# Science and Serendipity
# The Pink Lady's Slipper Project

*Richard Primack*

**What is the cost of reproduction?**
**An important hypothesis is put to the test.**

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The study of plants can sometimes lead to discoveries of an unexpected nature. For over a decade now, I have been investigating the reproductive biology of the pink lady's slipper orchid *(Cypripedium acaule).* When I was growing up in Boston in the 1950s and 1960s, my mother would point out the occasional pink lady's slipper as a special, rare plant to be held in respect, and I remember feeling almost reverential toward the exquisite flowers when I discovered them on hikes as a teenager. Hikers in the eastern United States still react with excitement when they encounter the plant during a late spring excursion, but although the flowers are beautiful and well worth seeking out, certainly they are no longer rare.

The idea that the pink lady's slipper is endangered stems from events earlier in this century when the species was indeed rare. At that time gardeners often dug up wild plants to transplant in their own yards, and this was perceived as a primary threat to the species' survival. Media campaigns to protect wildflowers targeted the pink lady's slipper, and the campaigns appear to have left a lasting impression on the public.

During the last few decades, however, the plant has achieved a tremendous increase in numbers in New England. Woodland areas that once had a few scattered plants now boast dozens, hundreds, or even thousands. In some areas of Boston, pink lady's slippers have become the most common woodland herb, forming dense populations. Although it is tempting to see a simple cause-and-effect relationship between the preservation campaign and the new abundance of the species, the truth is much more complicated. The success of the pink lady's slipper might also be explained by the recovery of its previously disturbed habitat. Much of the eastern United States was cleared for agriculture during the early history of our nation, and the landscape has only gradually returned to forest in the last century. It may be that the specific growing conditions it requires were not present earlier in the young forest.

One of the missing requirements may have been the species of soil fungi with which the orchid has a peculiar obligate relationship. These fungi form extensive networks of filaments in the soil that absorb water and nutrients. When an orchid seed germinates, the seedling is tended by the fungus filaments, which provide all the water, minerals, and carbohydrates the seedling needs to survive. In this early stage, the orchid seedling is just a small blob of white tissue below ground. Only after five years or so does the orchid begin to produce its first green leaves above ground. As the orchid increases in size over subsequent years, it finally becomes photosynthetically self-sufficient. At this point, the orchid begins to supply the fungi with excess carbohydrates in exchange for a continued supply of water and mineral nutrients. If the fungi had been extirpated from the soil after centuries of farming, then the orchid would not be present until the forest—and the fungi—had become firmly reestablished.

Yet another unusual characteristic may have limited the lady's slipper's ability to increase: Its flowers rarely become fruits. The reason for this peculiarity is that the flowers contain no nectar to attract pollinators. In fact, the flowers are mimic flowers that depend on the naiveté of bumblebees and other large insects searching for new nectar sources. If bees visit several flowers in a row in their search for nectar, they may transfer pollen from one plant to the stigma of another plant in the process. Once this happens, the petals droop to prevent further bee visits, the ovary swells, and over the next four months a grape-sized capsule containing tens of thousands of seeds develops. In the fall, the tiny seeds filter out of slits in the capsule and are carried away by the seasonal breezes. Unfortunately for the pink lady's slipper, there are not enough naive bees in the forests of New England. As a result, most orchid flowers remain unvisited, even in large populations. In a typical population, only one or two percent of the flowers develop fruits. Yet because of the large number of seeds per fruit, the populations can increase over time even with low rates of fruiting.

Under different circumstances, however, the orchid has the potential for prolific reproduc-

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Cypripedium acaule *(pink lady's slipper orchid) rendered in pen and ink by Blanche Ames Ames (1878–1969)*

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*The young orchid fruit expands rapidly following pollination. The grape-sized capsule contains tens of thousands of seeds.*

tion. When flowers are artificially pollinated by researchers and volunteers, the rate of fruit set can easily increase to ninety percent. In 1984, I realized that this property made the pink lady's slipper orchid an ideal subject for testing an important but unproven hypothesis: namely, that reproduction exacts a cost from plants and animals. Most biologists accept the idea that each organism has a finite supply of resources available for use in growth, survival, and reproduction. Thus, any individual organism that devotes a large portion of its resources to reproduction will have a slower growth rate and a reduced probability of survival and subsequent reproduction. These reductions are collectively termed the cost of reproduction. The hypothesis is supported by observations that trees grow slowly in years when they fruit heavily, and that pregnant animals lose weight and suffer higher mortality. However, a crucial missing element was experimental evidence: if individuals were manipulated to increase or decrease their levels of reproduction, how would the change affect the rate of growth and survival in a particular species?

Because the orchid readily makes fruits when artificially pollinated, I decided to test the hypothesis using large natural populations in Massachusetts—at the Hammond Woods in Newton and the Case Estates in Weston. With the help of volunteers, experimental plants at these two Boston-area sites were hand pollinated, while other plants in the populations were left untouched as controls. Hand pollination of the pink lady's slipper orchid involves gently spreading open and bending back the petal pouch with one hand, then inserting the index finger of the other hand into the resulting gap between the pouch and stigmatic column until the finger contacts the sticky yellow pollen mass. If one does this just right, the entire pollen mass sticks to the finger in one grainy clump. At the next flower, the process is begun in the same way, but this time one must bend the petal pouch back further to expose the stigma surface. The pollen mass on the index finger can then be rubbed onto the glistening green stigma. If the pollen and the stigma are at the right stage of stickiness, the entire stigma surface will be coated with a covering of pollen.

By the spring of 1985 I was eagerly awaiting the flowering season to see the results of my experiment of the previous year. To my surprise, I found no difference between the control and the experimental plants at the Hammond Woods site, either in the number of plants flowering or in the average size of the plants. At the Case Estates a few more control plants than experimental plants were flowering and the experimental plants seemed smaller, but the difference was not substantial. At this point I had a real dilemma: should I write up the results right away, boldly announcing that there was no cost of reproduction in the pink lady's slipper orchid? Or should I continue the experiment to see if the cost showed up after a second reproductive episode?

Because the two populations seemed to be showing somewhat different patterns, I decided to continue the experiment using a "press"

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*Hand pollinating required physical contortions of the volunteers, but nonetheless it proved to be one of the most popular aspects of the project.*

design. That is, every experimental plant hand-pollinated in 1984 was pollinated for a second time in 1985 and was thus given a chance to make a second fruit. The hand pollinations were successful, with most experimental plants making a second fruit by the fall. As in the previous year, the control plants did not make fruit. By the spring of 1986, the results proved worth the wait. A tremendous difference in plant size appeared between the experimental plants and the control plants, particularly at the Case Estates, where the experimental plants had lost twenty-five percent of their size in comparison with the control plants and far fewer of the experimental plants were in flower.

The results were striking, but I decided to put off writing up the results for publication in favor of conducting the experimental pollinations one more time. By 1987, after the experiment had been repeated three times on individual plants, the cost of reproduction was clearly evident at both sites. The results were slightly different, however, due in part to a contrast in plant health at the two localities. At Hammond Woods, the control plants grew larger over the course of the study, while the experimental plants stayed the same average size. At the Case Estates, however, a disease turned the leaves black every summer and probably killed many plants outright. The average size of control plants at this site declined over the years, but the experimental plants declined in size more precipitously. Clearly, the extra cost of producing fruits added to the stress of disease had major effects on the plants.

The experiment allowed us to test a related hypothesis as well. Scientists have speculated that plants may partially offset the cost of producing fruit by increasing their photosynthetic rate, thereby capturing more light energy. In this scenario, perhaps the chlorophyll might process light energy more rapidly, or the stomates


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*The pollen mass sticking to the index finger of a young volunteer is about to be rubbed onto the stigma Hand pollination results in almost every flower becoming a fruit.*

chamber and then measuring how rapidly the leaf absorbs carbon dioxide from the enclosed volume of air. The experiments showed, however, that the rate of photosynthesis did not depend on physiological changes to the plant, such as fruiting or removal of one of its two leaves, but did vary according to microenvironmental differences. Plants in full sun, whether control or experimental plants, have higher rates of photosynthesis than plants in shady areas.

Four years into the fieldwork I had a good story to write for publication, so I enlisted another of my graduate students, Pamela Hall, to perform all the elaborate statistical calculations needed to demonstrate the exact cost of reproduction. In the meantime, we continued the experiment, repeating the pollination and adding a third site at Broadmoor Audubon Sanctuary in Natick, which had much larger and apparently older plants than the other sites. Though we published our initial findings in 1990, I decided to continue the study, and by 1994, after further analysis by a third graduate student, Elizabeth Stacy, we had found several intriguing patterns. Of the control plants, which had never been hand pollinated, 73 percent had not produced even a single fruit over the entire duration of the

on the leaf surface might stay open wider or longer to absorb more carbon dioxide. Some limited laboratory evidence supports this idea, but we decided—given our success thus far—to test it in the field. With the aid of an infrared gas analyzer, my former student Miao Shili and I examined the experimental and control plants to discover if the experimental plants were absorbing more carbon dioxide than the control plants—a sure indicator of higher rates of photosynthesis. The analyzer works by enclosing a living leaf inside a transparent


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*To measure the rate of carbon dioxide absorption, a living leaf is enclosed inside the transparent chamber of an infrared gas analyzer.*


*The larger plant, on the left, is an experimental plant that never made fruits. The smaller plant is an experimental plant that has made many fruits and has become exhausted.*

study, whereas every experimental plant had produced at least one fruit. At the Hammond Woods and Broadmoor, 50 percent of the plants had produced five or more fruits over the years of the study. At the Broadmoor site, three of the largest experimental plants had produced thirteen, fifteen, and seventeen fruits over ten years in contrast to control plants with no fruit at all. At each site, the effects of fruit production were seen in the lower probability of flowering and much smaller leaf area in subsequent years. However, these effects peaked three to seven years after the start of the experiment. After several successive years of fruiting, the experimental plants seemed exhausted to the point where many were very small and unable to flower. The inability to flower, however, gave the plants a rest, which after a few years allowed them to recover and flower again.

By the summer of 1995, I felt it was time to wrap up this project, as we had clearly established the cost of reproduction. Through the years, I had come to know the characteristics of individual plants, almost regarding them as special summer friends. After eleven field seasons, I leave this project with a great sense of satisfaction: my initial love for and curiosity about this beautiful and unique wildflower species had blossomed into a full-scale scientific investigation yielding new insights into the natural history of the species.

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## Along the Way

Many unusual incidents occurred over the course of the lady's slipper project, but probably the strangest was the appearance of carefully arranged rock gardens at the Hammond Woods field site beginning in 1987. Groups of ten to twenty stones arranged in concentric circles, often planted with native wildflowers and carefully tended by unknown gardeners, simply appeared from time to time. The gardens varied in size from one only three feet across to a miniature Stonehenge six feet across made of elongated stones set upright in the ground. Curiously enough, none of the gardens interfered with my research plants. Over the years, I occasionally noticed cut flowers, bowls of rice and incense, and red-painted Sanskrit letters on the garden rocks. Though I still didn't know who had made the gardens in the midst of my study population or why they had done so, I began to appreciate them for their beauty and even tended them from time to time, removing dead leaves and fallen branches. Finally, in the spring of 1990 I observed an elderly man standing quietly behind a tree. I introduced myself and asked if he knew anything about the rock gardens. He told me that he had built them with a community of Buddhists and that they often came here to worship. Knowing from my wire tags next to the plants that someone was studying the orchids, he had not disturbed the plants and had even helped to keep them free of fallen branches, just as I had cleared his rock gardens. After that one meeting I never saw him again, and the gardens have gradually fallen into disrepair.

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*A Buddhist shrine reminiscent of a miniature Stonehenge at the Hammond Woods site.*