Life in the Freezer

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FRANCO PUPULIN

IT IS TEMPTING TO GIVE WAY TO pessimism about the future prospects for orchids. Today, many ecotourists visit the tropics on a mission that Douglas Adams, author of *The Hitchhiker's Guide to the Galaxy*, poignantly described as being a "last chance to see." The choice before us is clear. Are we prepared to allow many orchid species to go extinct, or will we rescue those at risk from oblivion using *ex situ* techniques? Already, the known distribution of *Paphiopedilum rothschildianum* has been reduced to just three localized populations on the slopes of Mount Kinabalu in Borneo, and many other slipper orchid species are classified as being critically endangered (Cribb, 1998). Dave Roberts, PhD, of the Royal Botanic Gardens, Kew, recently determined that *Angraecum longicalcar* had declined to a population of probably no more than 25 plants in its natural habitat in Madagascar (Seaton, 2007). In Australia's state of Victoria alone, 208 of the 372 orchid taxa are classified as being either threatened or extinct according to the IUCN (International Union for Conservation of Nature) 2001 Red List categories and criteria. However, little is known about the status of the vast majority of orchid species in the wild, and it is likely that many species are teetering on the edge of extinction (Koopowitz, 2001). It is our contention that many orchid species will either be preserved in living collections or, in all probability, they will suffer the fate of the thylacine.

The last captive Tasmanian tiger or thylacine (*Thylacinus cynocephalus*) died in Hobart Zoo on September 6, 1936. Despite the setting-up of a national park to preserve this marsupial, with its enormous gaping yawn, it has not been seen since the 1930s, its ignominious fate to be remembered as a collection of stuffed specimens displayed in natural history museums around the world, plus seven sad short, grainy, black-and-white film clips available on the Internet. The hope remains in some circles that DNA technology will enable us to bring this remarkable creature back to life in the future, but in all probability this will not be. The thylacine has gone forever, as have a small, but increasing number of orchid species and varieties, where all that remain are perhaps some illustrations, including paintings that record the past-awarded plants of the United Kingdom's Royal Horticultural and North of England Orchid Societies, and a few pressed specimens in herbaria. Whatever happened to all the beautiful awarded varieties of *Odontoglossum crispum*, for example, so meticulously recorded by Nellie Roberts in the later 19th and early 20th centuries, is a mystery.

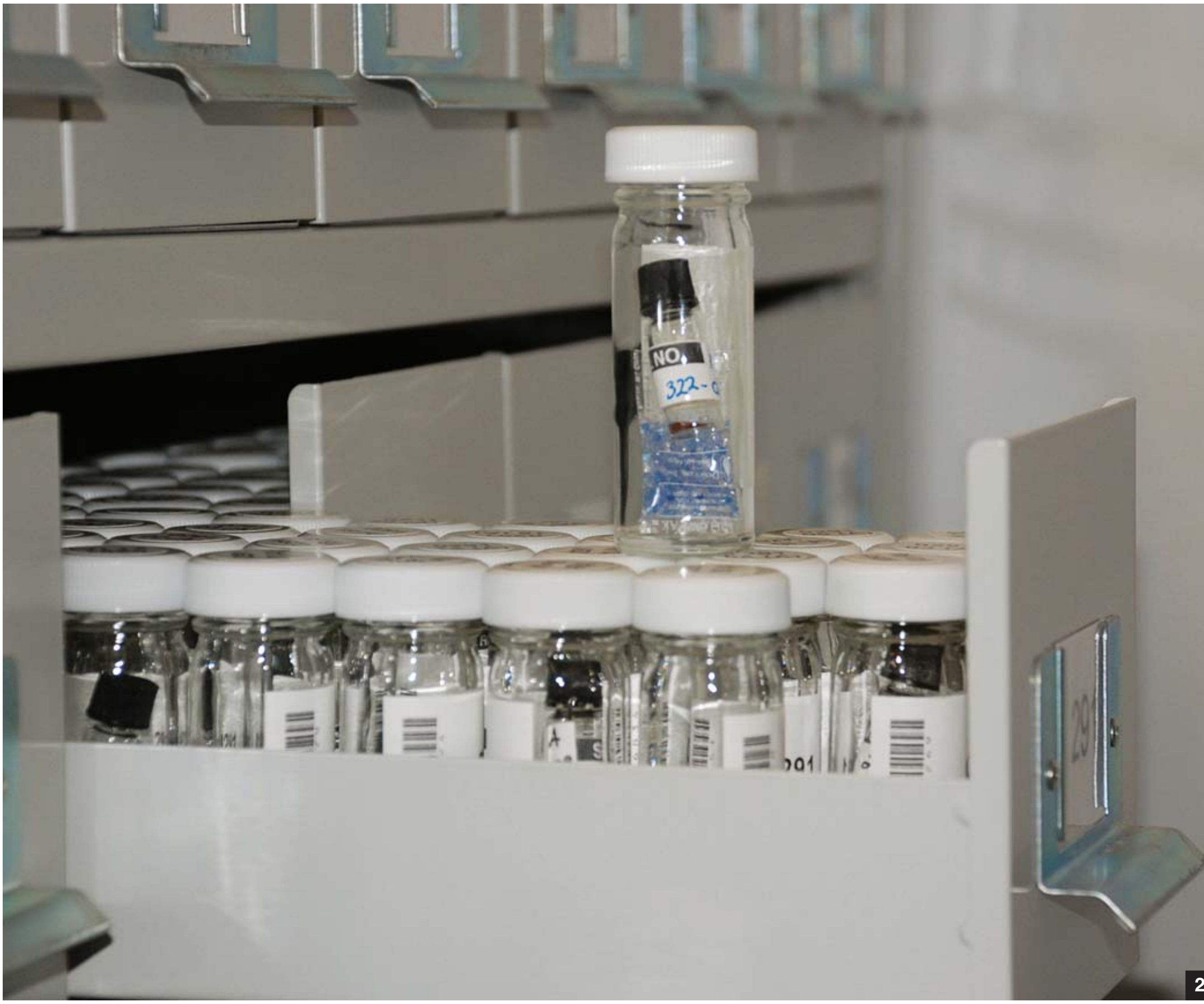
In contrast, the tale of spix's macaw (*Cyanopsitta spixii*) is one of a last-minute recovery, although this bird's long-term survival remains in the balance. Native to woodland galleries in the caatinga of northeastern Brazil, this exquisite blue parrot was collected to the point where only a solitary male remained in the wild (Juniper, 2002). In 2000, it also disappeared. From an original handful of birds (just 11 were known in captivity in 1999), a captive breeding program has increased numbers to the present 73 in collections around the world.

Happily for orchid enthusiasts, *ex situ* conservation, especially when compared with the problems inherent in breeding rare birds, is usually relatively easy. Most orchid species can be raised from seed with little more equipment than can be found in the average kitchen (Seaton and Ramsay, 2005). Instead of a slow recovery in numbers, they can be raised by the thousands in a few years.

Nevertheless, as burgeoning human populations combined with increasing material aspirations commandeer an ever-increasing proportion of limited global resources, pressure on wild populations is mounting. It is our opinion that we need

Orchid Seed Banking for the Future

BY PHILIP T. SEATON AND HUGH W. PRITCHARD



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to take advantage of the remaining window of opportunity to preserve and multiply species in *ex situ* collections, in the hope and expectation that opportunities may arise in the future for species recovery and possible reintroductions. The need for action is urgent.

INTERNATIONAL POLICY Attitudes have changed since the days when Victorian collectors removed plants from tropical forests by the thousands to satisfy the demands of orchid growers in temperate countries. Today, orchids are recognized as being an important component of many countries' natural resources. Article 15 of the Convention on Biological Diversity (CBD) recognizes the sovereign rights of states over their own flora and fauna, declaring that access to genetic resources and associated fair and equitable benefit sharing should be on "mutually agreed terms." Removal of orchids without prior agreement of the relevant authorities is an infringement of the CBD. Orchid seeds are covered by both the environmental laws of the host country and by international regulations. Before seed is collected, it is necessary to obtain permission from the relevant authorities. Likewise, seed from cultivated plants must be derived from legally collected plants. CITES

[1] Each participating country will choose one or more flagship species to use to promote the seed-storage project. One chosen by Costa Rica is *Cattleya dowiana*. Populations of the warm-growing "*guaria turrialba*" are found in a reserve in Talamanca, Costa Rica.

[2] At the Millennium Seed Bank, small vials of orchid seed are placed within larger tubes to ensure no moisture enters, and the seeds remain dry. The tubes are stored in drawers in cabinets in the cold room at -4 F (-20 C).

(Convention on International Trade in Endangered Species) regulations clearly state that illegally collected plants cannot be used as parents to generate legally valid seedlings.

In case the above sounds rather draconion, it should be remembered that there is a clear commitment on behalf of the global community to plant (including orchid) conservation, and that, in 2002, the Conference of the Parties to the CBD adopted the Global Strategy for Plant Conservation (GSPC) (www.bcgi.org/worldwide/gspc/). The objective of the GSPC is to halt the current and continuing loss of plant diversity through 16 outcome-orientated global targets for 2010. Target eight is of particular relevance to *ex situ* conservation: stating that 60 percent of threatened plant species should be in accessible *ex situ* collections, preferably in the country of origin, and 10 percent of these species be included in recovery and restoration programs. Indeed, in 2001, at the first International Orchid Conservation Congress (IOCC), the orchid community agreed to adopt and enhance four resolutions from the GSPC: 1) considering that 90 percent of threatened orchids should be secure in *ex situ* collections; 2) 50 percent should be in active recovery programs; 3) no orchids should be threatened by unsustainable harvesting; 4) and that every child should be aware of plant diversity (including orchids) by 2010 (Dixon and Phillips, 2007).

Further support for *ex situ* strategies for conservation came with the Gran Canaria Declaration II in 2006. This asserts that "*ex situ* collections have a key role to play in securing the conservation of wild plant species as natural resources, as an insurance policy for the future, as a basis for restoration and reintroduction programs and as support for adaptation of livelihoods to climate zones."

Orchid-seed banking provides what Richard Fortey, senior palaeontologist at London's Natural History Museum until his retirement in 2006, has described as "an insurance policy against the destruction of habitats that wise stewardship of the planet should never let happen." We believe that seed banks are an invaluable component of any conservation tool box. Weighing a few micrograms at most — a single seed of *Stanhopea oculata*, for example, weighs about 3 micrograms (Arditti, 1992) — a collection weighing 1 gram may contain as many as 350,000 individuals, and occupy a volume of a few cubic centimeters. Theoretically, a representative sample of seeds from all of the world's orchid species could be stored in a space no larger than that occupied by a few domestic refrigerators. Thus, seed banks can provide a means of preserving maximum genetic diversity in a minimum space, and at relatively little cost (Seaton and Pritchard, 2003).

ORCHID SEED STORES The Darwin Initiative is the United Kingdom's response to the 1992 Rio Earth summit, and addresses concerns over the security and exploitation of the world's biological diversity. It is administered by Defra (Department for Environment, Food and Rural Affairs) with the aim of funding joint projects between United Kingdom institutions and partner institutions in countries rich in biological resources. Since 1993, it has invested 65 million pounds in 601 projects in 148 countries (http://darwin.defra.gov.uk/). Orchid Seed Stores for Sustainable Use (OSSSU) is a three-year Darwin Initiative project, headed by Professor Hugh W. Pritchard (project leader) and Phil Seaton (project manager) of the Royal Botanic Gardens, Kew's Seed Conservation Department at Wakehurst Place. In many respects, this project is the realization of a dream, one of a global network of orchid seed banks. At this facility, white-coated technicians withdraw samples from cold storage to monitor the viability of their precious cargo. Gleaming white rooms are filled with emerald seedlings growing in sparkling glass vessels under bright fluorescent lamps.

The reality of OSSSU is perhaps a little more down to earth, but just as optimistic. The project began officially

Taking a Look Inside the


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A grey steel door set into a yellow wall leads to the seed bank.

LOCATED in the green rolling hills of the English countryside, about 40 miles (64 km) from central London, the Millennium Seed Bank, the seed conservation arm of the Royal Botanic Gardens, Kew, resides within the gardens of Wakehurst Place. Kew's Plant Physiology Unit was originally housed in the Elizabethan mansion, but was moved into its iconic new purpose-built premises nearby in 2000. The Welcome Trust Millennium Building, with its distinctive barrel-vaulted roofs, houses both the Millennium Seed Bank offices and laboratories, as well as a centrally-placed public exhibition area with a wealth of visual and interactive interpretation designed to engage people of all ages. From the latter, visitors can watch scientists at work through windows running alongside.

The principal aims of the Millennium Seed Bank Project are to conserve all of the United Kingdom's bankable species by the year 2010; to collect and conserve 10 percent of the world's plants by 2010 (some 24,000 species) principally from the drylands; and to develop bilateral research, training and capacity-building relationships world-wide in order to support and to advance the seed conservation effort.

On entering the public area of the building, the overall impression is one of light and space. The hive of activity seen through the windows, and the hushed atmosphere within, denotes serious science taking place. When they first arrive, all seeds, including orchid seeds, are placed in the dry room for processing, where they are held at 59 F (15 C) and 15 percent relative humidity. Reemerging from these conditions is reminiscent of on October 1, 2007 (a great deal of groundwork was carried out before this date) with the aim of promoting a self-sustaining network of orchid-seed banks around the globe using conventional seed-banking techniques. Its primary objective is the collection, storage and *in vitro* propagation (conservation biotechnology) of seed of a minimum of 250 species, representing approximately one percent of the species in the Orchidaceae, focusing on orchid hotspots in the Asian and the Central and South American regions, and bringing together orchid biotechnologists from 16 participating countries.

The initial focus is on plants that are available in living collections in botanical gardens, and commercial and private collections. Although acquisition of seed derived from species in the wild may be desirable, and such seed collections are certainly not precluded by the project, they were not included in the original application because of potentially high collection costs. Raising plants specifically for seed collection in living collections, with their advantages of enabling controlled pollinations and the ability to monitor seed capsule development, is frequently a more practical option. Likewise, it was decided that, at least in the initial stages, it was not necessary to focus on rare species. Indeed, what is common today all too often has the nasty habit of becoming rare tomorrow. There is also the problem of deciding which species are rare. Although it could be argued that Red Lists would be the ideal tool for assessing the conservation status of species, presently there are few orchid species included in the 2004 Global Red List. Brazil and Ecuador, however, are completing their National Red Lists (and we are hopeful that there are others of which we are currently unaware), and one can only hope that other countries will follow suit soon.

Working with just 250 species in the first instance may be modest, but our objective is to significantly exceed this one percent target, and to establish a firm foundation for long-term storage of orchid germplasm around the world, with the eventual aim of involving an ever-increasing number of countries. Indeed, the names of Kunming, Yunnan Province, China, and the Botanical Gardens of Universidad Autonoma de México (UNAM), Mexico City, have been added to the list of project participants since the two workshops took place. There is much more involved in establishing an orchid seed-banking network than simply collecting and placing seeds in a freezer. It has taken 20 years to reach this stage of development, while the scientific basis for action has been improved and confidence built internationally.

ORCHID HOTSPOTS When making our grant application, a number of decisions had to be made. Which countries should be invited to participate in establishing an orchid-seed-banking network; which institutions within those countries would be most appropriate and whom should we invite? Although all three questions were intimately entwined, the most important was whom to invite. Margaret Mead, the famous anthropologist, when asked if it was really possible for a single person or a small group of people to change the world, responded, “It is the only thing that ever has changed the world.” We were looking for a special group of individuals, those with both the expertise and the commitment to carry the project forward in suitable institutions within countries with high levels of orchid biodiversity.

The obvious starting point for such a project appeared to be within the biodiversity of “hotspots,” the brainchild of British ecologist Norman Myers. His idea was to identify the most biologically diverse regions so that, with limited financial resources, conservation efforts could be applied where they would make the most difference. But how do you define a hotspot? One method is to rank areas by the number of species that live there; another is to rate how threatened an area is; and yet another uses regions that have high levels of endemism (many species confined to small geographical ranges). Conservation

Millennium Seed Bank

disembarking from a plane in a tropical country: with that characteristic warm moist earthy aroma. Although far from tropical, the everyday levels of atmospheric humidity in the United Kingdom are not conducive to the longevity of seeds.

After processing, the seeds are transferred to the seed bank. Descending the gleaming stainless-steel spiral staircase to the underground vaults visitors enter another world. Swinging back the heavy leaden door set in a canary yellow wall, you step from an atrium flooded with light from above into the artificially lit cool white space of the bomb and radiation-proof concrete bunker that is the seed bank. All around, the seeds are being processed ready for storage at –4 F (–20 C). To enter the cold rooms, first you have to fight your way into a fetching blue fur-lined all in one, best achieved by sitting on the floor. Fur boots, fur mitts and a peaked hat with ear muffs complete the outfit, so that you resemble someone who wouldn’t look out of place embarking on an Arctic expedition. I hadn’t realized that –4 F (–20 C) was so cold. However, you are reassured, as upon entering the cold rooms an alarm system is activated, so that you will be retrieved before you are conserved for the future.

The seed accessions are stored in labeled bottles, double contained within Kilner jars. The labelling relates to a comprehensive database collating information on aspects of the collection: from where it was collected to how many seeds are in the jars. The state of the seeds and their vessels are monitored. That this generally takes place once every ten years demonstrates the confidence placed in the workings of the seed bank.

One of the most important services offered to MSBP partners worldwide is the safe duplication of seed collections at the Millennium Seed Bank. This vital insurance policy ensures that seeds will be stored under the best possible conditions so that they are available in the future to be used for re-introduction, habitat restoration and sustainable use projects, particularly in the countries of origin. But this is only part of the work within the seed bank. There is tireless effort spent in developing the scientific understanding of processes involved in seed storage and germination, and in characterizing their properties. No one has ever looked in so much detail before at the vast majority of seeds that are currently being examined at the Millennium Seed Bank. — *Philip Seaton and Tim Marks.*

International currently recognizes 34 distinct biodiversity hotspots, defining such areas as containing at least 1,500 species of vascular plants as endemics, and having lost at least 70 percent of their original vegetation cover (Synge, 2005). The question for those concerned primarily with orchid conservation is: Are the equivalent orchid hot spots found at the same locations as those for other species? Cribb considered this key issue in his opening address at the 18th World Orchid Conference in Dijon, France (Cribb and Govaerts, 2005), where he came to the conclusion that orchid distribution mirrored that of flowering plants as a whole. A map showing where they are most dense included the South American Andes, Madagascar, the Malay archipelago, Australia and China, for example. In the end, the decision was made to initally focus the OSSSU project on Asia and Latin America, thereby including as well as the more obvious tropical orchid hotspots, the temperate orchid floras of China and Chile.

ORCHID SEEDS The seeds of orchids are tiny, having evolved for long-distance dispersal on the merest breath of air. The security of a food reserve has been sacrificed in favor of seed miniaturization and, in their natural environment, a potentially dangerous liaison with a compatible fungus to facilitate access to nutrients early in germination. How members of the Orchidaceae developed such a risky strategy is perhaps a mystery, but certainly the sheer number of species testifies to the family's success. Perhaps because of these special characteristics, and despite Lewis Knudson's findings in the 1950s (Knudson, 1954) that dry seeds of at least some orchid species could be stored for at least 20 years at refrigerator temperatures, the view has persisted in some circles that orchid seeds are short-lived. Detailed research over the past 20 years has, however, revealed that while seeds of some orchids are relatively short-lived, the benefits of seed drying are quantifiably similar to those of crop seeds. Thus the basic principle of drying seeds for banking is as pertinent and valuable for orchids as for many other species.

With the exceptions of *Vanilla* and *Selenipedium* species, whose seed capsules are berries and whose seed-storage responses remain to be characterized, for orchid species with seeds that are wind-borne, that is, the vast majority of the family, it is highly likely that they are capable of tolerating dry storage, probably for many decades (Seaton and Pritchard, 2003). Although they tolerate cryopreservation (liquid nitrogen), –4 F (–20 C) and 11 F (5 C) storage the optimum temperature for storage of orchid seeds is not totally clear, as some species have seeds that appear sensitive to storage at –22 to –58 F (–30 to –50 C). To date, however, attempts to freeze-store orchid species have been limited to a relatively narrow taxonomic, ecogeographical and potentially genetic range. Thus, one of the main objectives of OSSSU is to test seed banking on a wide range of orchid species in a living experiment. What follows is a précis of those protocols developed and agreed on at the two workshops (Seaton *et al.*, 2008).

A Resumé of Protocols Established for Setting Up Orchid Seed Stores for Sustainable Use

IDENTIFICATION OF SPECIES Correct identification and accurate, robust record keeping is crucial to enable tracking from parent plant to stored seed and progeny. It is important to ensure that the parent plants have been identified correctly. There is often an interval of several years between germination and first flowering, and the discovery that the species is not what it was expected to be is to be avoided at all costs. Plant names often change as taxonomic understanding improves over time and require checking, as do collection records, and a search should be carried out for an existing herbarium specimen within the institution, thereby enabling linkage between pollen and seed parents, and herbarium specimen and collection records. Where no herbarium specimen exists, either a pressed specimen will be made, or a flower preserved in spirit (a mixture that includes alcohol). At a minimum, specimens will be photographed or drawn. Where seed is harvested in the wild, photographs will be taken of the habitat, and field data recorded.

POLLINATION AND HARVESTING SEED Quality of seed depends on a number of variables. These include the age of the flower (and, therefore, of the pollen) at the time of pollination, whether harvesting mature rather than immature seed, parentage, and environmental conditions during maturation of the seed capsule. If storing wild-collected material, it is important to collect a representative sample of mature seed. Out-crossing between different genotypes is preferable, but where only a single plant of a rare species is available, or one wishes to preserve the integrity of specific varieties, self-pollination may be appropriate. Where possible, a number of flowers should be pollinated, and when a species carries multiple flowers, using 10 percent of available flowers. Freshly opened flowers are more likely to be successful than older blooms, and flowers pollinated as soon as they are fully open. The older the flower the older the pollen, and old pollen is itself likely to be less viable (Pritchard and Seaton, unpublished). To maximize the number of full seeds per capsule, a full pollen load (i.e., all available pollen from one flower) should be applied to the stigma of the seed parent. Label the capsule parent with the date of pollination, recording in a notebook how many flowers were pollinated, and how they were pollinated (by hand or naturally occurring pollinators). Good culture of the capsule parent is important, as unsurprisingly, healthy, well-grown plants produce larger capsules than their less fortunate neighbors.

Although sowing of immature embryos ("green pod" techniques) has advantages in terms of reducing the time a capsule is carried by the parent plant, avoiding problems of surface-sterilization of seeds during the sowing procedure and, in the case of some hardy species, circumventing dormancy mechanisms, the likelihood is that such seed cannot be stored as successfully as mature seed. Drying is a key component of successful orchid seed storage. Seed is, therefore, best harvested at, or just prior to, splitting of the seed capsule. This implies a prior knowledge of the detailed life history of the species in question or regular observation of the capsule development process. Seed should be collected in paper envelopes: bagging of seed capsules (to prevent loss of seed) is not recommended, as the seed capsules tend to "sweat." Date of seed collection and whether the capsule had begun to split should be recorded, and will allow calculation of the time to maturity of the seed capsule, so that this can be related to seed quality. Data on orchid capsule development is relatively limited in the literature (for a current table see Yam *et al*., 2007), and the OSSSU project collaborators aim to add significantly to baseline knowledge in this area.

Once harvested, seed can be cleaned, separating the seed from any debris arising from the seed capsule, for example. To reduce contamination and debris, the ends of seed capsules should be first trimmed and, to remove seed from capsule, the capsule gently squeezed from both ends. One of the project aims is to determine how many seeds are present in the capsules of each of the species in the project.

SEED DRYING AND STORAGE

At one extreme, moist seed will support the growth of fungal and bacterial spores, which will multiply rapidly and kill the embryo. In addition, infected seed soon becomes almost impossible to sterilize and to sow in a sterile flask without contamination. At the other end of the spectrum, life processes depend on the presence of moisture. Thus, reducing seed moisture contents to the extreme can dramatically shorten life-spans: removing moisture entirely will result in the death of embryos.

Seed moisture content is dependent on the relative humidity of the surrounding atmosphere. Although the advantages of storing at low temperatures are understood almost intuitively, the same does not appear to be true for reduced moisture content. Yet the benefits of reducing seed moisture content can be greater than those of reducing seed storage temperature. If placed in a humid atmosphere, seed will gradually absorb moisture; likewise, in a dry atmosphere, seed will lose water to the air. In either case, over time, the seed and the atmosphere will reach an equilibrium, at which point moisture is neither lost nor gained. The aim is to obtain a seed-moisture content as close to the optimum as is practical (neither too high, nor too low). For the project, the recommendation is to dry/equilibrate seed over a saturated solution of lithium chloride, which produces circa12 percent relative humidity at 68 F (20 C). The seed should be equilibrated as a thin layer in the desiccant, well above the saturated salt, for seven days at approximately 59 F (15 C). Orchid seed takes up moisture from the atmosphere rapidly, and to avoid change in seed-moisture content seeds should be transferred to hermetically sealed glass tubes quickly (glass tubes are preferable to plastic, where seeds tend to adhere to the sides of the tubes). Tubes should be as full of seed as possible, thereby minimizing the head space above the seeds.


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[3] At the Millennium Seed Bank, cases along a hallway house seeds of many plant families (not just orchids) where they are stored in cabinets in a cold room at –4 F (–20 C).

A potential problem arises with the long-term integrity of any closure on a storage jar, vial or other container. Glass storage jars, with their combination of a natural rubber seal and a clamp, have been shown to be the best available option by the Millennium Seed Bank Project (MSBP) at Kew, where the additional precaution is taken of renewing the seals at 10-year intervals. However, none of this design are small enough for orchid seed storage. Consequently, we are supplying participants with small tubes that can be placed within the larger, hermetically sealed jars. Sachets of Silica Gel Orange can be included in the larger tubes to act as an indicator (not as a desiccant) to demonstrate that seals have not been broken, and moist air has not entered from outside the storage vessel. Prior to use, the sachets of silica gel should be equilibrated over the desiccant along with the seed. If the seal begins to leak, the silica gel orange crystals will slowly change to green as the relative humidity rises. The first visible signs take place at a relative humidity of approximately 19 percent, when the crystals begin to turn khaki color. In this eventuality, the seal should be replaced, and the seed should be redried. The seed accessions themselves are stored in Kilner jars. Following the Millennium Seed Bank guidelines, the rubber seals will be changed every 10 years.

That reducing storage temperature improves seed longevity is appreciated. Good-quality (high initial germination) dry orchid seed stored in airtight vessels at a suitable seed moisture content, will maintain its viability at a temperature of 40 F (5 C) in a domestic refrigerator for many years. Further reducing storage temperature from refrigerator temperature to the temperature of a domestic freezer (around –0.4 to –4 F, –18 to –0 C) leads to additional increases in seed longevity (Pritchard and Dickie, 2003) and, where such facilities are not already in existence, project partners have been supplied with a dedicated freezer for the project.

Monitoring orchid seed during the storage period will be carried out immediately after drying, and after one month, one year and two years of storage, if possible. However, the aim is for sufficient seeds to be stored for assessments to be made at four years, eight years and beyond at regular intervals. Every time a tube of seeds is opened to withdraw a sample, the seed will begin to reequilibrate with the moisture in the atmosphere. Thus, the remaining seeds should either be re-dried after opening the container before returning to storage, or seed should be stored in a number of individual tubes that will be opened once only. The choice will depend on the number of seeds per storage vessel. Seed lots will be removed randomly each time a sample is due to be sown.

QUANTIFYING GERMINATION

All seeds will be scored and recorded as belonging to one of the following three categories: 1) empty, 2) potentially incompetent (i.e., an abnormally small embryo) and 3) potentially viable (i.e., with a full embryo). Potentially viable seed can be recognized by the presence of a plump embryo, although this is, in itself, no guarantee that the seed will germinate. It may, for example, require a special medium or a compatible symbiotic fungus or, in some instances, a dormancy-breaking procedure. However, the presence of such an embryo in

A Gallery of Flagship Species

VIETNAM *Paphiopedilum delenatii*
Endemic to southern Vietnam, *Paph. delenatii* was lost to cultivation after its discovery in 1922, until its rediscovery in the early 1990s. Its story in cultivation demonstrates the positive role growers can have in orchid conservation.

COSTA RICA *Psychopsis* (syn. *Oncidium*) *krameriana*
Growing from Costa Rica south to Ecuador, and commonly known as the butterfly orchid, with its antennalike appendages and striking yellow and orange pattern, the flowers of *Psychopsis krameriana* hover in the air like exotic insects.

ECUADOR *Dracula* (syn. *Masdevallia*) *simia*
This epiphyte grows in cloud forests.

CHILE *Chloraea gavilu*
Conservation of temperate orchid species such as this Chilean endemic is just as important as conservation of tropical species.

SINGAPORE *Grammatophyllum speciosum*
Plants of this orchid have been raised from seed at the Singapore Botanical Gardens and successfully transplanted into trees within the city.

Prepared by Philip T. Seaton

PHOTOGRAPHS COURTESY PHILIP SEATON EXCEPT FOR *PAPHIOPEDILUM ARMENIACUM* BY HOLGER PERNER

Species that may be showy, endangered or interesting for another reason that a participating country will use to promote the seed storage project

BRAZIL *Sophronitis* (syn. *Laelia*) *sanguiloba*
A representative of the rupicolous or rock-dwelling laelias.

CHINA *Paphiopedilum armeniacum*
Discovered in 1982, this golden-yellow-flowered species is easily raised from seed.

BOLIVIA *Oncidium lykaiosii*
This pretty endemic is rarely seen in cultivation.

COLOMBIA *Cattleya candida*
Endemic to Colombia, *C. candida* (syn. *quadricolor*) is being raised from seed by committed hobbyists as part of a reintroduction program.

GUATEMALA *Prosthechea* (syn. *Encyclia*) *cochleata*
The cockleshell orchid is the national flower of Belize.

INDIA *Paphiopedilum fairrieanum*
Now scarce in its natural habitat due to over-collecting, forest fires and the predations of goats.

THAILAND *Rhynchostylis gigantea*
An example the enormous amount of genetic diversity often present in a species is illustrated in this species with its many color forms (right).

CUBA *Encyclia phoenicea*
Endemic to Cuba and the Cayman Islands, the flowers of the chocolate orchid (left) have the aroma of vanilla and chocolate.

freshly harvested seeds generally suggests the seed is viable. A key criterion for germination is the splitting of the seed coat (testa), but additional criteria may vary among species. For example, the criteria for epiphytic species may be the appearance of rhizoids and the greening of the protocorm. Terrestrial species may or may not develop rhizoids, and are normally germinated in the dark and, therefore, do not turn green at this stage. To ensure that the procedure can be repeated at a future date, criteria for germination will be drawn or photographed.

Many people choose to store seeds in paper envelopes in a suitable airtight jar together with dried rice and sachets of Silica Orange as a practical (but short-term) alternative. Silica Orange acts as an indicator to monitor the integrity of any seals. If any leaks occur, and the indicator changes colour, the seed should be re-dried and the seal replaced. Waxed paper will not take up moisture, and the seed does not stick to it. However, for long-term storage (a number of years), after equilibration to a suitable moisture content, seeds are best placed within hermetically sealed tubes. Glass tubes are preferable to plastic (where seeds tend to adhere to the sides of the tubes).

A minimum of 100 to 200 seeds will be sown per Petri dish, although lower numbers (e.g., 50 seeds) will be acceptable where insufficient seed is available. This may be the case where species have small capsules from which the seed yield is low (e.g., some *Lepanthes*). Germination will be scored at one-month intervals for a minimum of three months, and up to nine months or longer where germination continues to increase, so that complete progress curves can be recorded. Tropical epiphytic species will be allowed to germinate at 77 F (25 C) under cool white fluorescent lights using a 12-hour photoperiod. Temperate terrestrial species should be germinated in the dark at 68 F (20 C).

Orchid Seed Banks Around the World and the Role of the Hobby Grower

Perhaps the largest number of species are stored at Kew's Millennium Seed Bank, with currently more than 900 accessions. But there are a number of other orchid seed-storage facilities, notably those in Australia and Singapore. At King's Park and Botanic Garden, Western Australia, the Orchid Seed Bank Challenge is to seed bank all the orchids from a global biodiversity hotspot. They are now 65 percent toward the 404 target taxa. This includes endophytes for each species as well as efficacy testing. Their hope is that this could become a model for other regions to follow, such work being carried out in tandem with orchid seed bank physiology studies (Kingsley Dixon, PhD, personal communication). Likewise at Singapore Botanical Gardens, possible advantages of liquid nitrogen storage (cryopreservation) are being explored.

[4] One of Darwin's finches from the Galapagos Islands is shown in this logo for the Darwin Initiative.

A SIMPLE METHOD The hobbyist grower, of course, is not likely to have access to equipment in a laboratory. Nevertheless, it would be difficult to overemphasize the importance of drying seed before storage. There can be few more-certain ways of consigning your seed to a rapid death than storing in a refrigerator in vessels that are not airtight. Access to laboratory chemicals such as calcium chloride or lithium chloride that have commonly been recommended for drying orchid seed, however, tends to be a problem for the hobbyist, and we have been looking for a suitable alternative. For the amateur, who may have difficulty accessing calcium chloride, dried rice is a suitable alternative (Seaton and Ramsay, 2005), and this method of drying seed is being used successfully by the United Kingdom's Hardy Orchid Society. The dry seed is stored in a domestic refrigerator at 41 F (5 C), and is available to members for a small fee to cover costs. In my grandmother's days, a few grains of rice were always placed in the salt cellar to keep it free-flowing. Table salt is hygroscopic, that is to say that it takes up moisture from the atmosphere and the rice (when dry), in turn, is used to absorb the moisture from the salt. It may seem strange to use one seed to dry another, but toasted rice has been used as a desiccant for a wide range of both temperate and tropical seeds. Any supermarket brand will do. Simply spread the rice as a thin layer (no more than one or two grains thick) in the bottom of a baking tray, and dry in the oven at around 212 F (100 C) or slightly higher for two hours. It is important to remember that the rice will require regular regeneration as, with repeated use, it will gradually become increasingly moist. The drying capacities (i.e., how much moisture it is capable of absorbing) of rice is also generally unknown, so you should use plenty of it, filling the desiccator at least three quarters full of dried rice.

You may be tempted to use silica gel as a desiccant. This is particularly appealing because it is normally purchased incorporating cobalt chloride as an indicator, which turns from blue to pink when it is moist and in need of regeneration (although the use of cobalt chloride as an indicator is gradually being phased out due to the potential toxicity of cobalt, and is being replaced by Silica Orange). However, as with dried rice, unless it is regenerated each time it is used, silica gel slowly absorbs moisture from the atmosphere, its water absorbing capacity gradually declines, and it produces a different, higher, relative humidity. A second, and potentially serious, problem with using dry silica gel as a desiccant is that when freshly regenerated it can produce low moisture contents, indeed so low that they are potentially damaging to the embryo and actually reduce seed longevity. The use of silica gel as a desiccant is not recommended for long-term storage.

Once harvested, seeds should be examined to check their viability. There is no point storing dead or empty seed. Ideally a sample can be examined under low magnification using a microscope, but a 10× hand lens will often be sufficient for at least a cursory examination. Potentially viable seed can be recognized by the presence of a plump embryo, although this is no guarantee that the seed will germinate. It may require a special medium or a compatible symbiotic fungus or, in some instances, a dormancy-breaking procedure; the presence of such an embryo in freshly harvested seeds generally suggests the seed is viable.

When it comes to choosing a container, many people store seeds in paper envelopes. Waxed paper will not take up moisture, and the seed does not stick to it. For long periods of storage (a number of years), however, hermetically sealed tubes are much better. Glass tubes are preferable to plastic (where seeds tend to adhere to the sides of the tubes). If seeds are being stored in paper packets, a practical (but short-term) alternative is to store the packets in a suitable air-tight jar together with dried rice. Tubes can be stored within storage jars. After equilibration to a suitable moisture content, seeds are placed within hermetically sealed tubes. As described above, sachets of Silica Orange can be used as an indicator to monitor the integrity of any seals if any air leaks occur. The aim is to enable us to judge if moist air is leaking past the seal into the jar. If it is, the seed should be redried and the seal should be replaced.

A number of short-term seed banks already exist in the world of the hobbyist grower and, because the seed is stored for a few years at the most, these are termed active seed banks. As with any bank account, there are both deposits and withdrawals. Members donate seed to the seed bank as soon as possible after it has been harvested. This is especially important if the member is not going to dry it first. Sending seed to the seed banker in paper envelopes is fine. However, franking machines are death to orchid seeds; they simply crush them. Seed should be sent within some sort of crush-proof container. It doesn't have to be fancy, but something reasonably sturdy inside a padded envelope is good. Clearly correct identification of seed is crucial and, if possible, donors are asked to supply a digital photograph of the parent plant(s). This can then be included in a suitable database, alongside other relevant information, including information on suitable germination media, and seed viability where the information is available. Particular attention should paid to accurate labeling. There is nothing more frustrating than flowering a plant for the first time, only to discover that it is not the species that you expected.

Every time a tube of seeds is opened, the seed will begin to re-equilibrate with the moisture in the atmosphere. There is a choice to be made between re-equilibrating the remainder of the seed lot over an appropriate constant humidity solution before returning to storage, or storing seed lots in a number of individual tubes which will be opened once only. If the intention is to remove seed samples for sowing at regular intervals, the latter option may be the most appropriate as long as the seed lot is thoroughly mixed at the start, ensuring that as far as possible, each tube is representative of the whole seed lot.

FINAL WORD As supported by the Darwin Initiative, OSSSU is only the beginning: we wish to secure the species' seed over the long term. Implicit in this statement is a recognition of the need for stable, long-term funding for seed-storage facilities. In addition, we are aiming to expand the seed conservation network to include additional institutions in more countries. Mount Kinabalu in Borneo, for example, has 850 species of orchids (in comparison, the total United Kingdom flora is around 1,400 plant species, including 50 or so orchids); and Madagascar has been described as the "eighth continent" due to its high degree of endemism. We wish to open a dialog with the managers of commercial operations and owners of private collections. We aim to set a much more ambitious target of more than 1,000 orchid species incorporated into conservation biotechnology programs by 2012. This relatively inexpensive insurance policy, with seeds stored under the best possible conditions, would ensure that orchid seeds would be available in the future to be used for reintroduction, habitat restoration and sustainable-use projects in their countries of origin. As a desirable spinoff, they could also be used as a reservoir of material to encourage the raising of orchids from seed, thereby reducing the pressure on wild populations from unscrupulous collectors. Moreover, the intention is for OSSSU to branch out into Africa within the current round of three-year funding by holding an orchid workshop in an African country.

Although many species, and particularly varieties, have doubtlessly disappeared, many valuable plants probably still reside in public and private collections. Such plants deserve to be propagated, and both made more widely available to experienced growers, and their germplasm preserved for the future. In our view, orchid conservation should be seen as a great cooperative venture. If we are to make a real impact — and rescue our botanical riches for future generations — we need to begin to involve a much wider range of people. There is something special about orchid people, especially their dedication and their enthusiasm. Here is an opportunity for the orchid community — hobbyists, commecial growers and scientists — to work together and form a network. In the end, conservation is about people who take the action, and it is for our children and their children that we wish to preserve this irreplaceable treasure.

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MORE ON LINE Go to www.aos.org, click onto *Orchids* October 2008 Preview, and join Philip Seaton and Holger Perner, PhD, on a field trip they took to see orchids in Huanglong, China.

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Philip Seaton and Professor Hugh Pritchard have worked together on various aspects of ochid seed research for more than 20 years. Phil is currently project manager of the Darwin Initiative, Orchid Seed Stores for Sustainable Use (OSSSU). (e-mail p.seaton@kew.org). Professor Pritchard is head of research in seed conservation and project leader for OSSSU.

The Workshops: China and Ecuador

THERE is something special about sitting around a table, eating and drinking together with people from different countries, cultures and perspectives. In total, more than 40 people representing 21 institutions and 15 countries took part in the two workshops. Representatives of the participating institutions reflected a wide range of experience in the laboratory, horticulture and the field, and were able to exchange experience and expertise in orchid seed storage and germination techniques; to develop common protocols for seed harvesting, storage and germination, to set targets for numbers of species handled each year; develop ways of sharing the data produced; and identify flagship species to promote the project. Participating institutions were also offered the use of the facilities of the Millennium Seed Bank (MSB) at Wakehurst Place.

CHENGDU WORKSHOP The first five-day workshop took place in October 2007 in Chengdu, the capital of Sichuan. China was chosen as one of the two hubs for the project because of its large orchid flora (more than 1,300 species in its large territory) and its highly diverse temperate orchid flora.

In addition to our hosts representing Sichuan Hengduan Mountains Biotechnology in cooperation with the Huanglong National Park Administration, participants arrived from the Botanical Garden of Indian Republic (BGIR), Bogor Botanic Garden and Purwadadi Botanical Garden, Indonesia; the University of the Philippines Los Baños (UPLB); Singapore Botanical Gardens; Mahidol University, Thailand; and the Dalat Institute of Botany, Vietnam; as well as from Beijing Botanical Gardens, the Institute of Botany, the Chinese Academy of Sciences, and Hainan University within China. You can imagine our delight when we were greeted by an enormous red banner proclaiming the event strung across the entrance the Sun Joy Hotel, our conference venue for the week. Our hosts, Holger and Wenqing Perner, told us that this was a normal occurrence in China, but for us it was exotic and wonderful. Would everyone arrive? We waited nervously in the hotel foyer as each of our guests appeared, and escorted them across the road for our first evening meal together.

Everyone had arrived by late evening, and we all appeared the following morning for the opening ceremony,

Participants in the Chengdu Workshop (left to right and front to back): Yu Zhang, Monina Siar, Lilian Pateña, Dwi Murti Puspitaningtyas, Thaya Jenjittikul, Mrs Lucy Wang, Wenqing Perner, Tim Wing Yam, Djauhar Asikin, Duong Tan Nhut, Nguyen Van Binh, Xiqiang Song, Songquan Song, N. Odyuo, Kanchit Thammasiri, Hugh Pritchard, Sudhansu Sekhar Dash, Phil Seaton, Holger Perner, Mr. Long.

which was attended by Mrs. Lucy Wang, officer of the State Forestry Administration, Beijing (the Chinese governmental agency for protection of plants and animals), and Mr. Long from the Sichuan Forestry Department. Also present were members of the media, the event appearing on the front page of the Sichuan Economic Daily the next day, and being introduced on the Sichuan television evening news. In addition to the discussions and individual presentations at the hotel, delegates were able to visit both Perner's laboratory and his nursery in the outskirts of Chengdu. Here we were able to learn more about his propagation techniques, and particularly his considerable success in raising *Cypripedium* and *Paphiopedilum* species from seed. He was also able to offer a post-conference trip to Huanglong National Park to see some of the orchid habitats in the mountains of northern Sichuan.

JARDÍN BOTÁNICO DE QUITO WORKSHOP The second workshop took place in November 2007 in Quito Botanical Gardens, where we were given a warm welcome by the garden's director, Carolina Jijon. The Latin American contingent included participants from BIOFAN, Universidad Autonoma Gabriel Rene Moreno (UAGRM), Bolivia; Universidad do Oeste (UNOESTE), Brazil; Instituto de Investigaciones Agropecuarias (INIA) and Jardín Botánico Nacional, Viña del Mar, Chile; Jardín Botánico de Cali, Colombia; Jardín Botánico Lankester, Costa Rica; Jardín Botánico Orquídeario Soroa, Cuba; and Universidad del Valle de Guatemala, as well as from Universidad de Cuenca and Universidad Tenica Particular de Loja in Ecuador. The opening ceremony was attended by Patricia Galiano, representing the Ministerio del Ambiente (Ministry of the Environment). As far as possible, the workshop was delivered in Spanish and English, with participants aiding one another.

Located in Parque La Carolina in the center of Quito, possibly the best-preserved Spanish colonial city in South America, pretty Jardín Botánico de Quito is an oasis of peace and tranquility in the midst of a busy city. A relatively new garden, it is currently developing its laboratory facilities. The workshop was held in the new lecture theater close by the greenhouse, which, with its signature dome, houses a large display of orchids in bloom set in a natural setting along winding pathways among orchids. Behind the scenes, both supporting the displays and functioning as a research collection, was an extensive assortment of Ecuadorian *Masdevallia*. Like many gardens around the globe, it is in demand for other events and during the workshop the greenhouse and surroundings were being used for the preliminary stages of La Reina de Quito (The Queen of Quito). — *Philip T. Seaton and Hugh W. Pritchard.*

Participants in the Jardín Botánico de Quito Workshop (left to rigth): Vicente Perdomo, Nelson Neto, Miguel Santiago Tascón Castro, Mayra Maldonado, Pedro León, Margarita Palmieri, Hugh Pritchard, Patricio Novoa, Jorge Warner, Yunelis Perez, Lily Santa Cruz, Phil Seaton, Francisco Merchán, Eduardo Sánchez, Fabian Quispe, Emily Serrano, Ingrid Morales, Yadira Cevallos.