Species Seed Propagation

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When I was a young boy I used to follow my grandfather Opa around in his greenhouse, and at the end of every days work we would collect a handful of flowers and take them up to the house to Oma. He didn't grow orchids, but he was a masterful gardener and a gentle loving man. He taught me many things about plants, most of which I don't remember, but he did leave one part of his skill with me forever, the wonderful art of observation. He also left me with his passion for flowers.

All of my work with species orchids has evolved from observing results. From pollination to the harvest of seed, and even effects of different media on germination, my work is based on results. It is result oriented data. What I have done in the last five years with Hoosier Orchid Company is not as important as how I have done it. I've done it simply by keeping good records and changing one variable at a time. I've tried to look at the different problems associated with species, analyze the results we have obtained, and end up with the simplest series of steps to conserve orchids through asymbiotic propagation.

The first step in species propagation is blooming your species orchid. Many authors have written wonderful works on the cultivation of orchid species and I will add just a few points. The main thing to keep in mind is that it is natural for a species to bloom. A flowering plant will try to bloom even with the poorest of treatment because its evolutionary existence is based on its blooming and being pollinated and preserving its genes for another generation. The other recommendation I have is to get educated; periodicals, books, orchid societies all exist for your enrichment. Take good advantage of them. Regardless of how good a grower you are or how well your plants are growing, if you do not impose a proper rest period, or low night temperatures, or a myriad of other cultural practices, certain species will refuse to bloom.

Darwin once wrote, "I never was more interested in any subject in my life than this of Orchids," and his main interest was with pollination. Orchids are the most highly evolved of all flowering plants and many of their pollination mechanisms are very specialized and specific to their pollinators. Co-evolution is a complicated theory but we must in many cases look at a species pollinator for clues to help us pollinate our plants. Orchids with white flowers, sweet fragrances and sugary nectaries are pollinated by moths nocturnally, and this is when we should expect the best results. Many orchids intensify their scents as the heat of the day increases correlating to an increased metabolism of their cold-blooded insect visitors. There are whole groups of orchids which will not accept self pollination. This is understandable with such an advanced group of plants, knowing inbreeding is a genetic dead end. Orchids are pollinated when the pollinia from the anther is placed onto the stigma, but orchids are equipped with a self pollination protective tissue called the rostellum, located in between the anther and the stigma. In Masdevallias this tissue is enlarged into a translucent flap which lies directly over the stigma. The unsuspecting pollinator forces the pollinia down on top of this tissue and the pollen never reaches the intended female receptacle. Traps and deceptions abound in the higher orchids. An amazing phenomenon called dichogamy takes place in the genus *Mormodes* of the *Catasetum* subtribe, and in many members of the *Stanhopea* subtribe. Here the flowers open in the male phase unwilling or unable to accept pollination. It is only after the pollinia are removed by a bee, and air dried riding around on its back, that the stigma has time to swell and will accept the pollinia, thus ensuring cross pollination. These are just a very few examples of how natural pollination can guide our artificial reenactments. Almost without exception the higher orchids have adapted to cross fertilization, to the extreme in the Catasetums, exhibiting sexual dimorphism. These plants can bloom male, female, or perfect flowers all on the same inflorescence or at different times on different inflorescences. With the sexes being separated by space (instead of time as in the last case) the pollinator must visit another flower on a separate individual to effect pollination.

We try to copy these natural processes and whenever possible we make an outcross between two distinct individual clones, selecting for vigor above color or size. This ensures the largest genetic diversity in our seedling population. Self pollination is advantageous when a unique or desirable parent exists or when only one plant of the species is in the collection. It is also a good idea to make multiple fruits on both parents. The lower flowers usually accept this burden best. If multiple fruits are set, you are then able to harvest one as an immature fruit and test the ripeness of the seed. Flowers should ideally be pollinated shortly after anthesis (opening), although it is several days in some cases before the fragrance cue for the pollinator is noticed, and this is an adequate waiting period. Look for fragrance and a swollen sugary stigma as clues to begin pollination. Flowers in the *Stanhopea* subtribe, some Dendrobiums, and most Sobralias are ephemeral, and are only in bloom for a few hours to a few days, and therefore must be pollinated immediately upon opening. Use all of the pollinia from one flower to pollinate another, because one pollen grain fertilizes one ovule which produces one seed. Using all the pollen from a flower increases your chances for success. Many times the first attempt at pollination will not be accepted, and you are left trying to pollinate flowers past their prime. A seedling blooming for the first time may die trying to produce seed, or not be vigorous enough to hold the seed capsule to maturity and will abort seed production. Choose only healthy well established individuals as mother plants. Orchid viruses are transmitted through their pollinia. If a plant is suspected of virus, it is best to use the infected plant as the mother or "pod" parent. The seed should then be harvested as dry seed. Immature seed from virused parents may be infected by placental tissue of the fruit during flasking. Unripe fruits which show obvious rot may internalize these pathogens and contaminate the developing seed inside.

If all goes well, fertilization of the egg and formation of the zygote will follow pollination. From this point on we wait and observe and treat the plant normally. We must remember that production of viable seed is a vital step in preservation of the plant's genome. Orchids which require a rest period after blooming should be rested. Undo stress can cause plants to dehisce unnaturally. Excessive light or heat, overfertilization, and overwatering are common causes for early splitting. When a fruit undergoes these stresses it may ripen the seed more quickly or it may abort the maturation process. Self pollinated fruits may linger on for some time and eventually abort seed production. Even if seed is carried to term the viability is usually reduced and the seedlings are slower to develop.

Some species of *Stenorrhynchos* may split open as early as thirty days while species in the *Vanda* and *Paphiopedilum* alliances will not mature for a year. Some early warning signs of natural dehiscence are soft, or yellowing fruits. If the diameter of the fruit has not increased for several weeks, after a reasonable period for the genus, the seed should be ready for immature seed culture. This period is normally two thirds of the way through its natural dehiscent time. With daily observation, using the table provided in the appendix of harvest dates for "green capsule culture", no seed should be lost. Green capsule culture, or harvesting the fruit in an immature state of development, does have advantages over the natural splitting of the fruit. The seed remains sterile inside the protective capsule and does not come in contact with the infecting pathogens in the air. If the seed is allowed to mature most of the way through its normal development, the seed germinates quickly, saving valuable time in a lengthy juvenile period. If we are not patient and harvest the fruit too soon, the embryos are slow to develop, and germination is slow or aborted altogether. Our other alternative is to let the capsule develop to maturity naturally. In most instances this is the preferred method. First and foremost we know the parent plant has done all it can to produce good viable seed. By staining the seed with Tetrazolium stain, which stains for living tissue, we are able to tell not only whether the seed has viable embryos but also what percentage per sample should germinate. We are able to make multiple sowings, and therefore correct any possible earlier fungal infection or oversterilization.

Excess seed can be dried and stored for an extended period of time. By routinely checking the viability of stored seed, a shelf life can be predicted for different groups of orchids. *Stanhopea, Zygopetalum*, and *Masdevallia* allies have delicate testa (seed coats), and are killed easily by oversterilization or extended storage by conventional methods.

Orchid seeds in nature are very small and dust-like, and are distributed by the wind upon drying of the capsule. After swelling from water uptake and possibly limited metabolic activity, the seeds rely upon infection by a specific symbiotic fungi to breakdown stored food reserves in the embryo and facilitate the uptake of minerals and sugars. This mycotrophic (dependance on fungi for survival) relationship is very complicated and not completely understood. In cultivation, we bypass this association entirely and grow the seed asymbiotically (without the fungi) on a standard nutrient media preparation. At Hoosier Orchid Company, we have successfully germinated heavily mycotrophic dependant terrestrial species in the genera *Aplectrum, Calochilus*, and *Cypripedium*, as well as many tropical epiphytic species all on a simple media formulation. Obtaining good viable seed to start with is the key to success with flasking, not a complicated media inundated with complex additives. If dry seed is the method of choice, as the fruit begins to split, the first priority is to decrease the moisture content in the seed by air drying. Fungal spores will breed around moist seed and kill it quickly. Once dried, each small seed must be sterilized individually. In this sterilization process, a narrow window exists when external pathogens are killed and when some viable seeds are also destroyed. An extended sterilization period or too high a concentration of the sterilant will greatly reduce the germination percentages.

The seed is sown in small batches and multiple sowings. If a flask contaminates or does not germinate properly, an adjustment can be made accordingly. With immature green seed the outside of the fruit is sterilized and the seed inside is extracted free from contamination. Regardless of the method used, eventually germination ensues and the flask of protocorms is placed in a culture room with warm temperatures and moderate light. The seedlings will need to be transferred to a new flask when overcrowding takes place and the media starts to break down. On each transferral to a new flask, each seedling should be given more room for growth. Choosing the proper time for transflasking is much more important than the formulation of the nutrient media.

After one to three years the seedlings should have well developed leaves and roots and be able to survive on their own out of flask. When deflasking orchid seedlings try to disturb their roots as little as possible. Wash the plantlets in tepid water and plant in community pots according to their size. Do not add any special fungicidal or fertilizer solutions for an unforeseeable problem in the future. Give the immature plants a sheltered environment until new root growth is noticed. Remember that a young plant needs as much air to the roots as an adult and guard against overwatering. During the first few months the seedlings will lose most of the "water" roots developed in flask and develop the "air" roots typical of normal adult epiphytic orchids. Two to eight years later we can expect the juvenile period to end and we may see the more vigorous seedlings bloom for the first time.

At Hoosier Orchid Company we hope species seed propagation lessens the burden on wild orchids through overcollecting. Saving seed and pollen for a gene bank even on a small scale could also play a vital role in conserving species. We may never be able to preserve the wild orchids because of habitat destruction, bureaucracies, laws of ignorance, and overpopulation demands. We will probably never reintroduce a cultivated species back into the wild. But our hope is to save the species for another generation, because, after all saved in cultivation is better than not saved at all. The outlook for orchids in the wild is not very good but hopefully I will still be able to show my grandchildren species orchids and leave them with an appreciation of nature just as I learned as a young boy.

Selected references:

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