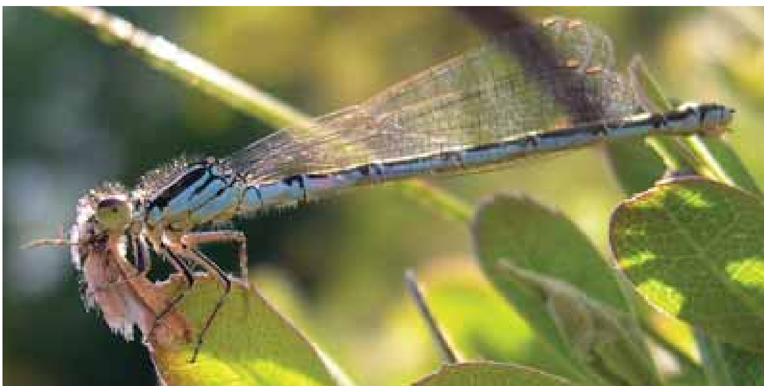
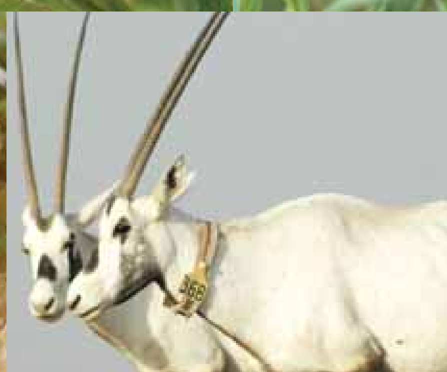
IUCN

Global Re-introduction Perspectives: 2010

Additional case-studies from around the globe

Edited by Pritpal S. Soorae





IUCN/SSC Re-introduction Specialist Group (RSG)

RSG

هيئة البيئة - أبوظبي
Environment Agency · ABU DHABI

DENVER ZOO

The designation of geographical entities in this book, and the presentation of the material, do not imply the expression of any opinion whatsoever on the part of IUCN or any of the funding organizations concerning the legal status of any country, territory, or area, or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The views expressed in this publication do not necessarily reflect those of IUCN.

Published by: IUCN/SSC Re-introduction Specialist Group & Environment Agency-ABU DHABI

Citation: Soorae, P. S. (ed.) (2010) GLOBAL RE-INTRODUCTION PERSPECTIVES: Additional case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE, xii + 352 pp.

ISBN: 978-2-8317-1320-5

Cover photo: Clockwise starting from top-left:
i. Damselfly, UK © *PC Watts*
ii. Corn crake, UK © *Andy Hay (rspb-images.com)*
iii. Western prairie fringed orchid, USA © *Margaret From*
iv. Arabian oryx, Saudi Arabia © *M. Z. Islam*
v. Corroboree frog, Australia © *D. Hunter*

Cover design & layout by: Pritpal S. Soorae, IUCN/SSC Re-introduction Specialist Group

Produced by: IUCN/SSC Re-introduction Specialist Group & Environment Agency-ABU DHABI

Download document at: www.iucnsscrsg.org

Conservation Status and re-introduction of Bermuda's Governor Laffan fern-an endangered endemic

Margaret M. From

Director of Plant Conservation, Center for Conservation & Research at Omaha's Henry Doorly Zoo, 3701 South 10th Street, Omaha, Nebraska USA 68107-2200 (psl@omahazoo.com)

Introduction

Bermuda's most endangered plant species; *Diplazium laffanianum*, is of great historical significance to the island nation. The species has not been reported from the wild since 1905 (Britton, 1918) and was reduced to only five living specimens housed at the Bermuda Botanic Gardens by 2003. It is listed as an endangered species by IUCN but is not on the CITES App. I. The species was a personal favorite of one of Bermuda's early governors and was later named the Governor Laffan fern in his honor. Long periods of isolation from other continents have resulted in unique island plant species which are particularly vulnerable to extinctions. Islands that are densely populated by humans such as Bermuda face extraordinary challenges to conservation for their endemic flora and fauna. The Bermuda Botanic Gardens provided tiny samples of spores collected from the remaining specimens for *ex situ* plant research at Omaha's Henry Doorly Zoo where the spores were germinated and grown under sterile laboratory conditions. Subsequently, many cultures containing hundreds of juvenile ferns were returned to Bermuda. The timing of the initiation for the first cultures was particularly fortuitous since Hurricane Fabian crashed into Bermuda shortly after the spores were sent to the United States for germination and the last adult specimens remaining in Bermuda sustained sea water damage in the hurricane. The very existence of the last known adult plants was threatened and research producing additional young ferns was the only means to prevent its final extinction.

Close up of *Diplazium laffanianum*

Goals

- Goal 1: Bring the last known fern specimens into the reproductive stage to provide viable spores for *in vitro* germination.

- Goal 2: Develop a successful protocol and medium for micropropagation of the fern spores.
- Goal 3: Provide young ferns for Bermuda's Botanic Garden that would be used to rehabilitate the species in its host country.

Success Indicators

- Indicator 1: Successful germination and production of young ferns from the spore samples.
- Indicator 2: Produce large numbers of ferns for return to the host country Bermuda.
- Indicator 3: Re-introduce the young ferns to the local environmental conditions at the Bermuda Botanic Gardens for conservation research in Bermuda.

Project Summary

The Bermuda islands consist of more than one hundred islands and outlying islets, many of which are not inhabited by humans. However, the main islands are among the most densely populated places on earth and natural areas have been reduced to tiny fragments, pushing the endemic flora to the verge of extinction in some cases. Tectonic plate movement deep within the Atlantic Ocean separated the Old World and the New World approximately 200 million years ago and Bermuda was formed by volcanic activities along the fault line left by the tectonic plate movements. Bermuda sits on this volcanic base that is covered by a cap of limestone formed from countless marine organisms. The native topsoil is only a thin layer of reddish soil supporting its plant life. Most of the endemic flora and fauna probably arrived through natural dispersal from North America but many non-native species arrived with successive waves of human colonization that began arriving in the 1500s. The Governor Laffan fern (*Diplazium laffanianum*) is Bermuda's most threatened native plant species. *Diplazium laffanianum* is now extinct in the wild and the last specimen was reportedly seen in the wild in 1905. The last known plants languished in an obscure corner of the Bermuda Botanic Gardens until the 1970s when the last five plants were moved to the government's Tulo Valley Nursery for safeguarding. The fern was reduced to only five living specimens by 2003 and a protocol for propagating the species had not been developed prior to this time. The species historically occupied areas at the entrances to Bermuda's limestone caves but development and habitat deterioration had altered the areas so drastically that today the original habitat no longer exists. With such a small population left on earth these last few individuals may have limited genetic diversity but the decision was made that whatever genetic diversity the species may still posses was worth saving.

A collaboration between the Bermuda Botanic Garden and Omaha's Henry Doorly Zoo was initiated in order to save the species from disappearing altogether. The plant research laboratory at Omaha Henry Doorly Zoo was enlisted to propagate the species *ex situ*. Cloning one of the remaining specimens was not considered a good conservation practice and an alternative plan was developed to carefully tend the adult plants until they would begin producing spores in hopes of retaining what genetic diversity the last specimens might possess. After several months of care at the Bermuda nursery two of the remaining specimens produced small amounts of spores which were sent for culturing to Omaha's Zoo. After several attempts at culturing them the fern spores began to germinate rapidly and *in vitro* cultures containing hundreds of little ferns were hand carried back to Bermuda for growing on at the Bermuda Botanic Garden. A series of four shipments containing many young ferns have been returned to Bermuda over the last six years. The area where the species was last seen in the wild no longer exists as a natural area. Therefore the species must be cultivated and maintained *ex vitro* at the botanic garden for the foreseeable future or until a restored habitat location can be provided. Nursery personnel at Tulo Valley Nursery and the Ministry of the Environment in Bermuda reserve responsibility for monitoring the young plants and growing the ferns on to a mature stage. Any re-introductions made to the natural habitat are determined by the same Bermudian authorities.

Major difficulties faced

- Careful consideration must be made for the return of the ferns to the host country in order to coincide with the best growing months.
- Limited source materials from a small population.
- No published culture methods are available for the species.
- *Ex vitro* acclimatization methods are complex for this sensitive species.
- *Diplazium laffanianum* spores have a very limited viability period making propagation timing critical.

Major lessons learned

- Spore condition, type and age have critical impact on propagation of the species.
- Re-introduced ferns require careful monitoring.
- Adaptive management methods must be employed for the plants due to their specific growth requirements.
- Cooperation between collaborators is necessary for successful re-establishment.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- A species was saved from final extinction.
- The project continues to produce many young ferns *in vitro* for future re-establishment in Bermuda.
- Successful protocols were developed for propagation and acclimatization of this fern that are useful for the collaborators as well as to other plant re-introduction projects.

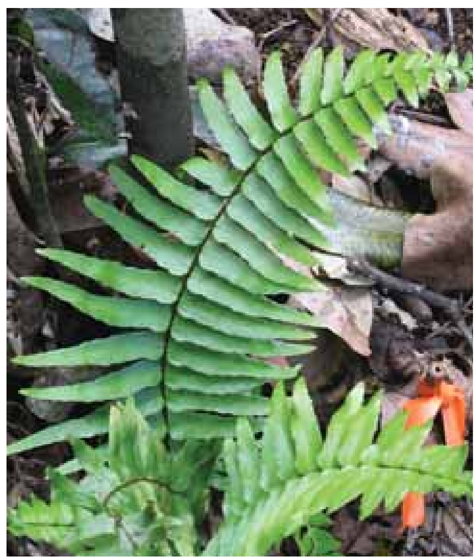
Reinforcement of the populations of critically endangered endemic fern *Diellia pallida*, Kaua'i, Hawaiian Islands, U.S.A.

Ruth Aguraiuja

Senior Researcher, Tallinn Botanic Garden, Kloostrimetsa Rd. 52, Estonia 11913 & Research Associate, National Tropical Botanical Garden, Papalina Rd., Kalaheo, Hawai'i 96741 USA (ruth.aguraiuja@tba.ee)

Introduction

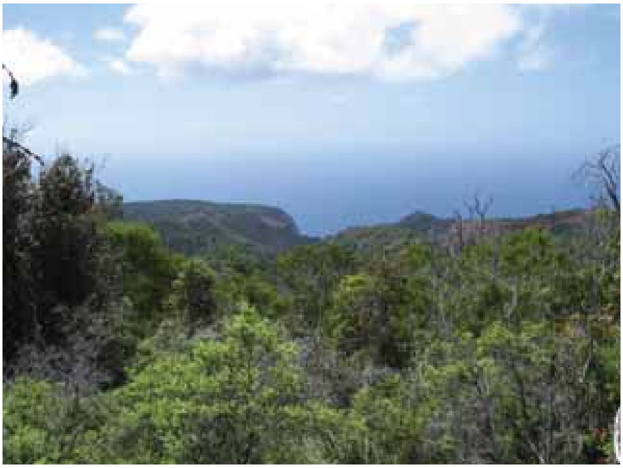
Diellia pallida (Aspleniaceae) is the endemic fern to the island of Kaua'i. It is a species of the lowland diverse mesic forest or Diospyros/Metrosideros mesic forest communities on the northern slopes of the western ridges of Koke'e Mountains. The natural population of *D. pallida* consisted of 13 mature individuals on three sites, only eight of these were reproducing (Aguraiuja, 2004). *Diellia pallida* is federally listed as Endangered (U. S Fish & Wildlife Service Species List, 25th February 1994). Corresponding to the IUCN criteria (IUCN, 2001) this species belongs to the category of globally critically endangered plant species (Aguraiuja & Wood, 2002). Due to very small number of existing individuals, their very narrow distributions, obligatory out crossing and slow recruitment, *D. pallida* is a subject to increased likelihood of extinction through any stochastic extinction event. The major threats to extant local populations of *D. pallida* are habitat degradation by animals (substrate erosion caused by trampling of goats, pigs, mule deer, red jungle fowl) and direct disturbance (trampling, uprooting and browsing). Trampling and erosion are the major factors of high mortality in gametophyte generation and sporeling stage, what in long term may lead to the depletion of natural spore bank.



The endemic fern *Diellia pallida*

Goals:

- Goal 1: Protection of all extant individuals as the main spore source for the habitat.
- Goal 2: Establishment of experimental population patches within known historical distribution area.
- Goal 3: Establishment of new natural generations, population recruitment.
- Goal 4: Species survival on the landscape within its natural communities; self-sustaining persistence of the populations and their normal evolutionary process.



Diellia pallida habitat in Mahanaloa Valley in Kokee Mountains on the island of Kauai

- Goal 5: The model method for the conservation of critically endangered fern species of same growth form and life cycle specificities, in the tropics and elsewhere, where the habitat conditions allow the recovery/restoration of the species.

Success Indicators

- Indicator 1: Survival of out planted individuals, increased number of mature spore producing individuals in the site.
- Indicator 2: Establishment of new generation.
- Indicator 3: Colonization and establishment in new empty patches of suitable habitat, extended distribution.

Project Summary

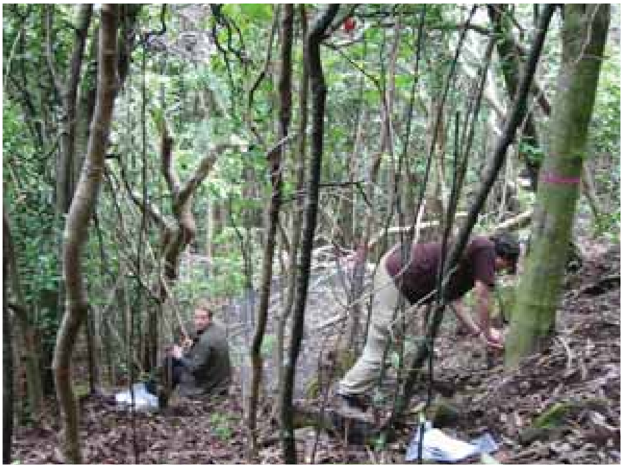
The conservation oriented research of *Diellia pallida* was started with the condition assessment of extant individuals and populations (Aguraiuja, 2001). As emergency, the cages were put on last mature individuals protecting thus the last spore source against browsing by feral animals. The cages also helped to stabilize the soil around the plants. Few rhizomes, uprooted by feral pigs and goats, where planted into the neighboring fenced ex-closure. With these activities, detailed observations and documentation of the changes in population structure, the research was continued (Aguraiuja, 2005). Protection of mature individuals and coincident more favorable weather conditions during 2003-2006, resulted in a drastic increase of gametophytes and sporelings of *D. pallida* in two local populations, demonstrating that habitat conditions are still suitable for germination and establishment of younger stages. The analyses of population regeneration still showed very low survival of younger developmental stages. Regardless that hundreds of sporelings developed during the winters of more favorable years, less than percentage of these survived till next winter, mainly because of the trampling, wash out and erosion.

In 2006, the preparations for propagation and reinforcement experiment were started in collaboration with National Tropical Botanical Garden (NTBG). The encouraging factors were: the high natural germination in the habitat; *D. pallida* was tolerant to the replanting; empty patches of suitable habitat in the historical distribution area; some fenced ex-closures with suitable microhabitat conditions within historical distribution area. As the trampling and erosion were documented as main factor for the death of the individuals in younger stages, it was also decided to learn rescue the eroded sporelings and boost them up as emergency method for increasing the number of individuals. The main idea of recovery experiment was to reinforce the natural population by increasing the number of individuals and spore source of the habitat; to test if gradual imitation of natural population recruitment would influence the efficiency of population reinforcement efforts; and to test if establishment of experimental populations within fenced exclosures would affect the dispersal of the species via colonization of new patches of suitable habitat within the whole historical distribution area.

Two parallel but complementary lines of research were conducted: 1) propagation efforts in the laboratory and nursery conditions; and 2) reinforcement experiment and observations of natural and experimental populations in the field. The propagation trials were started with the sowing of single individual spores (N. Sugii, Lyon Arboretum). It was learned then that *Diellia* gametophytes may perenniate and live three to four or more years, and that they may be obligatory out-crossers. The germination tests with the mix of spores originating from different individuals resulted in first sporelings and showed also that *Diellia* ferns are very slow in their younger life stages. It took a year from the sowing to the emergence of the first sporeling of *D. pallida*. The stock *ex situ* conservation collection was created and divided between the propagation nursery in Lawai'i (NTBG) and the restoration area of Limahuli Garden (NTBG). Considering the patchy distribution and availability of safe and protected sites, seven experimental population patches were established in 2007. According to the recovery criteria for the Kauai Plant Cluster (USFW 1995) and available micro-sites, 30 individuals were planted into each. Simultaneous surveys of population dynamics of natural and experimental populations were started. Since the survival has been highest in two sites, indicating that habitat conditions must be still suitable for *D. pallida* in there. Based on the analyses of the survival rates and microhabitat conditions of this first out planting, the optimum number of individuals will be worked out for the plantings during several consecutive years.

Major difficulties faced

- The uncontrolled disturbance by introduced game and feral animals in the habitat.
- The uncontrolled erosion in the habitat, scarcity of safe sites.
- The uncontrolled insect damage and fungal disease.
- Stressed mature individuals staying sterile, extreme population fluctuations during longer dry periods.
- Difficult to propagate the species, obligatory out-crosser.
- The native forest community loosing its structure: increased smothering effect of the leaf litter, less ground vegetation, drier soil.
- Only very small patches of the native forest community were protected by fences, most of natural germination areas were located outside of fenced areas.
- The current and historical distribution area is divided between several landowners.



Soil sampling for soil spore bank tests in the natural habitat

Major lessons learned

- The population monitoring showed that population dynamic followed the local climate pattern of the cycle of wetter and drier years, where the germination and establishment of new individuals was successful during the period of more favorable conditions and could completely fail during the drier period. The best timing for more successful population reinforcement activities would be during the winter months of wetter years.
- For more successful reinforcement and population recovery additional research of micro habitat conditions is needed, as the choosing the appropriate microhabitat site is crucial for the survival of the fern individuals.
- The boosting of disturbed individuals of *Diellia pallida* and propagation in ex situ conditions should be short-term and in toughening conditions for future planting into natural habitat, decreasing thus the after planting environmental stress and increasing the potential survival of the individuals in the site.
- The individuals of fern species *Diellia pallida* need after-care and site management during their first year in the site.
- The single out planting into the habitat equals to a single occasional natural distribution event. It may take very long time until structured self-sustaining population evolves naturally, particularly in relatively hard conditions of mesic forest on steep slopes, and probability for this event to happen is as big as that of extinction. It was learned that the out planting should be gradual during several consecutive years or by the cycles, imitating thus the natural colonization, population growth and establishment of structured self-sustaining population.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- The appropriate habitat conditions still exist and support the establishment of new individuals, if protected against introduced animals.
- The methods and plan for further bigger population reinforcement within the whole distribution area on the landscape have been worked out and tested.

The initial results show that the recovery of the fern species *Diellia pallida* is possible. Since, the study of ecology, population monitoring, and restoration tests have been conducted within the framework of scientific research project initiated by the researcher. For further success in landscape level recovery of the species, the institutional collaboration for the protection of the sites, propagation of the plants, population reinforcement, conservation management activities and population monitoring, needs to be build up.

References

Aguraiuja, R. 2001. Population status of five Hawaiian endemic fern taxa within the genus *Diellia* (Aspleniaceae). CBM Skriftserie 3: 7-24.

Aguraiuja, R. & Wood, K. R. 2002. The critically endangered endemic fern genus *Diellia* Brack. in Hawaii: its population structure and distribution. Fern Gaz. 16 (6, 7 & 8): 330-334.

Aguraiuja, R., Moora, M. & M. Zobel. 2004. Population stage structure of Hawaiian endemic fern taxa of *Diellia* (Aspleniaceae): implications for monitoring and regional dynamics. Canadian Journal of Botany (*in press*).

Aguraiuja, R. 2005. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Disseratationes Universitatis Tartuensis 112. Tartu University Press. 111 p.

Endangered and Threatened Wildlife and Plants; Determination of Endangered or Threatened Status for 24 Plants from the island of Kauai, Hawaii. U.S. Fish and Wildlife service Division of Endangered Species. Department of the Inferior. Fish and Wildlife Service. 50 CFR Part 17. RIN 1018-AB69. (Adopted from the Federal Register for Friday, February 25, 1994).

IUCN. 2001. IUCN Red List Categories: Version 3.1. Prepared by the IUCN Species Survival Commission. IUCN, Gland, Switzerland & Cambridge, UK.

U.S. Fish and Wildlife Service. 1995. Recovery Plan for the Kauai Plant Cluster. U. S. Fish and Wildlife Service, Portland, OR. 270 pp.

Conservation introduction of the parasitic plant dactylanthus at Waipapa, New Zealand

Sebastian (Avi) Holzapfel [1] & John Dodgson [2]

[1] – Department of Conservation, Research & Development Hamilton, P.O. Box 516, Hamilton 3240, New Zealand (aholzapfel@doc.govt.nz)
[2] – 71 C Waiwera Street, Kawhia, New Zealand (dodgson@ihug.co.nz)

Introduction

Dactylanthus *(Dactylanthus taylorii* (Balanophoraceae), pua-o-te-reinga, wae-wae -atua) is New Zealand's only fully parasitic native flowering plant. A root parasite, it lives as a usually subterranean tuber attached to the root of several native angiosperm tree and shrub host species often associated with secondary (regrowth) broad-leaved forest (Moore, 1940; Ecroyd, 1996 & Holzapfel, 2001). Fossil records dating back 23 million years show the species in the past distributed over both main islands of New Zealand and some off-shore islands; today it is only found in ~85 populations on the North Island and one offshore island, though still over a wide range of climates and altitudes (Ecroyd, 1996 & Holzapfel, 2001). Dactylanthus is listed in New Zealand as nationally vulnerable (de Lange *et al.*, 2009); populations have severely declined mainly because fruit production is prevented by introduced browsers, in particular brush-tailed possums (*Trichosura vulpecula* Kerr), kiore (*Rattus exulans* Peale) and ship rats (*R. rattus* L.) (Ecroyd, 1996). Conservation management is guided by a recovery plan (La Cock *et al.*, 2005) and includes the establishment of new populations at sites where introduced browsers are managed or absent. Here we describe the first fully monitored and quantified experimental establishment trial at Waipapa, central North Island.

Dactylanthus habitat in the ecotone between open area & mature forest © S. Holzapfel/DoC

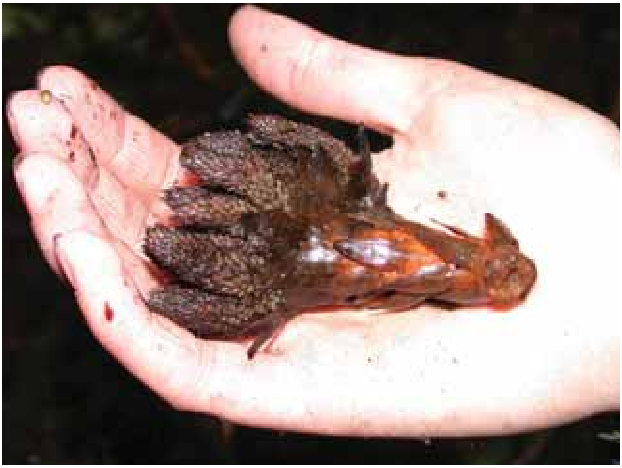
Goals

- Goal 1: Establishment of dactylanthus from seed.
- Goal 2: Robust evaluation of the effectiveness of two different sowing densities.
- Goal 3: Preliminary evaluation of the effect of dominance of host species and site exposure.

Success Indicator

- Indicator 1: Dactylanthus established and flowering long-term in at least one experimental plot.

- Indicator 2: Key data of sufficient quality and quantity collected and analyzed annually to allow robust evaluation of success and effect of variables.



Maturing fruit head of dactylanthus
© S. Holzapfel/DoC

Project Summary

Preliminary trials had indicated that dactylanthus could be established from seed, provided a suitable host tree was present (Ecroyd, 1996 & Holzapfel, 2001). In 1999 we established trial plots at Waipapa, central North Island, in the ecotone between mature podocarp forest and open frost flats, at sites with suitable host species but no natural population of dactylanthus. Twenty-four permanently marked seeding plots (50 x 50 cm) were established across four sites differing in host species dominance (3 main hosts), host age, and exposure ('open' or 'closed' canopy). Each plot received about 1,500 seeds of the same seed mix, the equivalent of a single fruiting head with moderate fruit set. Seeds were either sown over the entire plot area ('broad' sowing) or all seeds sown into a single, central 5 x 5 cm grid ('central' sowing). Three plots of each sowing density were established at each site. Over ten years, monitoring was undertaken annually late in the flowering season, when most inflorescences had emerged through the forest floor. Data on the total number of inflorescences, their position and their sex were recorded. Dactylanthus is considered dioecious, i.e., each individual plant produces only inflorescences of one sex. Because dactylanthus individuals are long-lived, establishment within each plot was largely cumulative over the monitoring period. To ensure that establishment could only occur from the initial seed sown, plots were kept covered throughout the year with fine mesh cages to prevent entry by known pollinators, i.e., native short-tailed bats (*Mystacina tuberculata* Gray), and introduced ship rats (Ecroyd, 1996) or mice (*Mus domesticus* Sage & Sage). See Holzapfel & Dodgson (2004) for further details on methods.

Establishment of dactylanthus was confirmed for the first time four years after sowing, at one plot in each of two sites. The number of plots with dactylanthus increased steadily in the following years; ten years after sowing dactylanthus had established at all four sites and in 22 of the 24 plots. Total number of inflorescences and mean number of inflorescences per plot increased each year for most years, though successively smaller increases each year indicated that plots were nearing their maximum flowering capacity.

Central sown plots established plants earlier, while inflorescence numbers (and, by proxy, number of plants) per plot were higher for broad sowing, probably due to lesser crowding of plants compared to central sown plots. Inflorescence numbers per plot were as high or higher as in wild populations. A striking result was the high proportion of female inflorescences compared to males, ranging from 100 % to 69 %. This was in direct contrast to wild populations where on average males outnumber female inflorescences five to one (Ecroyd, 1996). The male proportion increased, however, each year for the four most recent years of monitoring and it is conceivable that over time the sex ratio at the trial site would become similar to that of wild sites. Whether these skewed and changing sex ratios mean that individual plants change sex, or that the species is not dioecious but monoecious and plants produce both male and female inflorescences in varying proportions over time, is an area of ongoing research. Establishment rates and inflorescence numbers were similar at the three sites that had closed canopy but different host species dominance and age. The open canopy site showed delayed establishment and only about a tenth of the number of inflorescences per plot compared with the closed canopy sites, and contained the only two unsuccessful plots. This might indicate that exposure influences establishment of dactylanthus, e.g., drier soil leading to higher seed mortality or fewer host roots being available for establishment. Because this particular site was the first of all four sites to be set up, however, it cannot be discounted that a suboptimal sowing technique at that early stage of the project (‘starter-effect’) rather than site conditions was responsible for the lesser success.

Major difficulties faced

- Ensuring that monitoring was carried out late in the flowering season each year so that most inflorescences could be counted. As flowering times varied from year to year this necessitated repeat visits in some years.
- The unexpectedly high number of inflorescences developing in the latter years of the study meant monitoring effort increased four-fold over the time of the project, putting pressure on resources and capacity required to maintain the same level and quality of data acquisition.
- The inability to distinguish whether the lesser success at the open-canopy site was due to micro-site conditions or because the sowing technique had not been optimized when the site was sown.

Major lessons learned

- Dactylanthus can be successfully established in the wild from seed, both through broad and central sowing.
- Establishment success was not correlated to a particular host species or -age but might be correlated to microclimate or other microhabitat conditions at the site.
- Sex ratio of inflorescences was opposite to that of wild populations, but similar to those in earlier pilot trials. Understanding this difference and the observed trend towards increasing ‘maleness’ over time is an important area for ongoing research.

- Success at this site has so far not been replicated at other sites using the same methods, meaning that not all conditions required for establishment have yet been identified.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Establishment occurred at all sites and in most plots, with the overall population vigor, size and flowering output comparable to or exceeding that of wild populations.
- Data of sufficient quality were obtained to robustly analyze the key components of the trial.
- Results have further advanced our understanding of the establishment and reproductive biology of dactylanthus.
- The success at the specific site has not been able to be replicated to the same extend at other sites, therefore a description of required standards for its use as a conservation management technique is still incomplete.

References

de Lange, P. J., D. A. Norton, S. P. Courtney, P. B. Heenan, J. W. Barkla, E. K. Cameron, R. Hitchmough & A. J. Townsend. 2009. Threatened and uncommon plants of New Zealand (2008 revision). New Zealand Journal of Botany 47: 61-96.

Ecroyd, C. E. 1996. The ecology of *Dactylanthus taylorii* and threats to its survival. New Zealand Journal of Ecology 20: 81-100.

Holzapfel, A. S. & J. Dodgson. 2004. Experimental seeding trials for the root parasite *Dactylanthus taylorii*. DOC Science Internal Series 173. Department of Conservation, Wellington.

Holzapfel, S. 2001. Studies of the New Zealand root-parasite *Dactylanthus taylorii* (Balanophoraceae). Englera 22: 7-176.

La Cock, G. D., S. Holzapfel, D. King & N. Singers. 2005. *Dactylanthus taylorii* recovery plan, 2004-2014. Threatened Species Recovery Plan 56. Department of Conservation, Wellington.

Moore, L. B. 1940. The structure and life-history of the root parasite *Dactylanthus taylori* Hook.f. New Zealand Journal of Science and Technology 21: 206B-224B.

Conservation and re-introduction to augment threatened orchid populations in Madagascar

Margaret M. From

Director of Plant Conservation, Center for Conservation & Research at Omaha's Henry Doorly Zoo, 3701 South 10th Street, Omaha, Nebraska 68107-2200, USA (psl@omahazoo.com)

Introduction

Madagascar's orchids comprise approximately 1,000 currently classified species with nearly 80% endemism and many species that are on the verge of extinction due to the rapid conversion of forests to slash and burn agriculture. The island nation is a biodiversity hotspot of the highest concern according to IUCN. It is home to many animal and plant species found nowhere else on earth. The orchids in Ranomafana National Park are representative of orchid species that were once widespread in Madagascar's eastern rainforest region. The park offers a measure of protection to the orchids. However, most of the surrounding landscape has been burned and converted to crops in order to feed an impoverished human population. Malagasy orchids are among the island's most vulnerable plants partly due to the highly specialized niche they occupy in the forest which disappears quickly when the trees are destroyed by human activities or from the frequent cyclones that hit the island. A single downed tree may have provided support to dozens, and possibly hundreds of individual orchid plants, along with other epiphytic species. Illegal exploitation is another threat to the orchids.

Orchid seeds were collected in Ranomafana National Park under permits in collaboration with the Association Nationale Pour la Gestion Des Aires Protogees (ANGAP), the Madagascar Institute Pour La Conservation Des Environments Tropicaux (MICET), the University of Madagascar at Antananarivo and The Lab for Rare & Endangered Plants at Omaha's Henry Doorly Zoo, Omaha, Nebraska, USA. Concurrent propagation studies were conducted at the University in Antananarivo and at the Omaha Henry Doorly Zoo's plant research laboratory. The orchid plants resulting from the project were used for re-introductions

Re-introduced orchid on tree trunk

in Madagascar. Small samples of the remaining uncultured seeds were cryopreserved in liquid nitrogen at the zoo's frozen germplasm bank for use in future research projects and re-introductions in order to provide a back-stop to species extinctions.

Goals

- Goal 1: Conduct field surveys of the orchids found in Ranomafana National Park.
- Goal 2: Develop successful micropropagation protocols for the seeds.
- Goal 3: Provide biotechnology training and conservation education for Malagasy graduate students, professors, park guides and local residents near Ranomafana.

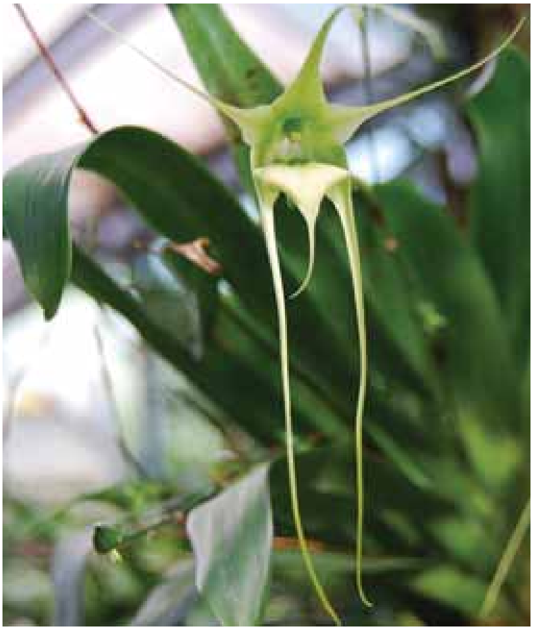
Success Indicators

- Indicator 1: Increase the number of orchid plants used for return to the native habitat, and re-establish them in areas where the seeds were originally collected, in order to augment current populations.
- Indicator 2: Long-term monitoring of success/failure rates for each of the orchid species re-introduced.
- Indicator 3: Develop a useful model for propagation and re-introduction to be used in Madagascar and other plant research projects conducted elsewhere.

Project Summary

The habitat where this project originated is a mountainous region characterized by high humidity, high rainfall and dense jungle that provides shady growing conditions. Eleven orchid species representing five genera were propagated *ex situ* and returned to Madagascar for re-introduction to the forest in Ranomafana National Park. The collection of 839 juvenile orchids represented members of Aeranthes-6 species, Aerangis-1 species, Bulbophyllum-2 species, Calanthe-1 species and Cryptopus-1 genera. Orchids are over-exploited, often illegally, all over the world and Madagascar has seen its share of illegal collection of orchids that wind up being sold in the marketplace. Political instability in the country and a burgeoning population searching for any means to support families often results in pressure upon plants with economic value. The collaborative project enlisted local residents and graduate students in orchid propagation and re-introduction to promote regional conservation. Students and professors from the Madagascar University were given biotechnology training for micropropagation, cryopreservation and re-introduction techniques previously developed at the Omaha Henry Doorly Zoo plant laboratory in the United States. Juvenile orchids produced at the zoo were transported to Madagascar after a rigorous phytosanitary inspection in the United States and were also quarantined upon arrival in Madagascar prior to re-introduction in the forest.

The plants that were returned to Ranamofana National Park were transported, still in sterile cultures, and were acclimatized to the light and humidity regimes right at the re-introduction site. Transporting the plants in aseptic cultures prevented any pathogenic material being transferred between the two countries in order to avoid any environmental problems in the rainforest. A local resident was employed to


Aeranthes orchid

assist with the re-introductions and to make monthly monitoring expeditions to the park for follow up with the plants. Epiphytic species were attached to the trees by using natural materials such as local mosses and vines. The Calanthe species is both an epiphyte and a terrestrial species that readily adapted to the natural habitat when planted in the detritus near the base of trees. The overall survival rate was greater than had been anticipated, particularly encouraging since this was a pilot project to test whether orchids could survive when re-introduced to the rainforest directly from sterile *in vitro* cultures. The Aerangis species survived at 75.86%, Aeranthes species survived at an average of 63.84%, the Calanthe species survived at 80%, Bulbophyllum species averaged 15.74% survival and the Cryptopus species survived at 100% after one year.

The Bulbophyllum species have roots that are thin and appear to have few reserves of nutrients or moisture and must be kept wetter than other species when they are reintroduced to the wild. Future re-introductions for that genus will be returned to the forest under improved techniques. All of the orchids continue to be monitored and after 5 years a number of the re-introductions have commenced blooming which indicates that those plants have now entered the reproductive stage, an important life stage for population sustainability. In order to engage Madagascar local schoolchildren in conservation they were taken on field trips to the park to observe orchids and other endemic plants in order to raise awareness of their own region's natural resources. An art contest sponsored at the local elementary school taught the children about the orchid structures and their particular role in forest biodiversity. Many of the schoolchildren had never before entered the park or been made aware of the importance the park's plant diversity holds for them and their country.

Major difficulties faced

- Timing of re-introductions with the proper season to increase survival rates.
- Choosing specific re-introductions sites and conditions that would allow orchid re-introductions to survive.
- The distance between the countries of the collaborators which increased costs.

- Concurrent studies at the Malagasy university were difficult due to inadequate facilities for aseptic micropropagation.

Major lessons learned

- *Ex situ* seed micropropagation allows for a large number of propagules produced. A single fruit removed from the wild produces many orchids with little or no impact on wild populations.
- Re-introductions are most successful for orchids when made at the outset of the rainy season to avoid excessive dehydration while plants are small.
- The host country will benefit most if project participants include local residents who have first hand knowledge of the local conditions needed for re-introduced plants.
- Each species has its own particular niche in the natural habitat which requires careful documentation and analysis to facilitate successful re-establishment.

Success of project

Highly Successful	Successful	Partially Successful	Failure
√			

Reason(s) for success/failure:

- This is the first known project of its kind to detail a successful re-introduction of orchids directly from sterile *in vitro* cultures into the natural habitat.
- Engaging local residents and students made the project successful and at the same time raised public awareness of the natural resources.
- The majority of the re-introduced plants survived past the five-year mark and have gone on to begin their reproductive cycle, indicating successful re-establishment in the wild.
- Seed samples were cryopreserved at Omaha's Henry Doorly Zoo to preserve germplasm that in some cases is irreplaceable.

Experimental introductions of the heath spotted and early marsh orchids into a restored ecosystem in Switzerland

Samuel Sprunger[1] & Grace Prendergast[2]

[1] - Curator, Swiss Orchid Foundation, Jany Renz Herbarium, Schönbeinstrasse 6, 4056 Basel, Switzerland (samuel.sprunger@unibas.ch)
[2] - Conservation Biotechnology Unit, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK (grace@kew.org)

Introduction

The heath spotted (*Dactylorhiza maculata*) and early marsh (*Dactylorhiza incarnata*) orchids are protected species in Switzerland and are listed on CITES App. II and the EU habitats directive. L'Etang de la Creule was constructed between 1993 and 1994, to channel run-off water from the banks of the A16 motorway to Courgenay. The basin is separated into two distinct areas by a dyke of large rocks, one part is for the water to settle and the other is of interest for development of flora and fauna. The south bank is managed to favor a diverse range of insect species. The north bank is steep and hot, and the waste water goes into a channel to the east. The sides of the pond are surrounded by a mixture of prairie flowers. The area is hidden from view by many native species of trees and bushes. Fifteen years after it was built, this artificial environment is home to more that 150 plant species. Many plant and animal species arrived spontaneously, without the intervention of man. The two orchid species have been introduced.

Goals

- Goal 1: *In vitro* seed germination from local plants of *Dactylorhiza maculata* and *D. incarnata*.
- Goal 2: Acclimatization of *in vitro* plants to cultivation in pots.
- Goal 3: Increased diversity of a restored ecosystem by introduction of two protected orchid species.
- Goal 4: Natural regeneration of introduced orchid species.

Success Indicators

- Indicator 1: Successful *in vitro* germination of *Dactylorhiza maculata* and *D. incarnata*.
- Indicator 2: Acclimatisation of plants to soil in Switzerland.
- Indicator 3: Survival and regeneration of introduced plants.

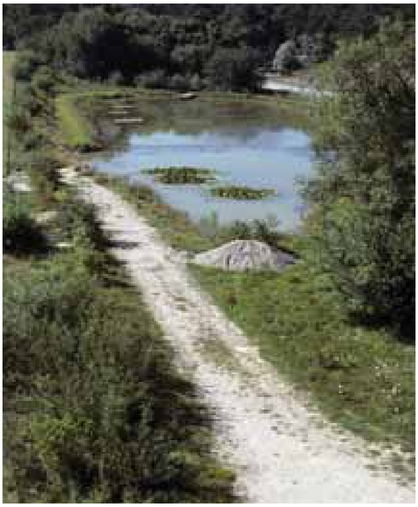
Project Summary

Feasibility: It is important to source plant material from a habitat similar to the introduction site and to cultivate plants under the conditions that they will encounter, in preparation for planting. In this experiment, plants were grown in

Flowers of *Dactylorhiza incarnata* (*left*) and *Dactylorhiza maculata* (*right*)
© Samuel Sprunger

pots using soil from the introduction site. The pots were kept outside, in the same region as the re-introduction site, so that the plants would be exposed to local environmental conditions. It was important to grow the plants to flowering size before planting out to verify the species, as it can be difficult to identify them when collecting seeds after flowering has finished. Local agreements were needed to collect seeds and to carry out the introductions: Permission was obtained from the 'Ponts et chaussée Delémont, séction route nationale', the Swiss equivalent of the Highways agency. The restored area is of great interest to local people as the pond Etang de la Creule is new to the region. The cultivation in pots, reintroduction and monitoring was carried out in Switzerland, by Samuel Sprunger. The germination protocol was developed at Royal Botanic Gardens, Kew and production of *in vitro* plants was funded by and performed at Royal Botanic Gardens, Kew. Some seeds and plants were retained at Kew for *ex situ* conservation.

Implementation: Seeds capsules of *Dactylorhiza maculata* and *D. incarnata* were collected between 1999 and 2001, at Chevenez and Porrentruy, with the land-owners' permission. These sites were close to the planned introduction site. Artificial pollination was carried out so that seed collection did not deplete the natural populations. CITES permits were obtained to transport the seeds from Switzerland to Britain. Phytosanitary certificates were obtained to return the *in vitro* plantlets to Switzerland. The seeds were collected as green capsules and transported by post. On arrival at Kew, they were surface-sterilised by dipping in 99% ethanol then passed through a flame. This procedure was carried out three times for each seed capsule. The capsules were cut open inside a laminar flow bench, and the seeds were sown onto petri dishes of modified Greenaway medium (Salman *et al.*, 2002) and incubated at 20^0C in the dark. Although the seed capsules were green and intact, the seeds inside were brown and fully mature and some were banked for later use. Germination was successful, using freshly collected seeds which did not need to be bleached. Seedlings develop more quickly if grown together with a symbiotic fungus but this presents problems when moving plants from one country to another, since the natural distributions of fungal species are largely unknown. Therefore these seedlings were grown asymbiotically to avoid introducing non-native fungi. Germinated seedlings were



Overview of habitat
© Samuel Sprunger

transferred to fresh media in 0.45 kg honey jars and when shoots developed the plantlets were moved into the light. Subsequently they were transferred to fresh media every six months. In late 2002, the largest plantlets were transferred to plastic containers for transportation. They were carried by air as hand-luggage to Switzerland, where they were potted up. Plants were considered large enough to pot up when they had ½ to 1 cm tubers and roots. In spring 2003 plantlets were removed from the agar and rinsed with tap water to remove any traces of agar from the roots. They were potted up into compost containing calcareous soil from the proposed destination at La Creule, with 5-10% organic matter. Sand and leaf mould were added in the following proportions: Soil (8), sand (1) and leaf-mould (1). Three or 4 plantlets were placed into each 10 cm, plastic pot , placed outside in semi-shade and watered with rain water when needed. Each plant produced 2 or 3 leaves in the first year, and 50% flowered by the third year after potting. In June 2005, 15 pots of each species were planted out at la Creule. At this stage the plants were in flower or had immature seed pods

Post-release monitoring: *Dactylorhiza* species are dormant during winter, but in the year following their introduction, between 70% and 90% of the plants produced new leaves and flowers. In spring 2008, many young plantlets of both species were observed, some flowering for the first time, showing that natural regeneration had taken place and confirming that the habitat chosen was suitable for survival and natural regeneration of these species.

Major difficulties faced

- CITES permits are needed for certain plant material to cross international borders and this has a cost implication.
- It is not always possible to identify species when they have finished flowering and produced seeds, especially in areas where similar species occur together.
- Poor results have been obtained if *in vitro* plants are sent by post or as air cargo, so transportation relied on the goodwill of staff travelling to Switzerland for other reasons.
- Asymbiotic propagation is relatively slow.
- Disturbance of introduced seedlings by birds, animals and people.

Major lessons learned

- *In vitro*, asymbiotically raised orchids can be successfully introduced to a man-made environment.
- Source plants could be mapped or marked when they are in flower and a voucher specimen could be collected, to facilitate species identification.

- If propagation could be carried out in Switzerland this would avoid the need for CITES permits and locally obtained fungi could be used for symbiotic germination.
- Several years are needed to carry out and assess the success of such a project.
- Protection from disturbance by birds and animals may be needed, such as wire cages. Local education and interpretive information may reduce human damage.
- Collaboration between our organizations was beneficial to share expertise at different stages of the project.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- The plantlets survived transfer from *in vitro* conditions to cultivation in pots.
- The plants survived introduction in to a man-made environment.
- The plants reproduced in their new environment.

Acknowledgements

We thank Kew students: Tim Blancpain, Barry Coetzee, Estelle Gill, Helenka Jurgielowitz and Bob McMeekin for assistance with preparing laboratory media and transferring plants to fresh media, Sara Redstone, Kew Plant Health and Quarantine Officer, for organizing phytosanitary certificates. Dr Ryan Cripps for transporting the plants to Switzerland and Dr Phillip Cribb for his help and encouragement. Special thanks to Mr Vincent Challet, Mayor of Courgenay for encouraging the continual improvement of natural areas in the vicinity of his town.

References

Lauber, K. & Wagner, G. 2000. Flora Helvetica, Haupt Berne.

Landolt, E. 1991. Rote Liste. Gefährdung der Farn und Blütenpflanzen in der Schweiz. Bern EDMZ.

Ritter, M. 1985. Contributions à la protection de la Nature en Suisse 7 LSPN, Bâle/ Inventaire des prairies et pâturages secs du Canton du Jura.

Salman, R. S., Prendergast, F.G. & Roberts, P. 2002. Germination of *Dactylorhiza fuchsii* seeds using fungi from non-orchid sources. In Kindlmann, P., Willems, J.H. & Whigham, D.F. (eds) Trends and fluctuations and underlying mechanisms in terrestrial orchid populations. Leiden: Backhuys Publishers.

Sprunger, S. 1998. Combe Vatelin, Pinéde de Courgenay et l'étang de la Creule–un ensemble de biotopes à sauvegarder et à protéger! Bauhinia 12(1/2): 23-31

Sprunger, S. 1999. Courgenay, un haut lieu de la flore en Ajoie. Société Jurassienne d'émulation, Actes, 43-64

Propagation and re-introduction of the western prairie fringed orchid in Nebraska, USA

Margaret M. From

Director of Plant Conservation, Center for Conservation & Research at Omaha's Henry Doorly Zoo, 3701 South 10th Street, Omaha, Nebraska 68107-2200, USA (psl@omahazoo.com)

Introduction

The western prairie fringed orchid (*Platanthera praeclara*) is an endemic orchid that is protected throughout its range in the Great Plains of the United States and Manitoba Province in Canada. The orchid is on the IUCN Red List for North America. The species had not been successfully propagated prior to 1999, when it was successfully micropropagated from seeds at the plant research lab at Omaha's Henry Doorly Zoo and subsequently re-introduced to augment an existing wild population. The research project was initiated in the sandhills of Nebraska where the largest known population in the state is located on the Valentine National Wildlife Refuge. The orchid species is sometimes found in widely scattered and isolated populations throughout the eastern two-thirds of the state but most of those populations have only a few individual plants. The species is associated with a specific pollinator which is believed to be in decline across the range and the orchid is also highly dependent on a symbiosis with specific soil fungi which facilitate uptake of soil nutrients for plant survival.

Western prairie fringed orchid (*Platanthera praeclara*)

The research project involved propagation and re-introduction of the propagated orchids, isolation of the fungi associated with the orchid's roots and rhizomes in the wild and soil nutrient analyses of the natural habitat in order to identify environmental conditions necessary for survival. The primary aim was to create a profile of some of the factors in the environment that support the orchid and to assist wildlife managers in decision-making regarding currently known populations and identifying potential re-introduction sites.

Goals

- Goal 1: Propagate the orchid *in vitro* for use in wild population augmentation.

- Goal 2: Develop successful micropropagation protocols for the orchid seeds.
- Goal 3: Isolate the symbiotic fungus(i) from underground tissues of the orchids found in the wild.
- Goal 4: Analyze soil nutrients present in the orchid's natural habitat.
- Goal 5: Re-introduce micropropagated juvenile orchids to the wild.

Succes Indicators

- Indicator 1: Successful propagation of the species that had previously resisted attempts at seed propagation.
- Indicator 2: Isolation and characterization of suspected fungal symbionts.
- Indicator 3:Determining nutrients and minerals in soils at orchid sites and comparing them with nearby non-orchid sites.
- Indicator 4: Monitor re-introduced plants for growth and survival.

Project Summary

Due to its protected status under the US Endangered Species Act permits were obtained from US Fish and Wildlife Service and the Nebraska Game & Parks Commission to collect seeds from *Platanthera praeclara* on the Valentine National Wildlife Refuge. The terrestrial species makes a very sporadic appearance from one year to the next and is believed to survive underground for some of its life stages, which may be part of a species survival strategy in a harsh environment characterized by broad swings in temperature and rainfall. The orchid's sporadic show may also be related to a periodic unavailability of the suspected fungal symbiont(s) during natural fluctuations of surface waters that peak and recede within the habitat over the course of the seasons and years. The habitat is characterized by arid hills with low-lying sub-irrigated meadows between the sparsely grass- covered sandhills.

The orchid seeds are smaller than a single grain of dust and have both physiological and chemical dormancies which must be understood in order to get them to germinate. A multi-step process was developed to scarify and surface sterilize the seedcoats without damaging the bare microscopic embryo within prior to *in vitro* culture on sterile agar-gelled media. The germination is very low and generally was less than 6% and the sensitive seedlings were prone to easy die-back even under sterile *in vitro* conditions and were slow-growing. Juvenile plants used for re-introduction trials were grown *in vitro* at the lab for two to three years prior to planting-out. The orchids were kept in vitro under sterile conditions to reduce any chance of introducing pathogens to their specialized microhabitats in the wild. More than one hundred-thirty juvenile orchids were planted back in the habitat near the adult plants which provided the seeds that were collected three years earlier. Re-introduced orchids survived at a low rate but were encouraging enough to warrant further re-introduction investigation for the species.

To identify potential symbionts, a small amount of root tissue was collected in the wild and the fungi were then isolated in the laboratory. A total of twenty-seven isolates were cultured *in vitro* and fourteen of them were targeted as possible symbionts for the orchid. A small number of the *in vitro* grown orchids were inoculated with the suspected symbionts. Inoculated orchids grew equally well as

Orchid habitat in the Nebraska sandhills

non-inoculated orchids for a few weeks but those inoculated were more likely to die before maturity than the orchids that were grown in the absence of fungal inoculation. Soils were analyzed from orchid sites and nearby non-orchid sites to determine whether there were nutrients more or less prevalent in the orchid microhabitats. The soil analyses may help make it possible to test potential re-introduction sites for their soil contents prior to planting out *ex situ* produced orchids. Soil core samples were taken near adult *P. praeclara* orchids and at non-orchid sites nearby which appeared to be similar to the orchid sites. Soil cores were taken in spring, summer and in the fall and soil samples were almost always totally water-saturated when taken near an existing orchid, regardless of the season, while samples taken in similar-looking non-orchid habitat within 50 m of orchids were not saturated, indicating that water availability is critical to the orchids' survival. As a result of the soil analyses a general profile of nutrient, soil textures and water availability have been delineated for future *P. praeclara* re-introductions if, and when, they are made in this part of the orchid's native range.

Major difficulties faced

- A complicated and prolonged legal permitting process and many restrictions placed on the project since the study took place on federal land.
- Determining the best time to re-introduce the orchids to the natural environment when repeated measures were restricted by governmental regulations.
- The orchid species is extremely sensitive to root disturbance making handling of the seedlings difficult.
- Identification of preferred re-introduction sites.
- A concurrent eight-year drought was assumed to have had a detrimental effect on the re-introduced plants and may have skewed the outcome to some degree.

Major lessons learned

- The orchid species can be successfully propagated in asymbiotic cultures.
- Re-introductions of inoculated orchids were no more likely to survive after re-introductions than orchids that were raised asymbiotically *in vitro* and re-introduced to the wild.
- The species shows a preference for soils that are generally nutrient-poor but the element magnesium is abundant near existing *P. praeclara* orchids.
- Re-introductions are best made in the very early spring when the soil and air temperatures are still cool and there is ample soil moisture.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- Survival of re-introduced orchids was generally somewhat lower than expected.
- Legal restrictions precluded adequate replications for the re-introductions which would have allowed a large study to be done.
- More than 98% of soil microbes are still unclassified by science, making fungal symbiont identification difficult.

Conservation introduction of a locally extinct fern species in Estonia during 1998-2008

Ruth Aguraiuja

Senior researcher, Tallinn Botanic Garden, Kloostrimetsa Rd. 52, Estonia (ruth.aguraiuja@tba.ee)

Introduction

Woodsia ilvensis (L.) R. Br. has a disjunct circumpolar distribution (Hultén & Fries, 1986). The species has not been evaluated for IUCN yet, its condition and conservation status within the whole range varies from locally common to locally extinct (Torleif *et al.*, 1993). In Estonia, *Woodsia ilvensis* was historically rare. It was found growing in northern and north-western Estonia representing the south-eastern border of its Scandinavian disjunction, were few areas offer suitable habitat. First found in 1887, it was documented only in four locations in different times and has not been found since 1977. Initially it was assessed as critically endangered (Lilleleht, 1998) and until 2004 it belonged to I category of protected plant species (RT, 2004). Since 2005, it has been considered as naturally extinct species in Estonia (Kukk & Kull, 2005).

Considering that habitat conditions may have changed on previous locations, an experimental project was started to test if it would be possible to successfully introduce *W. ilvensis* into new localities where suitable habitat conditions exist.

Goals

- Goal 1: Testing the habitat suitability for population establishment.
- Goal 2: Establishment of a viable experimental population.
- Goal 3: Creating a local spore source for further natural dispersal of the species.

Success Indicators

- Indicator 1: Long-term survival of out planted individuals.
- Indicator 2: Establishment of new natural generations, population recruitment.
- Indicator 3: Colonization and establishment in new empty patches of suitable habitat, extended distribution.

Re-introduced individuals in 2009

Project Summary

Woodsia ilvensis is known as a fern of dry sunny or half shaded rocky habitats, on thin acidic soils on many different types of metamorphic and eruptive rocks. The older findings from Estonia were from the north-eastern coastal cliff. The most recent findings were from old stone fences piled from the stones collected from surrounding fields. While checking previous locations in Estonia during the period of 1994-2006, not a single individual was found. The two older locations were dominated by calcareous substrate. In two most recent locations the habitat was degraded - the stone fences were destroyed or removed. Considering that substratum reaction may be limiting factor and that changed habitat conditions may not support the species survival in previous locations any longer, the search for new apparently suitable habitats for experimentation of conservation introduction was started. There are only few areas of almost 'calcium free' environment in northern and north-western part of Estonia where vendian layer is denuded. These areas, some northern islands, stone fences and siliceous erratic boulders were checked for possibly suitable habitat.

In many regional floras, *Woodsia ilvensis* is mentioned sharing the habitat with *Asplenium septentrionale* (L.) Hoffmann. Both species are represented in Finland and on easternmost islands of Finnish Gulch (Glazkova, 1996). On its most recent location in Estonia, *W. ilvensis* was also found growing together with *A. septentrionale* (Hein & Puusepp, 1962), both currently extinct on this site. There is a single natural population of *A. septentrionale* growing on the south facing stone wall of the old churchyard on small island of Prangli. It was decided to consider *A. septentrionale* as the indicator for suitable habitat conditions and choose the north -facing side of the same stone wall for re-introduction experiment of *W. ilvensis*. As there was no natural source of local spores, all plants were grown from the spores received via the seed and spore exchange of botanical gardens. Only spores of wild provenance were used for introduction experiment (Joensuu HB, North-Karelia, spont.; Helsinki HB, Uusimaa, spont.). The sowings were made in laboratory conditions and timed for late autumn. Next spring young plants were planted into the mix of leaf mold, drained peatland forest soil and coarse sand, and thereafter taken into the shaded sphagnum beds, where they were kept and handled with minimal maintenance until planted into natural conditions.

In 1998, two years old individuals were planted onto the old north facing stone fence on the island Prangli. In 2001, a group of five years old individuals were added to that site. In both cases, the planting was timed to late summer and early autumn according to more moist weather conditions, thus giving plants enough time to get rooted before winter. The plants were watered only after the planting and then left into natural conditions without any maintenance or site management. The introduction on stone fence was successful. Some plants died during two first years, the rest survived and continue growing. The ferns are well adapted to the conditions of dry and open habitat conditions. They are tolerant to long droughts, drying and curling up the fronds during the dry months and turning back green and growing on after the late summer or autumn rains. The spores are produced yearly. The germination tests show that they produce viable spores. No sign of regeneration, natural recruitment or spread has been discovered yet. The number of fronds and rhizome tips is bigger for these ferns which were out planted when younger and smaller (two years old plants). The monitoring is conducted yearly. The growth, condition and vitality of single individuals are assessed. During each visit the whole habitat patch is searched for regeneration.

The results confirm that if the spores of *Woodsia ilvensis* would land on suitable substrate within suitable habitat in northern or northeastern part of the Estonia, they could germinate, the individual plants could get established and persist at least for a certain period of time. The ferns have been out in the habitat for eleven years without any maintenance. For now, the age of experimental individuals is 13 years and they have been growing in natural habitat for 11 years. As the ferns of this experimental population still increase in size and produce spores yearly, one may conclude that the habitat conditions of the site may be suitable for the species. Since, the population recruitment has not been observed.

Major difficulties faced

- More research on microhabitat requirements and conditions is needed, for selecting the exact locations for planting the individual ferns into natural habitat. Not only substrate reaction, but the moisture content and régime in the soil of the microhabitat could be vital for long-term survival, particularly in case of ferns of dry and mesic habitats. The ferns do not have a deep root system as most of flowering plants do. Rather thin additional roots grow out of the rhizome, and get their water and nutrition from relatively smaller soil area.
- Many fern species are sensitive to repeated planting. They may get disturbed and need a longer period for the recovery and after-care. For this reason it is important to consider which developmental stages could be best for the successful establishment in the habitat. Theoretically, it would be good to plant out the fern individuals in as early developmental stages as possible, enabling the longer period for adaptation with natural conditions and the rhythm of natural changes in the habitat. The ‘right’ developmental stages for out planting may be specific depending of autecological characteristics of the species and habitat conditions.

Major lessons learned

- The growing process is the continuous adaptation to environmental conditions. The ex situ propagation should be toughening the plants for future planting into natural habitat, decreasing thus the after planting environmental stress and increasing the potential survival of the individuals in the site.
- The single out planting into the habitat equals to single occasional natural distribution event. It may take very long time until structured self-sustaining population evolves naturally, and probability for this event to happen is as big as that of extinction. It was learned that the out planting should be gradual during several consecutive years or by the cycles, imitating thus the natural colonization, population growth and establishment of structured self-sustaining population.
- The optimal number of individuals planted per year and the numbers of years, necessary to promote establishment of a viable population, may be specific to

the species or the group with similar life strategy. Analyzing monitoring data of natural and experimental populations helps to specify these numbers.

- As the number of individuals and amount of spores/seeds is limiting in case of endangered species, it would be more sustainable and more effective to start with smaller experimental populations, gradually increasing the size and number of patches in accordance to the intermediate survival analyze.
- If appropriate habitat with characteristic community and habitat conditions could still be found, then any after-management of the site will not be needed.
- The best indicators for selecting the possibly suitable habitat were characteristic species of the typical to the species natural community.

Success of project

Highly Successful	**Successful**	Partially Successful	Failure
	√		

Reason(s) for success/failure:

- Though limited and patchy, the suitable habitat conditions for *Woodsia ilvensis* still exist in Estonia. The thorough preliminary analysis of historical and current natural distribution, the distance from closest natural spore source, availability of suitable habitat and microhabitat conditions, life history characteristics of the species and the structure of natural community, are essential for the successful re-introduction/restoration/recovery efforts.

References

Aguraiuja, R. 2001. Eesti kaitstavate sõnajalgade uurimine Tallinna Botaanikaias. Tallinna Botaanikaia uurimused V. Taim ja keskkond, lk. 85-98. (Study of protected fern species of Estonia in Tallinn Botanic Garden. Studies of the Tallinn Botanic Garden V. Plant an Environment)

Aguraiuja, R. 2005. Hawaiian endemic fern lineage Diellia (Aspleniaceae): distribution, population structure and ecology. Dissertation, University of Tartu

Hultén E, Fries, M. 1986. Atlas of North European Vascular Plants North of the Tropic Cancer. I. Koeltz Scientific Books, Germany

Kukk, T. & Kull, T. (eds). 2005. Atlas of the Estonian Flora. Tartu

Lilleleht, V. (ed). 1998. Red Data Book of Estonia. Tartu

RT (2004) Riigi Teataja (State Gazette)

Torleif, I., Andersson, R. & Tjernberg, M. 1993. Red Data Book of the Baltic Region. Part I. Lists of threatened vascular plants and vertebrate. Swedish Threatened Species Unit, Uppsala

Juniper scrub restoration at the Cample Burn, Clyde Muirshiel Regional Park, Scotland

Alan Brown

Countryside Officer, Clyde Muirshiel Regional Park, Park HQ, Barnbrock, near Lochwinnoch, Renfrewshire, PA10 2PZ, UK (*alan.brown@clydemuirshiel.co.uk*)

Introduction

Juniper (*Juniperus communis* ssp. *communis*) is a UK Biodiversity Action Plan priority species that occurs in a number of habitat types listed under the EC Habitats Directive and Juniper scrub is recognised as a nationally scarce woodland type. The above species is globally the most widespread subspecies and is relatively common throughout lowland Britain on both limestone and acid soils, especially on the Chalk Downs of southern England and the Scottish Highlands. However, within Scotland juniper has been lost from 23% of areas in which it was formerly present and in a further 34% of areas its future is under threat in the short to medium term (Sullivan, 2003).

A survey of juniper by Plantlife recorded that of 453 sites around 40% had fewer than 10 plants (Long & Williams, 2007). There is concern for the viability of fragmented populations and that regeneration is likely to be limited as 67% of all plants recorded were mature, old or dead. This lack of juniper regeneration is a significant problem at the majority of sites across the British uplands and therefore planting schemes may be necessary to ensure the survival of this species at particular sites. In Scotland juniper may be found from coastal locations to high on the mountain tops. Although juniper is recorded close to the summit of Braeriach in Aberdeenshire at 975 m around 80% of all juniper are normally found closer to 400 m (Sullivan, 2003). The juniper within CMRP all occur at an altitude of between 300 to 450 metres. The juniper restoration project at the Cample Burn site is located within Clyde Muirshiel Regional Park, 30 km west of Glasgow and is also within a Special Protection Area for Hen Harriers.

Close up of juniper berries and leaves

Goal

- Goal 1: To safeguard the existing juniper.
- Goal 2: To naturally regenerate the juniper and propagate stock from locally provident plants.

Guided walk to a juniper exclosure

- Goal 3: To allow regeneration or to introduce mixed scrub woodland rowan, willow and birch) integrated with open heather moorland for nesting/hunting hen harriers.
- Goal 4: To encourage local involvement in the conservation and restoration of juniper woodland scrub.
- Goal 5: To promote the value of juniper scrub woodland in the natural heritage
- Goal 6: To extend Juniper scrub woodland across the Local Biodiversity Action Plan area.

Success Indicators

- Indicator 1: To safeguard all existing juniper stands within the Regional Park.
- Indicator 2: To plant out 1,000 juniper within a 17 ha exclosure.
- Indicator 3: To establish a second project area within the Clyde Muirshiel uplands for juniper scrub woodland habitat.

Project Summary

The Cample Burn site habitat is largely a blanket mire that contains *Erica tetralix, Calluna vulgaris, Scirpus cespitosus* and *Eriophorum vaginatum.* The land is farmed for sheep and was until recently also managed for grouse shooting by the rotational burning of small patches of heather. Anecdotal evidence has suggested that juniper in the Clyde Muirshiel hills was once relatively common, but written references only give a non specific description of it being frequent in woods and heaths. Juniper appears to have been lost through muirburn and overgrazing by sheep. In the Regional Park's 281 km^2 there are six mature juniper plants, two of which are female. In the surrounding council areas of Glasgow, covering over 6,000 km^2, there are only fifteen sites with juniper (Broome, 2008; *pers comm*). Fencing of the 17 ha moorland site was completed in January 2008 and the first thirty juniper were planted by volunteers at the Cample Burn site three months later form stock derived from local species. A group in the local village, the Lochwinnoch Community Garden, is nurturing the juniper cuttings that will be planted out over the next three years. However, the survival rate of the juniper from cuttings has been around 10% and only 60 juniper have been planted at the site so far. Juniper does grow better from seed, but berries have only been found occasionally within the Regional Park. It was suspected that the poor success rates of propagation may have been due to the mature plants and the small size of available cuttings that were less than half of the recommended length of 10 cm

(Broome, 2003). For the Cample Burn site there were only a handful of juniper bushes from upland sites in its seed zone.
Initially, the sourcing of appropriate juniper was limited by guidance on semi-natural planting that recommended the use of seed/cuttings from within the same seed zone. However, due to the high failure rate of propagated cuttings it became unlikely that enough plant material could be collected locally. A solution was reached through a working group (South Scotland Juniper Network) where it was suggested by Forest Research that cuttings or seed collected could include sites with environmentally similar conditions. For the Cample Burn site locations were matched using a set of parameters developed by Forest Research to indicate a cool wet climate and a high nitrogen soil (Weber & Broome, 2008; unpublished). Four main areas had similar environmental conditions to Clyde Muirshiel.

Park staff with community gardener: Gordon Nicol

However, two of these areas were not suitable as some of the species plants could not be confidently distinguished as sub-species *communis* or as *ssp. Nana.* Around 300 juniper cuttings were collected from bushes in the Pentland Hills, Edinburgh and over 1,000 seeds collected with the assistance of the Borders Forest Trust form a site near Peebles, in the Scottish Borders. Following forestry guidance the juniper seeds were treated with a 1% citric acid solution for four days, stored at 4^{o}C for 30 weeks and then planted out in seed trays (Broome, 2003). After two years the imported juniper will be transferred to the Cample Burn site.

Another aspect of the project has involved the promotion of juniper and this has been done through guided walks, treasure hunts, BBQs and interpretation panels. Leaflets on the restoration project have also been displayed at key sites across South West Central Scotland. Of the six juniper within the Regional Park area three are now fenced within the Cample Burn scheme, a small exclosure has been completed for one other site, one was already inside a fenced Reservoir and the sixth bush is relatively inaccessible in a steep sided gulley. All the planted juniper have shrub tubes to guard against roe deer and mountain hares and the plants are weeded twice during the summer. A few Rowan (*Sorbus aucuparia*) have regenerated within the exclosure and it is planned to introduce some eared willow (*Salix aurita*) to the site next year. After a wet summer the first year's planting appeared to be waterlogged and this may have led to the demise of fifteen juniper, while those planted the following year and in drier ground have displayed vigorous growth.

Major difficulties faced

- Commercially grown on stock was sold accidentally to another project.
- Due to the old age of the local juniper plants the cuttings were less than half the length recommended by the Forest Research.
- The juniper propagation had under a 10% success rate and the lack of local bushes severely limited the amount of cuttings available for propagation.

Major lessons learned

- Working with a cross boundary group helped to solve problems with lack of cuttings and seed and identified common propagation problems.
- The grant application benefited from being part of a joint partnership with Action for Mountain Woodlands.

Success of project

Highly Successful	Successful	**Partially Successful**	Failure
		√	

Reason(s) for success/failure:

- The project is not finished and the limited success in propagation is likely to be overcome through increased use of seed.
- Several elements of the project have been very successful such as fencing the site, promoting juniper conservation and partnership working.

Acknowledgements
I would like to thank Alice Broome from Forestry Research at Roslin, Edinburgh for her helpful comments on the text for the first draft of this article.

References

Broome, A. 2003. Growing Juniper: Propagation and Establishment Practices, Forest Research.

Broome, A. (pers. comm.) 2008. Forest Research, Northern Research Station, Roslin, Midlothian EH25 9SY

Long, D. & Williams, J. 2007. Juniper in the British Uplands: the Plantlife juniper survey results

Sullivan, G. 2003. Distribution and condition Extent and Condition of Juniper Scrub in Scotland. Report to Scottish Natural Heritage, Contract No. BAT/AC205/01/02/96.

Weber, J. F. & Broome, A. 2008. Unpublished. Forest Research, Northern Research Station, Roslin, Midlothian EH25 9SY