



Global Re-introduction Perspectives: 2010

Additional case-studies from around the globe
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IUCN/SSC Re-introduction Specialist Group (RSG)





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

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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 than 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

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, section 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.



Flowers of *Dactylorhiza incarnata* (left) and *Dactylorhiza maculata* (right)

© Samuel Sprunger

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°C 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 $\frac{1}{2}$ 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.

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