

Artificial Pollination of *Cypripedium* Species

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Introduction

The genus *Cypripedium* comprises around 45 species of hardy orchids. Members of this genus are mostly found in temperate zones in the Northern Hemisphere. The center of diversity of the genus lies in China where most *Cypripedium* species are found. Representatives of the genus are also present in North and Central America and in Europe. Their spectacular features and their hardiness make them a very popular garden plant. Most plants available in the trade, however, have been poached from wild populations, this holds especially for species native to China. Consequently, wild populations are under threat of extinction. Nowadays more and more *Cypripedium* species are artificially propagated from seeds, especially species endemic to North America. Artificial propagation from seeds and subsequent breeding allows for a sustainable supply of healthier and more robust plants for the gardener, as compared to plants poached from wild populations. Artificial propagation from seeds requires skill and specialized equipment and is carried out in laboratories by people that are mostly specialized in *Cypripedium* propagation. However, these people require seeds to start with. This article is the first of a series I intend to write about artificial propagation of *Cypripedium*s from seeds. This series will start with an explanation on how to pollinate *Cypripedium* flowers and is intended to encourage people having flowering *Cypripedium* plants to pollinate their flowers and either sow the resulting seeds themselves or send them to people who can sow them. In this way the reader can directly help to generate a sustainable supply of *Cypripedium* species and to take the pressure off wild populations.

Architecture and Natural Pollination of the *Cypripedium* Flower

The architecture and natural pollination of the *Cypripedium* is explained according to the sketches of a *C. parviflorum* ssp *pubescens* flower presented in figure 1A and B. The different parts of the flower are attached to the ovary. The ovary passes into the column that harbors the various sexual organs of the flower. In contrast to other flowers, the pollen grains in the orchids are glued together on two structures called pollinia. Insects that land on the lip are directed to its opening via the staminode shielding the pollinia from direct contact with the visitor, preventing self pollination. In the lip the insect tries to get out again and it is directed upwards where it has to wriggle its way along the stigma. Pollen grains of another *Cypripedium* flower on the insect body, acquired from a previous visit, will stick to the stigma. After it has passed the stigma, the only way to freedom is across one of the two narrow openings beside the pollinia. As the insect passes the pollinia on its way out, the sticky mass of pollen grains will adhere to the insect's body and can be carried to another flower. After a few visits, the insect usually loses its taste for *Cypripedium* flowers, as it is not rewarded for its pains by any nectar.

The pollen grains attached to the stigma germinate and grow into the stigma and into the ovary via structures called pollen tubes. Upon entering the ovary they fuse with

ovules to form embryos, a process taking around 5 to 12 weeks depending on the species.

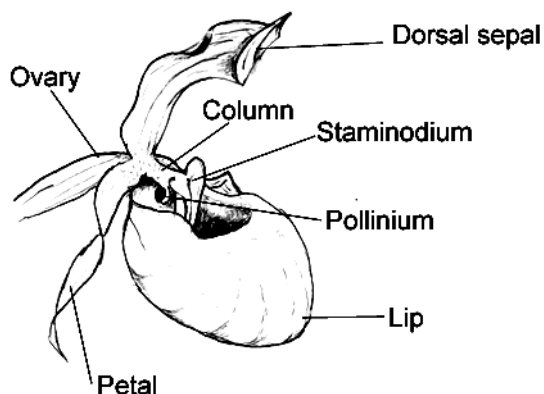


Figure 1A. Overview of a *Cypripedium* flower.

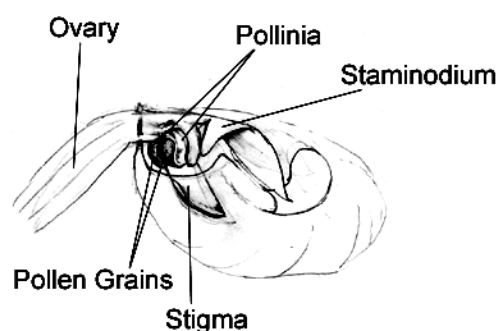


Figure 1B. Inner structure of a *Cypripedium* flower. The position of the lip surrounding the several organs is roughly sketched.

When to Pollinate a *Cypripedium* Flower

After successful pollination, the ovary develops into a seed capsule carrying the seeds. The development of the seeds requires extra energy and resources from the plant. It is therefore advisable to pollinate plants that are vigorous and well established. The plant usually compensates for the extra energy required for the development of the seed capsule by lengthening the active life of the shoot carrying the capsule. When the plants are appropriately fertilized and good cultural conditions are provided, the plants do not experience any penalties from carrying a seed capsule. A single shoot *C. parviflorum* ssp *pubescens* plant, which I pollinated, and subsequently carried a seed capsule, developed three flowering shoots and a minor shoot the next year. However, it is not advisable to pollinate all flowers on a plant carrying multiple shoots as this weakens the plant. This has probably to do with the fact that per shoot, less soil and light are available as compared to a plant having only one or a few shoots.

Artificial Pollination of *Cypripedium* Flowers

Artificial pollination has the advantage over natural pollination in that the grower can choose the parent plants and decide whether to pollinate the plant with its own pollen (selfing) or with pollen of a different plant of the same species (cross pollination) or even another species (hybridizing). To avoid accidental natural pollination by insects the flower may be protected by netting. Although the whole architecture of the *Cypripedium* flower is aimed at avoiding self-fertilization, selfings do not seem to result in a lesser quality of the seed and the subsequent offspring, provided that the plant itself is not bred in already. A selfing is to be preferred if one wants to preserve desirable characteristics of a plant, such as albino forms. This trait is lost in the

offspring when the mother plant is pollinated with the pollen of a colored plant. One may choose for cross-pollination or hybridization if one wants to mix the desirable characteristics of two different plants.

Artificial pollination also has the advantage that the pollen load that is brought on the stigma is much bigger as compared to natural pollination. A good time to pollinate *Cypripedium* flowers is 4 days after the flower has opened entirely. To pollinate the flowers, I use a hook that is used by dentists to test for dental cavities. The curve in the hook makes it the ideal instrument to reach the stigma in the interior of the flower without damaging it. A toothpick can also be used but it is more difficult to reach the stigma and subsequently get the pollen grains onto it. The procedure is depicted in figures 2A and 2B.

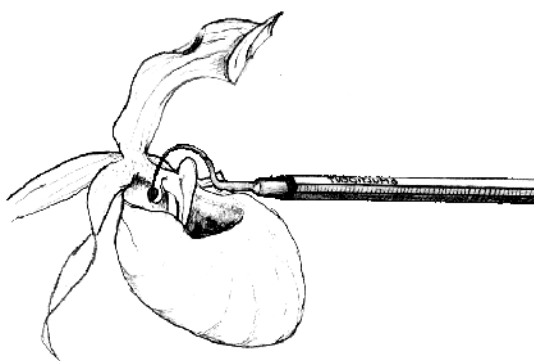


Figure 2A. The pollen grains are scooped from the pollinia with the inner site of the point of the hook.

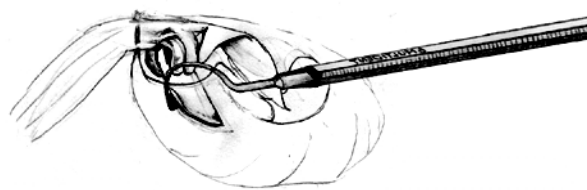


Figure 2B. The pollen (dark mass on the point of the hook) is gently rubbed over the stigma.

The pollen grains are gently taken up by scooping them up from one of the pollinia with the point of the hook (figure 2A). If the pollen mass is dry, the hook may be wetted with some saliva to make the pollen stick. It is best to have the pollen on the inner side of the point of the hook. If this procedure is performed carefully, the pollinia are not removed from the flower. The hook is then moved into the back of the interior of the flower and the pollen are put on the stigma by gently pushing and rubbing without damaging the stigma (Figure 2B and Figure 3). A skilled person can perform the whole procedure within half a minute after which the hook is cleaned with a cotton pluck drenched in 70% (v/v) alcohol. The alcohol will kill the pollen remaining on the hook. This is done to avoid accidental cross pollination. Make sure no alcohol is left on the hook when proceeding with the next flower. After



Figure 3. Photograph of a *Cypripedium smithii* flower being pollinated as described in this article.

pollination, put a label on the part where the ovary passes into the flower stem. The label should state the date of pollination and the names of the parents. After successful pollination, the ovary will start to swell after around two weeks and will develop into a seed capsule. In some areas weevils occur that may attack the seedpod and eat its contents from inside out, rendering it useless for propagation. To prevent this, the developing ovary may be protected by fine, thin netting.

Harvesting the Seedpod

There are two options to harvest the seed capsule. The first is to harvest the capsule at a stage that the fertilization has just occurred and is referred to as the “green pod method”. At this stage the seeds are not fully developed, and dormancy factors that prevent germination of the seeds are not yet present but will eventually accumulate as the seed capsule matures. Consequently, the capsule must be harvested after development of the embryos but before the onset of accumulation of dormancy factors. This requires a very strict timing. The time between pollination and harvesting the immature seeds is depending on the species and on the prevailing weather conditions*. After harvesting, the “green pod” is sterilized from the outside, cut open and the seeds are directly sown on an appropriate medium in the laboratory. As the seeds are not fully developed, they cannot be dried and stored and must be sown immediately. After sowing, the seeds can be examined with a magnifying glass or a microscope to see whether they contain embryos. When the seeds do not contain embryos, no germination will occur. It is advisable to perform the “green pod method” on plants carrying multiple seed capsules. When after examination the seeds do not contain embryos, another capsule can be sown at a later stage. When the “green pod” is harvested at the correct time, the percentage germination is often higher as compared to the method using mature seeds.

The second option is to leave the seed capsule on the plant until ripe. This is in most cases around September-October. However, some plants such as *C. formosanum* may require longer times. The pod is ripe when it changes color which may be from green to yellow/brown. Harvest the pod by cutting it off the plant before it opens. Store it in a coffee filter on a cool and dry place and let it dry out. When the pod is completely dry, it can be opened and the seeds must be stored in the fridge. This can be done by placing the coffee filters with the dry seeds in an airtight container. Ideally, seeds are stored in a glass vial in an airtight container containing calcium chloride that functions as a desiccant. It is advisable to put the calcium chloride on the bottom of the container and the vials on a “table” in the container. The container with the seeds can be stored in the fridge. Seeds of *C. parviflorum* ssp *pubescens* that were stored this way for 4 years still germinated profusely. Put labels on the vials mentioning at least the parentage and date of storing the seeds in the fridge. When sending the seeds to other people, put them in a small paper bag such as the ones used by stamp collectors. Make sure the seals do not leak by putting cello tape on them. Put a label on the baggie with parentage and date of cold storage and put it in a padded envelope to prevent the seeds from being crushed by the machinery handling the mail. The padded envelope is not a luxury but really essential.

I hope that this article will persuade other Cyp enthusiasts to pollinate the flowers on their plants to ensure propagation. In this way we all can contribute to sustainable Cyp breeding and take the pressure off from wild populations. Artificially propagated plants are mostly free of diseases associated with plants from the wild and are generally of a much better quality because the plants are raised from seedling to flowering sized plants under the artificial conditions we provide. As Cyp breeding goes on, the quality of the artificially propagated Cyps will increase and prices will drop, putting an end to poaching the plants from nature. If you are not able to sow the seeds yourself, or you don't know any people who can, you may send the seeds to the Orchid Seedbank Project, ask at the Cypripedium Forum for potential sowers, or contact the author (Addresses below). I will either sow the seeds myself or send them on to other sowers.

* I only have a very few flowering-sized *Cypripedium* plants myself at the moment. Therefore, I hardly use the "green pod method" and I am currently not able to provide the reader with the times required between pollination and harvest of the "green pod" for the different species. I would be obliged when other workers may provide me with these times so I may present them in an update of this article. I don't have many flowering-sized plants as I never buy any *Cypripedium* plants for reasons of conservation. Occasionally, I get divisions from friends in return for seedlings, but the great majority of my plants I have sown myself from mature seeds that were sent to me. Next year I expect the first flowers on the plants I raised from seeds. Hopefully these plants will yield seeds thereby completing the cycle.

Acknowledgements

I wish to thank Bill Steele, (Spangle Creek Labs, <http://www.uslink.net/~scl/>) and Paul Perakos for critically reading the manuscript.

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