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Ex Situ Conservation of Orchids in a Warming World

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Abstract Whilst there is overwhelming scientific evidence that dramatic changes in regional climates are likely to occur throughout the 21st century, the scientific community remains uncertain how the effects of global heating will combine with other environmental factors to affect wild orchid populations. It is, however, likely that many populations will be affected adversely and that *in situ* conservation techniques by themselves will not be sufficient to prevent the extinction of many species. A range of complimentary *ex situ* strategies are discussed. Amongst these orchid seed banking has been shown to be an invaluable tool for conserving the maximum amount of genetic diversity in the minimum space and has the potential to enable the conservation of valuable material for possible re-introduction and habitat restoration programmes in the future. The Darwin Initiative project, 'Orchid Seed Stores for Sustainable Use' (OSSSU), is currently establishing a global network of orchid seed banks focussing initially on countries with high orchid biodiversity in Asia and Latin America. Particular reference is made to *ex situ* conservation in China, together with the urgent need to gather more data to determine which habitats and species are most at risk of extinction in the wild in the immediate future.

Keywords Orchids · Seed Storage · Living Collections · Global Warming · China

Introduction

If climate changes due to global warming occur on the scale that has been predicted by many experts, the world will be a very different place by the end of this century. The observed increase in the concentration of greenhouse gases since the pre-industrial era has probably committed the world to a warming of 2.4°C (1.4°C to 4.3°C) above pre-industrial surface temperatures (Ramanathan & Feng, 2008). As climate change interacts with habitat loss and fragmentation, introduced and invasive species and population growth, many ecosystems are likely to undergo radical modification. It may be that some reserves set aside to conserve endangered orchid species will themselves change considerably and be unable to support those species for which they were established.

Impact of Global Climate Change

It is generally accepted in the scientific community that global climate change due to global warming is already taking place and that this is due to increased greenhouse gas emissions resulting from human activities. According to the most recent report of the Intergovernmental Panel on Climate Change (IPCC, 2007), "Warming of the climate system is unequivocal ..." and "... many natural systems are being affected by regional climate change, particularly temperature increases."

The outlook for the future is disturbing. In Asia climate change is, "projected to compound the pressures on natural resources and the environment associated with rapid urbanisation, industrialisation and economic development." In Latin America there is, "a risk of significant biodiversity loss through species extinction in many areas" and "by mid-century increase in temperature and associated decreases in soil water are expected to lead to gradual replacement of tropical forests in eastern Amazonia. Semi-arid vegetation will tend to be replaced by arid land vegetation". In Eastern Australia there will be an increased risk of drought and fire. Levels of precipitation are likely to change radically in many areas of the world (Solomon et al., 2009).

Effects of Global Climate Change on Orchid Populations

The potential effects of climate change on orchids are difficult to predict and some ecosystems are likely to be more vulnerable to climate change than others. There is evidence that vegetational zoning on tropical mountains is strongly controlled by temperature (Primack & Corlett, 2005). Increasing atmospheric temperatures may result in vegetational zones gradually moving vertically up mountainsides, both permitting lowland species to migrate upwards and eliminating species in the highest zones (Foster, 2001). In addition there may be changes in low level cloudiness. The Monteverde Cloudforest in Costa Rica already seems to be experiencing a reduction in cloud immersion (Foster, 2001). Orchid populations on or close to the tops of limestone mountains in the Yachang Reserve in Guangxi, China, may be similarly vulnerable to climatic warming (Liu et al., 2010, this volume).

Not only may orchids in forest canopies be exceptionally sensitive to desiccation (Benzing, 2004), they may also be affected indirectly. Orchids are one component in a complex web of interactions with other epiphytes in the canopy which in turn may be affected in different ways by changes in the availability of light, nutrients and moisture. Neither do we know whether any changes in plant phenology associated with changes in temperature and precipitation will be synchronous with phenological shifts of orchid pollinators (Liu et al., 2010 this volume). Resource needs of pollinators can be subtle and complex (Vereecken et al., 2010, this volume). Climate change is a major threat to pollination services and networks (references cited in Pemberton, 2010, this volume) and there is a need to conserve the plant communities in which orchids live (Pemberton, 2010, this volume).

Numbers of European terrestrial orchids have continued to decline dramatically over the past 30 years or more due to a combination of factors, including habitat loss and fragmentation. However, in the UK, *Himantoglossum hircinum* has recently begun increasing again, as has *Ophrys sphegodes*. It has been suggested that the increase in *H. hircinum* may be due to climate change (see references in Kull and Hutchings, 2006). Neither should we forget the potential effects of climate change on the orchids' fungal partners. As just on example of the level of complexity that may occur between orchids and other members of its environment, the underground orchid *Rhizanthella gardneri* of Western Australia has an obligate mycorrhizal relationship with *Melaleuca uncinata*, a fungus gnat and a termite as specialist pollinators and a specialist marsupial dispersal agent of their berrylike indehiscent seeds. The orchids long-term survival is threatened by droughts that are occurring because of climate change that are causing the demise of the *Melaleuca* (Swarts & Dixon, 2009b).

Not all orchids will react to global warming in the same way, and some may be better adapted to be able to cope with future changes. A comparison of species belonging to the closely related genera *Paphiopedilum* and *Cypripedium* in China reveals that they have different physiological adaptations and survival strategies (Hu Hong, unpublished data). The evergreen *Paphiopedilum* are adapted to lower resource environments, with a lower rate of photosynthesis and growth, whereas the deciduous *Cypripedium* species have higher rates of photosynthesis and show more rapid growth during the active period. An increase in atmospheric CO_2 could increase rates of photosynthesis, but at present the effects of changes in temperature remain uncertain and there is a need for more research in this area.

It has been forecast that the combination of higher temperatures and lower rainfall will make forests more susceptible to fire (Primack & Corlett, 2005). This may lead to local species extinctions, or to a change in species composition, or a combination of the two. A study of post-arson impact on two species of epilithic (Angraecoid) and three species of terrestrial (*Cynorkis*) growing on a granite outcrop in Madagascar indicated different responses (Whitman et al., 2005). The terrestrial species appeared to be much more fire-resistant than the epilithic species and the authors concluded that although non-severe fires enhance landscape diversity, an increase in frequency of fires may negatively effect epilithic populations. Twenty-two orchid species restricted to the mountain rainforest in the Montebello region of Mexico were lost when their habitat was completely destroyed by fire in an area where fire was used in the management of agricultural and cattle grazing (Soto Arenas et al., 2007). Vegetation damage caused by severe frosts in the winter of 1997–1998, combined with extreme drought in the following spring led to drying of the epiphyte load into suitable fuel load for the crown fires that had previously been unknown in such a wet habitat. It is not possible to determine whether such an event in itself is a result of climate change, nevertheless it is very likely that in the future some extreme weather events to will increase in both frequency and intensity as a result of global climate change (IPCC, 2007).

An Ex Situ Action Plan

Although *ex situ* conservation techniques should not be viewed as a substitute for effective *in situ* programmes, considering the likely increase in risks to *in situ* populations and the fact that conservation through reserves alone is unlikely to be able to provide adequate protection to many orchid species, *ex situ* conservation and the storage of germplasm clearly has a role to play and is an indispensable component in the conservation tool box. (Cribb et al., 2003; Swarts & Dixon, 2009a). The Gran Canaria Declaration II on Climate Change and Plant Conservation in 2006 (http://www.bgci.org/ourwork/gcdccpc/) 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 change and shifting climate zones".

The scientific orchid community produced a Conservation Action Plan more than 10 years ago (Hágsater & Dumont, 1996) which recognised the value of *ex situ* conservation techniques. The important role that *ex situ* conservation techniques are likely to have in the future has also been recognized in Target 8 of the Global Strategy for Plant Conservation (GSPC) which was unanimously agreed by all the parties to the UN Convention on Biological Diversity (CBD) in 2002 (www.bcgi.org/worldwide/gspc/). This is to have 60% of threatened plant species in accessible *ex situ* collections (preferably in the country of origin) by 2010, with 10% included in recovery and restoration programs. At the first International Orchid Conservation Congress (IOCC) the orchid community agreed that by 2010 90% of threatened orchids should be held in secure *ex situ* collections, with 50% of these in active recovery programs (Dixon & Phillips, 2007). So far as we are aware, it is unlikely that this target will be reached globally, although The Orchid Seed Bank Challenge in Western Australia has achieved achieved seed storage and mycorrhizal selection for three quarters of the 408 native terrestrial species in the southwestern Australian biodiversity hotspot (Swarts & Dixon, 2009a, b) and the global Darwin Initiative project Orchid Seed Stores for Sustainable Use (OSSSU) has, at the time of writing, already exceeded its initial 3 year target of 240 species (see below) by a considerable margin (Seaton & Pritchard et al., unpublished data) and a new interim target of 1,000 species has been set.

China

China is home to two of the worlds biodiversity hotspots (Myers et al., 2000), and is one of the most important Range States for orchids. The Hengduan Mountains Region or Mountains of Southwestern China hotspot is found entirely within its borders, and arguably hosts the world's richest temperate flora, including many orchids. It is a centre of biodiversity for the genus *Cypripedium* (Cribb, 1998; Perner & Luo, 2007).

The Indo-Chinese subregion which forms part of southern China, amongst other important orchid genera, is home to endangered species of *Paphiopedilum,* many of which have been classified as being critically endangered (Cribb, 1998). All Chinese *Cypripedium* species have been under heavy collection pressure for around 25 years (Perner, 2005). Populations of *Paphiopedilum* in China also remain under threat due to the pressures of collection for illegal international trade (Liu et al., 2009). This is part of a wider international problem. *Paphiopedilum vietnamense* for example, discovered in the neighbouring Vietnam in 1999, became extinct in the wild due to illegal collection by 2003 (Roberts & Dixon, 2008).

In addition to the collection pressures, recent rapid economic growth and rural development in China has resulted in shrinking and damaged orchid habitats (Liu et al., 2009). With a 2,000 year history of collection of certain orchids for traditional herbal remedies, this practice continues to exert pressure on wild populations, and as a result some of the species used in Chinese herbal medicine, such as *Dendrobium officinale*, are now very rare in their natural habitats, (Cribb et al., 2003).

In response to such threats to orchid populations, since 2001 China has implemented a National Program for wildlife conservation and Nature Reserve Construction. Under this framework orchids have been treated as one of the 15 key taxa in urgent need of protection. The Chinese government has also formulated a National Action Plan to develop objectives for orchid conservation by 2010. China is currently giving a lot of attention to both the *in situ* and *ex situ* conservation of orchids. Based on information garnered from the nationwide resources surveys for orchids from 1997 to 2002, 160 nature reserves and small protected areas harbour various numbers of endangered orchids and they are under protection.

A re-introduction of Chinese endangered plants workshop was held on September 8th, 2009. As a demonstration species, *Paphiopedilum armeniacum* seedlings were re-introduced into nature reserves as part of a program of population restoration. Local people are employed to help protect the re-introduced seedlings from illegal removal (Hu Hong, pers. comm.). Unfortunately *P. armeniacum* remains endangered due to its ornamental value. Local people can make an income through the illegal digging up wild plants, and yet there is enormous potential for the production and sale of locally produced artificially propagated material. Not only can it be propagated from seed, it is a weedy plant that can easily be increased by division of rhizomes or tillers. Artificial propagation would bring with it the added advantage of reducing pressure on wild populations. Wild orchid re-introduction programs both in China and other parts of the world do, however, need to begin taking projected changes in climate into account.

Seed Banking and OSSSU

More than 20 years ago, at the 1984 World Orchid Conference (WOC) held in Miami, it was proposed that the orchid community should begin banking orchid seed as an insurance against possible losses of species from their habitats in the wild (Anonymous, 1985). However, despite Knudson's report of 30 years earlier (Knudson, 1954) that dry seeds of at least some orchid species could be stored for at least 20 years at refrigerator temperatures, there remained a need for further data. Detailed research over the last 20 years has revealed that, although some orchid seeds are relatively short-lived, the benefits of seed drying are quantifiably similar to those of crops seeds and the principle of drying is as pertinent and valuable for orchids as for many other non-orchid species. Thus it is likely that the vast majority of species belonging to the Orchidaceae are capable of tolerating dry storage, probably for many decades when stored at −20°C (Seaton & Pritchard, 2003). Furthermore, because orchid seeds are small in size (0.05–6 mm) and weight (0.31–24 μg) (Roberts & Dixon, 2008) large numbers can easily be stored in a small volume, making them ideal subjects for seed banking without the need for large facilities. Indeed, a domestic freezer will have sufficient capacity to store seed representing the orchid flora of an individual country.

The Darwin Initiative project, ‘Orchid Seed Stores for Sustainable Use’ (OSSSU) is funded by Defra (the UK Government’s Department for Environment, Food and Rural Affairs). The aim of the Darwin Initiative (http://darwin.defra.gov.uk/) is to fund joint projects between UK institutions and partner institutions which are rich in biological resources and would benefit from some additional financial input and UK expertise. OSSSU is a 3 year partnership between the Royal Botanic Gardens, Kew’s Seed Conservation Department at Wakehurst Place and partner countries located initially in Asia and Latin America. The aim is to establish a global network of orchid seed banks, with an initial focus on countries with high orchid biodiversity in Asia and Latin America (Seaton & Pritchard, 2008).

OSSSU commenced in October, 2007 with a workshop in Chengdu for participants from within China plus representatives from India, Indonesia, the Philippines, Singapore, Thailand and Vietnam to exchange expertise and to develop common protocols and project targets. Since the initial workshop interest in participating in the project has steadily grown in China and a number of institutes are actively responding to the conservation challenge and in addition to the participation of Kunming Institute of Botany (KIB) and Sichuan Hengduan Mts Biotechnology, as the network has expanded Beijing Botanical Gardens, Xishuangbanna Tropical Botanical Garden and Kadoorie Farm and Botanic Garden have recently joined as the first of a group of newly created Associate Members.

A second workshop was held for participants from Latin American hot spots at Quito Botanical Gardens for participants from Ecuador plus Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba and Guatemala. The regional network is expanding to include Mexico, with Panama, the Dominican Republic and Peru expected to join as Associate Members. At the time of writing the organisers at Kew are looking for additional partners to expand the network and have recently recruited Estonia as an Associate Member in Europe, with the expectation that Italy and Spain will join shortly.

Mature (i.e. seed harvested at the point of dehiscence of the seed capsule) dry seed is stored at −20°C in hermetically sealed tubes. The value of OSSSU not only lies in storing orchid seed over the longer term, however, but also in generating data. Species are being germinated on at least two different media one of which is Knudson C (Knudson, 1946)—for the first time providing a comparison of the performance of a minimum of 240 species representing a broad range of species and genera on one medium.

Individual species are likely to have different germination percentages on different media. Cueva and González (2009) reported a higher percentage germination (90%) for *Cyrtochilum loxense* cultured on MS medium (Murashige & Skoog, 1962) than on Knudson C (60%). In contrast, 9 month old (age of seed capsule) seed of *Paphiopedilum delenatii* has been reported to give a higher germination percentage on Knudson C (90.1%) than either full strength MS (9%) or half strength (73%) medium (Nhut et al., 2005). Thus each species is being germinated on at least one other medium (not necessarily MS) based on the in-country experience with germinating seed of the particular local orchid flora.

Central to OSSSU is the web site (http://osssu.org/). In addition to information about participants, resources and news updates, partners can access a password protected database to find up to date information about who is storing what and where, and how the material is being germinated. The common protocols (for details see Seaton & Pritchard, 2003 and http://osssu.org/main/resources.html) allow comparisons to be made across a wide range of species. A key element has been agreement of a common definition of germination, which in this case is germination of full seeds (seeds containing a viable embryo); successful germination being defined as the point at which the swelling protocorm ruptures the testa. The ultimate aim is to capture the genetic variation present in wild populations. This must be performed within current legal frameworks, both within country and conforming to international regulations. Duplication of seed collections is good seed banking practise for security of collections. This can be achieved in country, but there is the additional option to store duplicate accessions of orchid seeds in the Millennium Seed Bank at the Royal Botanic Gardens, Kew under a separate agreement.

Growing Orchids from Seed and Other Micropropagation Techniques

Most orchids are relatively easy to raise from seed (Seaton & Ramsay, 2005), indeed it is frequently possible to produce more plants than remain in wild populations (Swartz & Dixon, 2009a, b). By making seed-raised material widely available, this may indirectly reduce collection pressure on wild populations. A wide range of material can be cultivated from green (i.e. undehisced) capsules. This has the disadvantage that immature seed cannot be dried and therefore it is unlikely that it can be stored over the long term (Seaton & Pritchard, 2003).

In response to concerns that, for terrestrial orchid seed in particular, we should also be considering storing the appropriate symbiotic fungi alongside the orchid seed, techniques have been successfully storing both orchid seed and its fungal partner in alginate beads at sub-zero temperatures (Wood et al., 2000; Sommerville et al., 2009). Somatic embryogenesis techniques have been proposed as an additional or an alternative conservation techniques where only a small number of clones remain as, for example, the critically endangered *Cyrtochilum loxense* from Ecuador (Cueva & González, 2009). Wounding techniques have been developed for propagation of *Paphiopedilum delenatii* (Nhut et al., 2005).

Living Collections

One could argue that an orchid species cannot be said to be safe unless it has been taken into cultivation. However, although some orchids in living collections in botanical gardens may be long-lived, some dating as far back as the nineteenth century, the majority are not. Plants growing in living collections are an important resource for educational and research purposes in botanical gardens, but cultivation specifically for conservation is demanding and only rarely practised effectively (Ramsay & Dixon, 2003). A potential problem with living collections that needs to be addressed is the restricted size of the gene pool of most species in cultivation (Nash et al., 2003). Botanical gardens (as well as amateur/hobbyist growers) tend to have either one specimen, or a very small number of clones of any one species. There is a tendency to cultivate the so-called “best” forms. From the perspective of conservation it would be more useful to grow large populations of at least some species, thereby providing a better representation of the wider gene pool and an insight into within species diversity as well as the diversity of orchid species.

More recently, the need to involve the wider orchid growing community has been recognised. Members of orchid societies have the potential to make an important contribution to orchid conservation. They are often a reservoir of largely untapped expertise which needs to be passed on to each generation. This knowledge base is vital to ensure that plants survive in cultivation (Nash et al., 2003). Collections are a dynamic resource. Even within the best maintained collections plants have a limited lifespan being subject to ageing, disease and accidental death. The assertion that, "A very small or very local population is ... set up for near-instant demise from any storm, flood, wildfire, drought or other natural disaster that comes along" (Wilson, 2002) could equally apply to an individual orchid collection. In the same way that small wild populations are vulnerable, so are living collections, plants may be lost due to heating failures in temperate climates (or indeed cooling in tropical climates), pests or disease, or poor culture. Thus there is a need to continually propagate species from seed within living collections. In order to minimise such losses there is a strong case to be made for increasing support for botanical gardens in the countries of origin and within each country to establish botanical gardens at different altitudes to accommodate the differing cultural requirements of individual species and there is no need for energy inputs for either cooling or heating. There is an opportunity for botanical gardens to co-ordinate their activities through organisations such as Botanical Gardens Conservation International (BGCI) and to begin to exchange information about what plants reside in their collections, and to exchange pollen of endangered species.

In the UK Plant Heritage (formerly the National Council for the Conservation of Plants and Gardens) plays an important role in recognising important plant collections (Oakeley, 2000). Its stated aim is to "conserve, grow, propagate, document and make available the amazing resource of garden plants that exists in the UK." There are currently 19 orchid collections ranging from a National Collection of *Anguloa*, *Lycaste* and *Ida* to a collection of *Stanhopea* (http://www.erythos.com/BOC/NatColl.html) held by amateur growers, commercial orchid nurseries and botanical gardens.

Looking Beyond 2010

There is cause for optimism about the future of orchid conservation. Seedlings of the endangered *Cypripedium macranthos* are being raised both symbiotically and asymbiotically at Beijing Botanical Gardens in China as part of a re-introduction project (Yu Zhang, pers. comm.). In Latin America another possible model is a project aimed at conserving and re-introducing *Cattleya quadricolor* (Seaton & Orejuela, 2009). A Red List has been published for the orchids of Colombia (Calderon-Saenz, 2007) and an Action Plan written for *Cattleya* species (Niessen, 2002; Niessen & Calderon, 2002). Members of the Asociacion Vallecaucana de Orquideologia in Cali have created a study group, pollinations have been carried out at two commercial nurseries (Orquideas de Valle and Vivero Medio Dapa) and seedlings raised by society members. Jardin Botanico de Cali (one of the partners in OSSSU) and is storing the germplasm. The eventual aim is to re-introduce plants into secure locations where they were previously present.

Liquid nitrogen storage may produce further extensions of life-spans for orchid seed, and is currently being investigated at a number of centres, including Singapore Botanical Gardens and Kings Park and Botanic Garden, Australia (Dixon & Batty, 2005). It has also been demonstrated that mycorrhizal fungi necessary for germination of orchids in their natural habitats could also be stored successfully at −196°C (Batty et al., 2001).

With limited resources, deciding upon priorities will always remain a problem for conservationists and the gathering of data for IUCN Red Lists has been seen as at least part of the solution. Although a number of countries, such as Singapore and Colombia, have completed their National Red Lists for orchids (Yam et al., 2010; Calderon-Saenz, 2007), there is an urgent need to gather more data and to transfer more orchid species to the IUCN Global Red List. This would enable decisions to be made concerning those orchid species that are at most immediate risk of extinction in the wild, with the aim of bringing them into cultivation and banking their seeds. Accurate identification of species is key to this endeavour. The current discussions and disagreements about exactly how many *Ophrys* species there are (see comments by Vereecken et al., 2010, this volume) is an extreme example of how difficult it can be for would-be seed bankers with limited resources to make decisions regarding which taxa to prioritise for long-term storage. The issue of whether scarce resources can be diverted into conserving genetically distinct populations within a species is likely to remain a matter of judgement.

Conclusions

Although the scientific literature on global warming makes sobering reading as we face the prospect of a changing world, the outlook for orchid conservation is not all bleak. There is a growing awareness of the need to take prompt action and an increasing number of *ex situ* conservation projects are being set up around the world. The establishment of OSSSU has induced a positive response and has the potential to be expanded into a key global facility. Living collections are currently under-utilised as a conservation tool, and there is a need to do more to include members of the wider orchid community. There remains an urgent need to identify populations which are particularly vulnerable and at imminent risk of extinction in the wild so that they can be brought into cultivation.

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