to appear above ground ("dormants"). For the HW and BM populations, these values were then compared to the mean sizes, pooled across years, of plants that survived and flowered in the next year of the study. CE was not included in the analysis as a disease at this site may have confounded the relationships among plant size, reproductive history, and apparent mortality. Combining treatment groups at HW, mean sizes of dropouts and flowering plants were $164.0 \pm 70.3 \text{ cm}^2$ ($N = 112$ cases) and $204.9 \pm 95.8 \text{ cm}^2$ ($N = 862$ cases), respectively. At HW, the subset of dormant plants that failed to appear above ground ≥ 1 yr averaged $125.9 \pm 87.3 \text{ cm}^2$ ($N = 57$ cases) in the year prior to going dormant. Just before going dormant, therefore, dormant plants were, on average 61.4% of the size of flowering plants.

After combining treatment groups at BM, mean sizes of dropouts and flowering plants were $237.5 \pm 135.3 \text{ cm}^2$ ($N = 96$ cases) and $318.9 \pm 210.5 \text{ cm}^2$ ($N = 581$ cases), respectively. At BM, on average, flowering plants were significantly larger than dropouts in all but the last 2 yr of the study. Of dropouts at BM, the subset of plants that failed to appear above ground in one or more year averaged $192.1 \pm 143.6 \text{ cm}^2$ ($N = 87$ cases) in the year prior to dropping out, only 60.2% of the size of flowering plants.

Dropouts were also examined for the total number of fruits produced in consecutive seasons prior to dropping out. At HW, experimental plants dropped out of the study after fruiting in 1.8 ± 1.3 (SD) consecutive years (range 0–7; $N = 111$). Of experimental plants at HW, only four (6%) plants produced fruits in > 4 consecutive years. At BM, plants produced fruits for an average of 1.6 ± 0.93

consecutive years (range 0–9; $N = 90$) before dropping out. Furthermore, of all experimental plants at BM, only three (5%) plants produced fruit in > 4 consecutive years. In both populations, the majority of plants in the experimental groups alternated fruiting periods lasting 1–3 yr with nonreproductive periods of approximately the same duration. In both populations, some control plants experienced dropout periods despite the fact that no control plants in either population produced fruits in consecutive years.

For experimental plants at both HW and BM, post-fruiting recovery periods (the number of years present with leaves but not flowering) ranged from one to several years, with an average duration of 2.1 ± 1.9 (SD) yr at HW, and 3.0 ± 2.3 yr at BM. Plant size in the year prior to dropout was a significant (and inverse) predictor of the length of the recovery period in both populations (HW: $P = 0.004$, $N = 111$ dropout periods; BM: $P = 0.016$, $N = 90$ dropout periods). Although significant in both populations, plant size prior to dropout only explained $\sim 7\%$ of the variation in the length of the recovery period. The relationship between the length of the postfruiting recovery period and the number of consecutive fruiting periods prior to dropout was significant and positive for HW ($P = 0.02$, $N = 111$), but not for BM ($P = 0.08$, $N = 90$).

DISCUSSION

These results demonstrate that there is a substantial cost of reproduction in the pink lady's-slipper orchid evident in an experiment lasting 11 yr. Reproductive costs were evident in a reduction in both the probability of flowering and plant size in subsequent years. Estimated per-fruit cost as seen in a reduced leaf area remained consistent with that estimated in the earlier 4-yr study (Primack and Hall, 1990). To summarize in terms of plant size, large plants were more prone to flower, but repeated

TABLE 4. Site-specific cost of reproduction as measured by leaf-area differences in the year following the reproductive event. Adjusted Differences represent the differences between the group means adjusted by the covariate. For significant comparisons, the reduction in leaf area for each fruit produced is also presented.

Comparison	Year	N	Initial leaf area	Fruiting history	Adjusted differences (cm ² of leaf area)	Adjusted differences (cm ² of leaf area) per fruit
Case Estates cost of fruits						
1 fl, 0 vs. 1 fr ^a	1985	124	245.07***	15.07***	30	30
2 fl, 0 vs. 2 fr	1986	73	46.57***	5.87*	38	19
3 fl, 0 vs. 3 fr	1987	43	51***	4.77*	46	15
4 fl, 0 vs. 4 fr	1988	27	16.13***	11.36***	60	15
Hammond Woods cost of fruits						
1 fl, 0 vs. 1 fr	1985	128	312.48***	2.26	8	NS
2 fl, 0 vs. 2 fr	1986	59	43.03***	6.18*	45	22
3 fl, 0 vs. 3 fr	1987	51	29.56***	15.68***	81	27
4 fl, 0 vs. 4 fr	1988	35	1.77	0.46	22	NS
Broadmoor cost of fruits						
1 fl, 0 vs. 1 fr	1986	80	211.53***	3.56	36	NS
2 fl, 0 vs. 2 fr	1987	44	24.84***	4.51*	59	29
3 fl, 0 vs. 3 fr	1988	30	8.98*	3	79	NS
4 fl, 0 vs. 4 fr	1989	15	0.02	0.62	71	NS ^b
5 fl, 0 vs. 5 fr	1990	9	3.91	4.11	275	NS ^b

^a Reproductive histories are indicated by the number of flowers (fl) and fruits (fr). Significance of reproductive costs was tested using ANCOVA, where current-year leaf area is the dependent variable (Year), Fruiting History is the categorical independent variable, and Initial leaf area is the covariate. Significant costs of reproduction are indicated by asterisks (* $P < 0.05$, *** $P < 0.001$).

^b Detection of significant effects may be limited by declining sample size. Furthermore, although plants with greater flowering and fruiting activity occurred (i.e., >4–5 fl, >4–5 fr), statistical tests were not possible for these plants due to insufficient sample sizes.

fruiting of flowering plants results in smaller plants over time. A cost of reproduction was not manifest in reduced survival rate.

Control plants can produce flowers for many consecutive years with no apparent cost. However, consecutive fruiting over several years reduces the probability of further fruiting. Experimental plants produced, on average, 1.7 consecutive fruits prior to cessation of flower production. Overall, very few experimental plants succeeded in fruiting in each of several consecutive reproductive seasons. Rather, about four consecutive years appears to be the maximum length of time that plants in these populations are able to sustain repeated annual flowering and fruiting due to hand-pollination. However, also important is that many nonfruiting control plants also failed to produce a flower or aboveground vegetative tissue in the following year. There does not appear to be a simple relationship between the number of successive fruiting episodes and the cessation of flowering. Instead, the trigger for cessation of flowering appears to be a significant loss in leaf area, probably indicative of a reduced energy budget. Nonflowering plants at HW and BM were smaller than flowering plants, averaging from 75 to 80% of the size of the latter. At some critical plant size, perhaps 60% of the average size of flowering plants, some plants may be induced to enter a dormant phase below ground. Such a tendency of plants to become dormant is seen in other populations of this species as well (Gill, 1996).

The length of the recovery period and return to flowering in experimental plants is highly variable, ranging from one to several years, with an average of 2–3 yr. The length of the recovery period is not a simple function of the number of consecutive fruiting episodes just prior to dropout, though this relationship was significant for HW. Rather, the length of the recovery period is inversely and

significantly (or nearly so at BM) related to plant size in the year just prior to dropout, that is smaller plants remain without flowers longer than the larger plants. Variability in the length of time plants need to recover and flower again probably results from a variety of environmental and intrinsic influences, including the physiological condition of the plant and the plant genotype. Many plants may fail to appear above ground in a given year simply because the young leaves are eaten by insects or rodents before they emerge above ground or are censused. Ultimately, these results, though not all clear, provide further evidence of the cost of reproduction in *C. acaule*. Plant size in a given year appears to be the most important determinant of the probability of that plant appearing above ground and with a flower the following year.

The ability to detect a cost of reproduction in these populations becomes obscured with time for at least two reasons. First, the cumulative death of plants over time reduces sample sizes. This necessitated terminating the work at the CE site, where almost half the plants had died after 7 yr. Second, the reduced probability of flowering with time for all plants meant that the treatments could no longer be given for many experimental plants.

The results highlight the variation within years and among sites that occurs in natural populations. Certain years were better than others for flowering and plant size at any given site, probably related to environmental conditions in the previous one or two years. Important factors are climatic variables, such as the amount of rain, and the degree of canopy closure, as affected by fire, insect damage to leaves, and succession (Gill, 1996; Whigham and O'Neill, 1988, 1991). At CE, for example, there was a gradual reduction in mean leaf area over time for all plants. Furthermore, disease-induced mortality of plants obscured the cost of reproduction over the latter half of

the study. In contrast, plants at HW and BM were more robust and variable from year to year. A fire damaged the leaves of many plants at HW in 1988 and may have released nutrients and killed competing shrubs, leading to strong growth and flowering in subsequent years. The cost of reproduction at the HW and BM sites was evident until the end of the experiment. The intensity, duration, and timing of the initial appearance of experimental effects followed a unique pattern in each population. This variation observed among populations serves to illustrate the need to use multiple populations in any experimental investigation of reproduction in natural plant populations. Unusual events or environmental conditions can create anomalous patterns in any one year or one site.

This experiment is useful for determining the length of time needed to conduct experiments of the cost of reproduction in perennial herbs. At all three sites, mean leaf area of the experimental plants is reduced relative to that of the control plants after a certain number of years of treatment (Fig. 3). After reaching this reduced leaf area, experimental plants remain within a range around 50–75% of the area of control plants for successive years. Plants showed this reduction in mean leaf area by year 3 at CE and by year 4 at HW and BM. The maximum reduction in the probability of flowering for experimental plants relative to control plants was seen in year 4 at CE, year 5 at HW, and year 7 at BM. An experiment lasting 7 yr would show the maximum effects on leaf area and flowering at all three sites. However, a 6-yr study of the cost of reproduction in a cycad concluded that additional years were needed to have a thorough understanding of the costs of reproduction (Clark and Clark, 1988). The value of continuing the present experiment for 11 yr is to provide a clear demonstration that this maximum cost of reproduction cannot be exceeded. When plants exhaust their energy budgets due to fruit production, they have a reduced leaf area and probability of flowering in subsequent years (Whigham, 1990; Zimmerman and Whigham, 1992). Presumably, the absence of flowers in a particular year allows the plant to build up the leaf area and associated energy reserves needed for flowering in subsequent years.

In conclusion, an experiment involving three populations and an initial sample size of over 100 plants at each site could unambiguously demonstrate a cost of reproduction. This cost could be calculated precisely in terms of the reduction in leaf area due to each fruit produced and a reduction in flowering probability. However, environmental variation and the random death of plants tend to obscure these experimental effects over time. Experiments lasting at least 4 yr are needed to reveal the full range of experimental effects at all three populations, with 7 yr needed for the maximum effects to occur. Continuing the experiment for the full 11 yr was valuable for demonstrating the variation among populations and the ability of individual plants to recover from repeated annual fruit production.

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