Science for Saving Species

Research findings factsheet

Project 4.3

Threatened Species Recovery Hub

National Environmental Science Programme

Understanding mycorrhizal fungi to propagate Australia's imperilled leek-orchids

In brief

Leek-orchids (*Prasophyllum*) are at risk of extinction due to habitat clearing, weed invasion, climate change and animal grazing, with 38 species currently listed as nationally threatened. Due to many species now remaining as only a handful of plants often from a single population, habitat protection alone will not be sufficient to prevent their extinction.

Ex-situ (laboratory and nursery) propagation has been highlighted as a high priority for leek-orchid conservation and recovery, but no breeding programs for leek orchids have previously been successful, leaving many Critically Endangered species teetering on the brink of extinction.

We identified 26 species of mycorrhizal fungi belonging to the family Ceratobasidiaceae growing in the roots of leek-orchids, most of which were new to science. Many of the mycorrhizal fungi were continentally widespread, however some appeared to be habitat specialists. Some leek-orchids used just one species of fungi to germinate their seed, while others utilized up to six species. We found that specific nutrients in the growing-media were required for the symbiotic germination of leek-orchids with their mycorrhizal fungi.

We achieved repeatable germination of the Endangered *Prasophyllum frenchii*, with seedlings surviving and flowering in nursery cultivation 18 months after sowing. We also successfully germinated another 18 species of leek-orchid.

Understanding orchid mycorrhizal fungi was an important step forward for leek-orchid propagation and conservation. These results will allow reintroduction programs for leek orchids to commence.

*LEFT: Coastal leek orchid (*Prasophyllum litorale*) growing near Portland, Victoria. Image: Marc Freestone*

Background

Leek-orchids are a genus of small native wildflowers that can be found in bushlands, native grasslands and seasonal swamps across southern Australia. They are named for their single spring onion–like leaf, which shoots up from an underground tuber each autumn. When rainfall is sufficient, they produce a spike of small brown, green or white flowers in spring. Nearly one-third of the 140 species of leek-orchid are at risk of extinction, due to habitat clearing and degradation, weed invasion, climate change and animal grazing. The genus has 38 species currently listed nationally as threatened, while some species are already extinct, such as the lilac leek orchid (*Prasophyllum colemaniae*).

Leek-orchids have tiny dust-like seeds that depend on colonisation by mycorrhizal fungi for germination in the wild. The symbiotic fungi live in their roots and the surrounding soil, and provide essential nutrients to the seeds that enable them to germinate. *Ex-situ* (laboratory and nursery) propagation has been highlighted as a high priority for leek-orchid conservation and recovery; however, large scale effective symbiotic propagation and breeding of these species has been hampered by lack of effective propagation methods, leaving many Critically Endangered species teetering on the brink of extinction.

Australian National University

Royal Botanic Gardens Victoria

University of Tasmania

Main aims of the research

We aimed to help prevent extinctions of threatened leek orchid species by understanding the identity and distribution of their mycorrhizal fungi and developing reliable symbiotic propagation methods for leek-orchids in cultivation.

To achieve this, we aimed firstly to identify the symbiotic fungi that grow within orchid roots in the wild. Secondly, we aimed to determine the optimal laboratory growing conditions for leek orchid germination and for supporting ongoing seedling development.

The Endangered Maroon leek orchid (Prasophyllum frenchii) occurs in sphagnum bogs in the Australian Alps, which were badly impacted by the 2019-20 wildfires. A project funded by the Australian Government's Wildlife and Habitat Bushfire Recovery Program has used the propagation method developed in this research project to successfully grow hundreds of the species at the Royal Botanic Gardens Victoria. Image: Marc Freestone

What we did

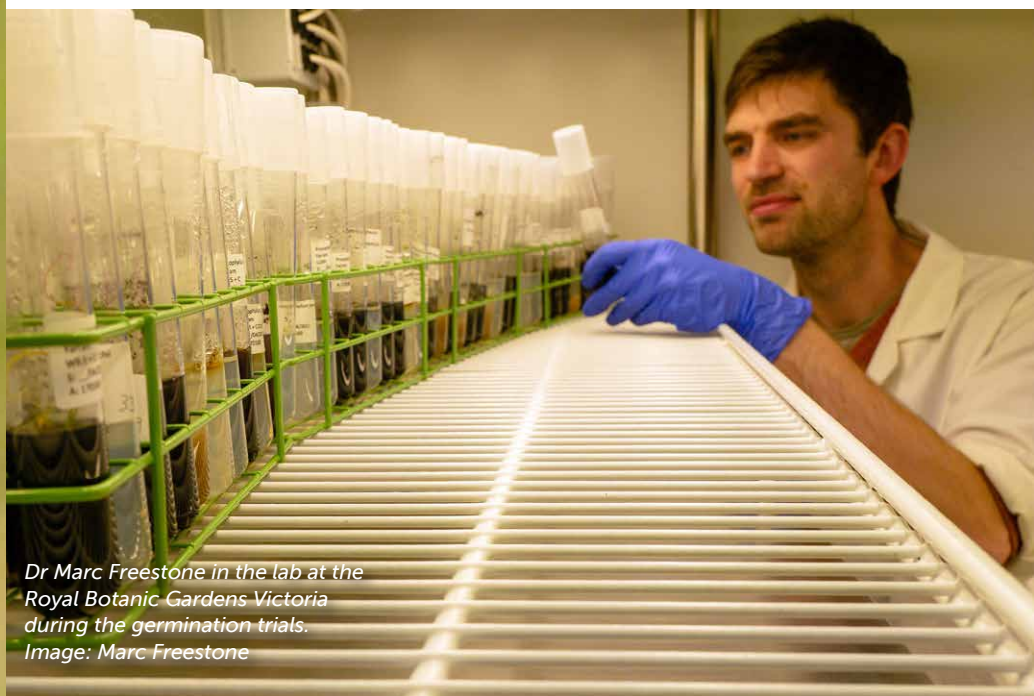
To improve the understanding of how leek orchid seeds germinate and survive in the wild, we collected root samples from 33 wild leek orchid species from across Victoria and south-eastern South Australia.

In addition, we wanted to identify if juveniles and adults used different mycorrhizal fungi or if leek-orchids utilised different fungi throughout a growing season. To answer these questions, we used seed baiting techniques, were we filled slide baits with orchid seed and buried these around wild plants at field sites near Yarram, Stawell and Benalla in Victoria, and observed the natural germination that followed. We also identified how many species of mycorrhizal fungi were associated with each orchid, throughout the growing season. To do this we extracted the symbiotic fungi living inside each orchid's roots, as well as the germinated seedlings from the seed baiting and then sequenced the mycorrhizal fungus DNA, to assist with identifying the different species of fungi.

We undertook a series of large germination trials in the laboratory to investigate the influence of different growing media on the germination of leek orchid seeds. We trialled agar-based growing media that varied in carbon and nutrient composition, with and without various additives. We extracted fungi from leek-orchid roots and grew them in petri dishes with the growing media. We then added the orchid seeds to the petri dishes and monitored germination.

We used the Endangered *Prasophyllum frenchii* as a model species, using mycorrhizal fungal isolates from the genus *Ceratobasidium*. We tested the rate of symbiotic germination using 15 different types of growing media. We also undertook a trial of different replate media to assess the best media for supporting the continued development of seedlings after germination.

Once the germination trials were complete, we then applied our new methods to germinate the seeds of 18 leek-orchid species.



Dr Marc Freestone in the lab at the Royal Botanic Gardens Victoria during the germination trials. Image: Marc Freestone

Key findings

We identified 26 species of *Ceratobasidium* fungi growing in the roots of leek-orchids, most of which are new to science. Seed burial trials around wild plants showed that some leek orchids required just one symbiotic fungus to germinate their seed, while others used up to six species of mycorrhizal fungi. Our research identifying the identity of these symbiotic fungi species and their distribution is a critical consideration for when leek-orchids are eventually introduced back to the wild.

We were able to successfully grow the mycorrhizal fungi extracted from the roots of the wild adult plants in petri dishes with a range of growing media. However, the nutrient composition of the growing media, was critical in determining whether the fungus would stimulate the seed to germinate. We obtained good germination results when we grew the fungus and seeds on high-macronutrient media and on media containing wheat bran as a carbon source instead of oatmeal.

After successfully developing these new laboratory-based methods for germination, we were able to transfer our methods to germinate seeds of 18 species of leek-orchid. These included the threatened species *Prasophyllum correctum* (two wild plants remaining), *P. fosteri* (eight wild plants), *P. anticum* (less than 50 wild plants) and *P. murfetii* (around 150 wild plants). Most seedlings survived when transferred to the nursery, and have formed the start of insurance populations, which are being held at the Royal Botanic Gardens Victoria. As these species mature and flower in the nursery, we hope that they will provide much-needed seed for future reintroduction programs. Overall, our studies have revealed that the mycorrhizal fungi–orchid relationship is complex. The nutrient profile of a site may play an important role in the ecology of the orchid–fungus relationship in the wild.

Implications and recommendations

We have developed a viable methodology for germinating threated leek-orchids, where none existed before. Understanding that the mycorrhizal fungi of leek orchids require a specialised nutrient medium to support ex-situ germination was a crucial step in developing a successful propagation strategy. Understanding the identity and number of species of symbiotic fungi associated with leek-orchids is also an important consideration for when leek orchids are eventually introduced back to the wild.

These germination techniques can be used by Australia's network of orchid conservation programs to implement reintroduction projects for the most imperilled leek-orchids. This will help to secure the long-term persistence of these species.

For some species of leek-orchid, this project has dramatically reduced the risk of extinction by creating a vital insurance population in cultivation. But while the prospects for many of our threatened leek orchids are looking brighter, their future now depends on the funding of urgently-needed propagation and reintroduction programs.

*Germinating Maroon leek orchid (*Prasophyllum frenchii*) seed on a high-macronutrient growing medium with symbiotic fungi. Image: Marc Freestone*

*LEFT: The new propagation method has allowed hundreds of Critically Endangered Fleurieu leek orchids (*Prasophyllum murfetii*) to be grown in a project funded by the South Australian Government at the Royal Botanic Gardens Victoria. Image: South Australian Seed Conservation Centre*

Implications and recommendations (continued)

Applying the method to fire impacted species

The 2019-20 bushfires extensively affected orchid populations in the Australian Alps (NSW), East Gippsland (VIC) and at Kangaroo Island (SA). To prevent the extinctions of 14 nationally threatened orchid species in these areas, a project was proposed by the Royal Botanic Gardens Victoria, the Botanic Gardens of South Australia, La Trobe University and received funding from the Australian Government's Wildlife and Habitat Bushfire Recovery Program. The project involves plant survival surveys, assessment of on-going threats to the wild populations, seed collection, mycorrhizal fungi collection and propagation.

The 14 orchid species targeted in the project included five nationally threatened leek orchids:

- Bago leek-orchid (*Prasophyllum bagonense*), Critically Endangered
- Maroon leek-orchid (*Prasophyllum frenchii*), Endangered
- Brandy Marys leek-orchid (*Prasophyllum innubum*), Critically Endangered
- Kelton's leek orchid (*Prasophyllum keltonii*), Critically Endangered
- Mignonette leek-orchid (*Prasophyllum morganii*), Vulnerable

The methods developed in this hub research project have been used to propagate these threatened fire-impacted species. There are now 200-500 seedlings of each of these species growing well in the laboratory at the Royal Botanic Gardens Victoria.

The plants will need to be at least three years to mature before they can be planted back in to the wild. Appropriate habitat and pollinator studies need to be conducted prior to introduction.

A separate collaboration between the Botanic Gardens of South Australia and the Royal Botanic Gardens Victoria funded by the South Australian State Government, has additionally used the new propagation methods to grow hundreds of seedlings of two South Australian leek-orchids, dramatically reducing the extinction risk for these species:

- Plum leek orchid (*Prasophyllum pruinosum*), Endangered
- Fleurieu leek orchid (*Prasophyllum murfetii*), Critically Endangered

Cited material

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National Environmental Science Programme

Cite this publication as NESP Threatened Species Recovery Hub. 2021. Understanding mycorrhizal fungi to propagate Australia's imperilled leek-orchids, Project 4.3 Research findings factsheet.

This project is supported through funding from the Australian Government's National Environmental Science Program.