8th INTERNATIONAL ORCHID CONSERVATION CONGRESS

Curtin University, Perth

Australia

3 - 6 September 2024

Curtin University

Australian Orchid Foundation

Orchid Specialist Group

Welcome to Perth!

Welcome to the 8th International Orchid Conservation Congress (IOCC).

The International Orchid Conservation Congress (IOCC) series started in Western Australia in 2000, and subsequent meetings were held in Florida, Costa Rica, the Czech Republic, La Réunion, Hong Kong, and at Kew. At the meeting at the Royal Botanic Gardens Kew, it was decided that IOCC8 should be held once again in Perth, where this series of conferences was first organized.

The "A Minute to Midnight" conference theme reflects the global rates of biodiversity decline. Never before in human history have so many wild species been faced with habitat loss and decline. Orchids have not been spared, and IOCC8 is dedicated to understanding the threats and the solutions that are needed to arrest the decline of orchids.

We are therefore delighted to welcome delegates from around the world to share their experiences of orchid conservation in a meeting that will inspire the next generation of orchid scientists.

We begin by acknowledging the Noongar people as the traditional owners of the land in which the 8th International Orchid Conservation Congress (IOCC8) will be held. We deeply respect their cultural, spiritual and educational practices and aspire to learn from their ways of living with the land. We are grateful to Curtin University for the use of the venue and for logistical support. The Australian Orchid Foundation provided financial support for bursaries for students so that they can attend the congress.

We have an inspiring and busy programme – with many talks and posters and other events including an ex-situ conservation workshop and a meeting of the Orchid Specialist Group.

After the conference we will have the field trip visiting the global biodiversity hotspot of Southwest Australia. The tour will take place during the peak orchid flowering season, and it is estimated that up to 100 species will be seen in flower, representing a range of genera including *Caladenia*, *Cyanicula*, *Diuris*, *Drakaea*, *Elythranthera*, *Ericksonella*, *Leptoceras*, *Microtis*, *Prasophyllum*, *Pterostylis* and *Thelymitra*. During the tour many different orchid habitats will be visited including forests, woodlands, shrublands, seasonally wet areas and granite outcrops.

The Organising Committee would like to take this opportunity to extend a warm welcome to Western Australia, and we hope you all enjoy the congress! Let the congress begin!

General Information

Tim Winton lecture theatres 1 and 2,

Curtin University, Building 213

Bentley, WA 6102

Directions to 213 Tim Winton Lecture Theatre (Bentley) with public transport

We suggest that you download the *Transperth* app for routes, planners and timetables.

The following transport lines have routes that pass near the 213 Tim Winton Lecture Theatre

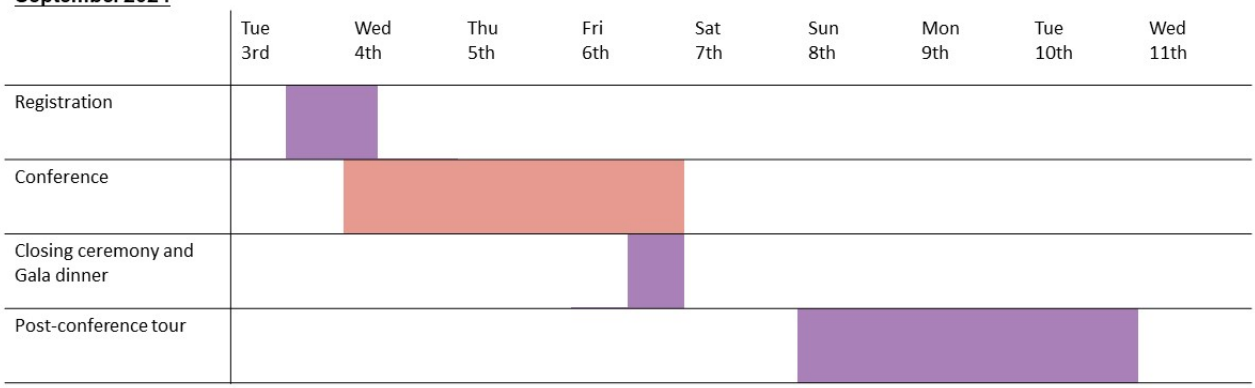
Buses:

100, 101, 72, 75, 960, 998

Note: the **960 runs every 10 mins** from the Perth Bus Station direct to Curtin

Train: MANDURAH LINE – with bus services to Curtin (can be irregular), but check timetables as connections can be infrequent

IOCC VIII PERTH 2024
a minute to midnight



Tuesday 3rd September

10:30 – 12:30	**Registration**	**Red Listing workshop** Organisers: Heidi Zimmer and Mike Fay
12.30 – 13.30	**Break**	
13:30 – 16:30	**Ex-situ workshop** Organiser: Emily Coffey and Jason Ligon	**Red Listing workshop, continued**

Wednesday 4th September

8:00 – 9:00	**Registration**
9:00 – 9:30	**Opening Ceremony & Welcome**
9:30 – 10:00	**Plenary Lecture** **Kingsley Dixon – A minute to midnight or a new dawn for orchid conservation?**
10:00 – 10:15	**Coffee/tea break**
10:15 – 10:45	**Plenary Lecture** **Michael Fay – Where are we up to with orchid conservation assessments?**
10.45 – 11:30	**Session 1: Conservation: Global Perspectives** Chair: Kingsley Dixon
11:45 – 12:00	**Group photo**
12:00 – 13:00	**Lunch**

13:00 – 14:15	**Poster Session**
14:15 – 15.15	**Session 2: Threats to endangered species** Chair: Michael Fay
15:15 – 16:00	**Coffee/tea break**
16:00 – 16:30	**Plenary Lecture: Amy Hinsley – From harvesters to consumers: taking a collaborative approach to understanding and reducing the illegal orchid trade**

Thursday 5th September

9:00 – 9:30	**Plenary Lecture** **Emily Coffey - The importance of ex situ conservation in preventing orchid extinction**
9:30 – 11:00	**Session 3: *Ex Situ* Conservation: Are we Making a Difference?** Chair: Jacopo Calevo
11:00 – 11:15	**Coffee/tea break**
11.15 – 12:00	**Session 3: *Ex Situ* Conservation (continued)** Chair: Jacopo Calevo
12:00 – 13:00	**Poster presentations**
13:00 – 14:00	**Lunch**
14:00 – 15:15	**Session 4: *In Situ* Conservation** Chair: Benjamin Crain
15:15 – 15:45	**Coffee/tea break**
15.45 – 17:30	**Orchid Specialist Group meeting and discussion (posters still available)** Organisers: Michael Fay and Amy Hinsley

Friday 6th September

9:00 – 9:30	**Plenary Lecture** **Jana Jersáková - Role of Fungal Interactions in Orchid Conservation: Lessons Learned and Future Directions**
9:30 – 10:45	**Session 5: Ecological interactions to Improve Conservation Outcomes** Chair: Brendan Jannisen
10:45 – 11:15	**Coffee/tea break**
11:45 – 12:30	**Session 5: Ecological interactions (continued)** Chair: Jana Jersáková

12:30 – 13:30	**Lunch**	
13:30 – 14:00	**Plenary Lecture** **Katharina Nargar: Reshaping our understanding of orchid diversity in the age of molecular genomics**	
14:00 – 15:15	**Session 6: Genetics and Molecular tools for Conservation** Chair: Katharina Nargar	**Session 7: Citizen Science and School Programs** Chair: Kingsley Dixon
15:15 – 15:45	**Coffee/tea break**	
15:45 – 16:15	**Australian Orchid Foundation – past, present and future**	
16:15 – 16:45	**Plenary Lecture** **Larry Zettler – Saving orchids in the 21st century: a reason for optimism**	
16:45 – 17:15	**Closing ceremony – announcement of IOCC9**	
18:30 – 21:30	**Gala Dinner: Awards**	

ORAL PRESENTATIONS

PLENARY LECTURE

A MINUTE TO MIDNIGHT OR A NEW DAWN FOR ORCHID CONSERVATION?

Kingsley Dixon*

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Abstract

For forty years botanic gardens and organisations from universities to schools have been active in the science and conservation of orchids. Over this time great strides have been made from the first steps when Kew Gardens in the 1990's invested heavily in the conservation and ultimately the reintroduction of the lady's slipper orchid (*Cypripedium calceolus*). In parallel other organisations around the world contributed an array of powerful scientific tools to arrest orchid decline. Ground-breaking work in mycorrhizal assisted propagation took the more difficult groups such as terrestrial orchids into the realm of being feasible to grow and reintroduce while epiphytic species were being successfully introduced into civic and wild areas. Molecular studies have revealed new taxa, new combinations and is a powerful tool in understanding orchid biology from pollinator interactions to mycorrhizal diversity. However the scorecard for the past forty years has not had the successes at the pace of the decline in orchid species and their habitats. Australia in particular has not delisted a single orchid species as a result of conservation efforts (in situ and ex situ) yet orchids now comprise the single largest family of endangered plants on the national list. Do we need a refresh on our approaches and what are the solutions as the clock keeps ticking.

1 - Conservation: Global Perspectives/Methods and Approaches that Make a Difference

ORAL PRESENTATION

BOTANICAL VOYAGING IN THE PACIFIC: FROM ECOLOGICAL IMPERIALISM TO PLANT SPECIES CONSERVATION

Michele D. Dominy *

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Abstract

Global conferences have emphasized the important role of botanical voyaging in the history of science for fostering a productive relationship between indigenous knowledges and plant technologies. In an advanced seminar on the anthropology of plants, students traced the arc from imperial natural history exploration to contemporary projects of species conservation and restoration. We focused on the 18th and 19th century voyages of Joseph Banks and Daniel Solander on HMS Endeavour, Charles Darwin on HMS Beagle, and Joseph Dalton Hooker on HMS Erebus. Our seminar first consulted archival sources – maps, naval logs, field journals, scientific correspondence, museum objects, and botanical illustrations. This “botanical imperium” provides insight into the relationship of ecological imperialism to botanic gardens, herbariums, seed banks, and trade nurseries. Students then met with archivists, herbarium specialists, plant biologists, and botanical artists to produce virtual exhibitions of a single plant species and to narrate their chosen plant’s movement from periphery to metropole and back. They each created a digital map, georeferencing plant provenance and tracking botanical circulation that was supplemented by primary source materials, herbarium specimens and botanical collections. Curated virtual projects represented living plants and their artistic and literary representations as well as integrating indigenous knowledge, and scientific conservation and preservation practices. Each student wrote a supplementary analytic paper that addressed the relationship between colonial and postcolonial ecologies for the species plant, focusing on its significance for biodiversity conservation. I profile two student projects that each tell the story of a single plant species – collected by Banks in Aotearoa New Zealand and Australia -- from the moment of collection to the present. Presenting plants as ethnographic subjects counters plant blindness, is valuable for public outreach in botanic gardens, and reveals the importance of the entwined relationship of plants and human ecosystems for ensuring our environmental futures.

1 - Conservation: Global Perspectives/Methods and Approaches that Make a Difference

ORAL PRESENTATION

ORCHIDS AND OCBILS: NEW CONSERVATION THEORY FOR OLD LANDSCAPES

Stephen D. Hopper AC*

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Abstract

OCBILs are old climatically buffered infertile landscapes found mostly in the Southern Hemisphere in more than half the world's Global Biodiversity Hotspots. They are especially common in the Southwest Australian Floristic Region (SWAFR), the Greater Cape of South Africa, and parts of Brazil. This review summarises what is known about orchids and OCBILs, especially those in the SWAFR. The orchids of granite outcrops, lateritic uplands, elevated peatlands and kwongkan (heathland) sandplains favour OCBILs. Comparisons with species in these genera that occupy YODFELs (young, often-disturbed fertile landcapes on lowlands) provide insights on new understandings for conservation managers. There are many threatened OCBIL species in genera such as *Caladenia* and *Drakaea* characterised by specialised pollination ecology and mycorrhizal relationships. These are examined in the context of hypotheses developed in OCBIL Theory regarding biological, cultural and conservation management attributes. The trends revealed are briefly examined for other OCBIL regions and their orchids.

1 - Conservation: Global Perspectives/Methods and Approaches that Make a Difference

ORAL PRESENTATION

SPATIAL PRIORITIZATION USING ORCHIDS AS TARGET SPECIES

Michele Lussu*, Michele Di Musciano, Leonardo Ancillotto, Luciano Bosso, Rocco Labadessa, Riccardo Testolin, Francesco Santi, Matteo Conti, Michela Marignani, Ilaria Bonini, Stefano Martellos, Antonio Pica, Paolo Laghi, Piero Medagli, Daniela Scaccabarozzi, Elisa Thouverai, Sara Magrini, Salvatore Cozzolino, Lorenzo Peruzzi, Alessandro Chiarucci

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Abstract

Prioritization of sites in which to expand protected areas (PAs) to limit biodiversity loss is a crucial challenge in national and international politics. Italy, thanks to its environmental heterogeneity and biological richness, can serve as a model for broader generalizations in biological conservation. This study aims to develop a comprehensive framework for prioritizing biodiversity conservation sites using orchids as target species. Utilizing a combination of biological, ecological and spatial indicators, we assess the conservation importance of 2x2km cells in sustaining diverse ecosystems. The prioritization model considers factors such as species richness, phylogenetic diversity, functional diversity and endemic taxa occurrences and rarity, By integrating georeferenced data and eco-evolutionary information, this approach ensures a holistic and sustainable approach to protect and enhance the natural heritage. The outcomes of this research will guide conservation efforts, enabling targeted strategies to safeguard Italy's unique biodiversity.

2 – Threats to endangered species

ORAL PRESENTATION

ORCHID WINNERS AND LOSERS AFTER FIRE IN WEST AUSTRALIAN URBAN BUSHLAND - A RESPONSE CONTINUUM REGULATED BY ORCHID BIOLOGY

Mark C. Brundrett*

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Abstract

The Southwest Australia Floristic Region is a global hotspot for plant taxonomic and functional trait diversity. This region includes many orchids with highly specialised associations with pollinators and mycorrhizal fungi. They also face threats from habitat loss, grazing, weeds and increasing fire frequency and severity due to climate change.

Aims: To identify short and long-term outcomes of fire on 17 orchids with diverse ecological strategies in an isolated urban banksia and eucalypt woodland.

Methods: Fire history maps revealed time since fire and fire frequency for 750 orchid locations. The mortality, plant size, flowering and pollination of orchids in post-fire areas was compared to long unburnt areas with 6-12 years of existing data. Key traits of orchid species such as tuber size and depth, seed production, clonality, phenology and population dynamics were also measured.

Key results: Fire history was very complex with 58 large overlapping major fires post 1972, averaging 8.7% of the 63 ha area annually. Correlating fire maps with orchid group sizes revealed four species usually lost to fire, seven preferring long unburnt areas and six fire tolerant species. Even the latter could be killed by unseasonal autumn fires. Sustained monitoring separated fire impacts from other factors and revealed a response continuum integral to the ecology of SWAFR orchids, and correlated with key traits like habitat preferences, tuber depth, seed production and clonal reproduction. Pollination varied from moderate decreases to substantial increases post-fire. One orchid produced most if its seed after fire and two had obligate fire promoted flowering, but the latter were highly clonal and rarely recruited from seed. Overall, impacts of fire greatly out way benefits to orchids, especially for seven species limited to, or preferring long-unburnt areas.

Conclusions: Fire history maps overlain with orchid locations and extended monitoring data revealed a spectrum of outcomes from catastrophic to highly beneficial, summarised as fire response indices. Fire responses are integral to the biology and ecology of SWAFR orchids and are correlated with key traits like seed production, tuber depth, and clonal or seed reproduction.

Implications: Fire history maps, comprehensive demographic data and fire response indices provide effective tools for sustainable management of orchids and their habitats. Some orchids are intolerant of major summer fires or unseasonal fires in autumn or winter, so their habitats must be carefully managed. Studies in urban areas provide essential knowledge for conserving less accessible rare species.

2 – Threats to endangered species

ORAL PRESENTATION

CALADENIA VS CLIMATE CHANGE – PREDICTION AND EMPIRICAL EVIDENCE FROM WESTERN AUSTRALIA

Jacopo Calevo*, Michael F. Fay, Kingsley Dixon

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Abstract

Understanding how species respond to ongoing climate change is crucial for their conservation. While extant species have persisted through historical climate cycles and temperature fluctuations, it is not clear how their ranges will be shaped by ongoing rapid rises in mean ambient temperature. We modelled the distributions of 26 *Caladenia* species native to the Southwest Australian Biodiversity Hotspot. We compared current range extents with past and future climate scenarios using a suite of consensus niche models to assess conservation risks. We also investigated ten species known to hybridize and how ecogeographical isolation will change as a premating barrier in future climates. Under historical warmer climate scenarios (last interglacial period and Holocene climatic optimum) most species had smaller ranges compared with baseline conditions (1970-2000). However, under both moderate and extreme future climate scenarios, only three species experienced range contractions, with the remaining species showing either no range change, or an increase in their range compared with current conditions. In species that hybridize, ecogeographical isolation will potentially increase, thereby acting as a stronger reproductive barrier than under present conditions. As *Caladenia* species naturally occur in seasonally dry conditions and have evolved in climatically buffered environments already subject to prior epochs of climate change, these results suggest that *Caladenia* are pre-adapted to survive in elevated temperatures under future climate scenarios. In vitro experiments aimed at testing the temperature tolerance of both seeds and fungi of *Caladenia huegelii* are in line with the suggested models and will be presented together with demographic studies of its main population over the past decades, and in situ seasonal fungal metabarcoding correlated to soil temperature and humidity.

2 – Threats to endangered species

ORAL PRESENTATION

COLLABORATIVE MAPPING OF COMPLEX ORCHID TRADE SUPPLY CHAIN FACTORS

Reshu Bashyal*, Amy Hinsley

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Abstract

International wildlife trade is a much-discussed conservation agenda. While legal and sustainable trade supports livelihood and provides food, medicines, and income, unsustainable wildlife trade threatens biodiversity. Wildlife trade management is crucial; it requires regular monitoring by enforcement. However, monitoring is complicated due to the involvement of multiple actors, trade routes, and strategies, which may frequently change to evade detection. Here we highlight this complexity and present a collaborative case of working with multiple stakeholders to understand medicinal orchid trade chains in Nepal; the orchid trade is illegal in Nepal that hosts >500 orchid species. We apply a participatory approach to trade chain mapping, involving 197 stakeholders involved in the medicinal orchid trade in Nepal. We triangulated stakeholder information with illegal trade seizure data (2010 to 2020) and legal trade data from the Convention on the International Trade of Endangered Species of Wild Fauna and Flora (CITES) database (2007 to 2016). We found that different orchid species can have different routes depending upon the parts in trade and key actors involved and that the secondary actors are as important as the primary actors. Our approach to mapping has greater significance as it includes collective views from a range of stakeholders involved in the trade or monitoring and planning (enforcement officials, herbal traders, trading associations, harvesters, intermediaries, and experts). Further, it will aid in planning and implementing national and international solutions at different points along trade chains to deter, detect, and prosecute illegal orchid trade. This method is likely relevant and scalable to other countries and taxa facing similar conservation challenges that are underrepresented in conservation literature, policy, and practice.

2 – Threats to endangered species

ORAL PRESENTATION

EXPLORING CHANGES IN ORCHID POLLINATION RATES AND THE IMPACT OF ANTHROPOGENIC FACTORS

Bofeng Song *

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Abstract

The ecological dynamics of pollination are critical to the survival and reproduction of flowering plants, yet these interactions are increasingly threatened by anthropogenic changes. Orchids, which represent one of the largest and most diverse families of flowering plants, are particularly vulnerable due to their specialized pollination strategies and often limited population sizes. Despite their ecological significance and extensive distribution, orchids face numerous threats including habitat loss, climate change, and other human activities that disrupt pollinator communities and interactions.

This study aims to investigate long-term trends in orchid pollination rates and the sensitivity of these rates to disturbance. Specifically, I am recording pollinia removal rates on in herbaria specimens of species in of the genus *Caladenia*, which is endemic to Australasia and known for its diverse pollination syndromes, including food and sexual deception. The study was conducted on ~10,500 specimens housed in the Australian National Herbarium, to assess and determine the pollination status of each orchid, I observed the specimens under a microscope and recorded the following five variables: Pollinia, Fertilised, Stigma pollen, Insect parts, and Accessible flowers. These variables allow me to determine the necessary information on pollinia removal rate, insect visitation rate, and pollination success rate.

The primary objectives are: (1) to quantify changes in orchid pollination rates over time, (2) to assess the impact of human activities on these rates, and (3) to evaluate the sensitivity of different *Caladenia* species, with varying pollination syndromes, to these changes. Preliminary results from this study include a linear regression analysis of the pollinia removal rates in *Caladenia* species. Additionally, I have compared the pollinia removal rates across different pollination syndromes. While these results are still preliminary and not entirely conclusive, they offer a promising direction for understanding the impact of different pollination strategies. I expect to complete all analyses in the coming months. This study will contribute valuable insights into the ecological dynamics of pollination and inform conservation strategies for maintaining orchid diversity and pollinator services in the face of ongoing environmental pressures.

4 – Threats to endangered species

ORAL PRESENTATION

THE ORCHID TRADE IN NEPAL AND NORTH EAST INDIA

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Abstract

The Himalayas host a wealth of orchid species with countless colours, shapes, sizes and ecologies. However, given the popularity of orchids not just as pretty flowers but also as medicine, the trade in many endangered species has grown exponentially, pushing orchid populations into decline.

My understanding of the trade in orchids is based on my experiences in Nepal and North East India, largely revolving around the illegal collection, processing and sale of various species, either for horticulture or medicinal purposes. Although both countries place all native species under a protected category, the rampant, unregulated removal and trade of wild plants have decimated a lot of habitats.

This presentation will be a report of the topic at hand, what is happening, what is being done to help orchids and what the future holds for these fascinating plants in peril. It is of utmost importance that we dismantle and study all the nuances and components of the trade to make proper judgments before it's too late.

2 – Threats to endangered species

ORAL PRESENTATION

MITIGATING ORCHID EXTINCTIONS IN A DIVERSE LANDSCAPE: HOW THE NSW SAVING OUR SPECIES PROGRAM IS HELPING ORCHIDS TO THRIVE IN THE SOUTHWEST

Anna Murphy*

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Abstract

The Department of Climate Change, Energy, the Environment and Water, NSW is working to secure the future of over 1,000 threatened plants, animals and ecological communities through the Saving our Species program. The diverse habitats in the south west region of NSW support a wide range of terrestrial orchids. Many of these species are highly threatened and some teeter on the brink of extinction. The Saving our Species program is securing them by taking a strategic approach to recovery. This involves using research and baseline data to implement evidence-informed on-ground actions to mitigate a wide range of threats impacting orchid populations.

3 - Ex Situ Conservation: Are we Making a Difference?

PLENARY LECTURE

THE IMPORTANCE OF EX SITU CONSERVATION IN PREVENTING ORCHID EXTINCTION

Emily Coffey*

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Abstract

Orchids, comprising one of the largest and most diverse families of flowering plants, are increasingly at risk of extinction due to habitat destruction, climate change, and illegal trade. Ex situ conservation efforts, particularly within botanical gardens, are critical to safeguarding these vulnerable species. Botanical gardens serve as vital centers for orchid conservation by leveraging their extensive horticultural and botanical expertise, advanced facilities, and extensive living collections. These institutions not only preserve genetic diversity but also play a key role in research, propagation, and restoration efforts. Moreover, botanical gardens are uniquely positioned to engage and educate the public on the importance of plant conservation, reaching more than 180 million people globally. By integrating ex situ conservation with research, public outreach and education, botanical gardens are indispensable in the global effort to prevent the extinction of orchids and ensure their survival for future generations..

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

ADVANCING ORCHID CONSERVATION: THE ROLE OF POLLEN BANKS IN PRESERVING GENETIC DIVERSITY AND SUPPORTING COLLECTIONS EX SITU

Jeremy A. Foster*

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Abstract

Pollen banking has become increasingly important in ex-situ plant conservation, especially for rare or threatened species. While the creation of pollen banks is widely used in crop breeding, pollen banking in plant conservation is an under-utilized tool. For example, stored pollen could be used when asynchronous blooming limits pollination, to restore genetic diversity, or overcome seasonal, geographical, or physiological barriers to plant reproduction. Pollen banking has considerable potential in the conservation of orchids, where pollination efficiency may be low, or flowers fail to set fruit. Here, banked orchid pollinia have the potential to make a valuable contribution to assisted reproduction. However, information regarding pollinia preservation, storage, and ability to remain viable over time is limited. Botanical gardens play a unique role in orchid conservation by maintaining and propagating species that are facing extinction in the wild. There is now the need to complement these efforts by developing effective protocols for species-specific pollen collections and long-term storage. At the Chicago Botanic Garden, our goal is to build on global pollen banking efforts to preserve orchid germplasm. Determining the optimal pollen storage conditions for orchids will allow us to improve the genetic diversity in living collections while also improving the restoration success in rare and endangered species.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

SYMBIOTIC GERMINATION OF THE CRITICALLY ENDANGERED COPPER BEARD-ORCHID (*CALOCHILUS CUPREUS*).

Jenny R. Guerin*, Dan J. Duval, Tiffany M. Harding, Noushka Reiter.

*Botanic Gardens and State Herbarium; jenny.guerin@sa.gov.au

Abstract

The Copper beard-orchid (*Calochilus cupreus*) is a critically endangered orchid endemic to the Southern Fleurieu region of South Australia. A single known extant population occurs in open coastal woodland (predominantly *Eucalyptus fasciculosa*) within the Aldinga Conservation Park on the Adelaide coastline. The number of individuals, monitored over several years, varies between 20 to 40 flowering plants depending on rainfall, climate, and management activities. Current threats to this population include weed incursion, trampling, and illegal collection. Maintaining the original population and establishing insurance populations at additional sites are the current conservation strategies to mitigate the risk of extinction. Symbiotic germination is a crucial step for establishing new populations of the Copper Beard-orchid; however, propagation of any species of *Calochilus* has not previously been reported. Seed collections maintained in the seed bank were used for propagation research at the South Australian Seed Conservation Centre. Fungal isolates obtained from lateral roots of wild plants were observed to be very slow-growing and have a distinctive morphology on growth media compared to other orchid genera. DNA sequencing showed association with the *Tulasnella* genus. A successful protocol for symbiotic in-vitro propagation was developed using novel growth media. This method has improved germination rates from negligible to >20% of seeds forming protocorms. Healthy plants were de-flasked and propagated in the shade-house last season, and this year over a hundred seedlings will be transferred into the nursery. Early results show that this growth media can be successfully used for other orchid genera. This propagation research offers new avenues for conserving the Copper Beard-orchid, thereby improving its prospects of long-term survival and potentially other threatened *Calochilus* species.

3 - Ex Situ Conservation: Are we Making a Difference

ORAL PRESENTATION

WARM STRATIFICATION AND OPTIMISED TEMPERATURES IMPROVE CONSERVATION OF THE ENDANGERED ORCHID, *CALADENIA ROBINSONII* (ORCHIDACEAE)

Brendan J. Janissen*, Ann C. Lawrie, Tien Huynh

*RMIT University; bjjanissen@gmail.com

Abstract

Maximising seed germination and seedling development is critical for conservation of endangered plants around the world. Orchidaceae is one of the most threatened plant families and can be one of the most difficult to propagate ex situ. Three critical, but potentially limiting, factors are important for orchid germination, namely, conducive conditions, 'ready-to-germinate' seed and effective mycorrhizal fungi. Our aim was to improve poor germination in vitro of a recalcitrant Australian endangered orchid, *Caladenia robinsonii*, and to predict the potential impacts of climate change on this species. Three experiments were conducted to optimise germination in *C. robinsonii*, with a focus on temperature. In Experiment 1, on the basis of meteorological data, three constant temperatures (15°C, 20°C and 27°C) were tested. In Experiment 2, the optimal constant temperature was compared with diurnally varying temperatures of 22°C/18°C (12/12 h), with and without warm stratification at 30°C/27°C (12/12 h) for 1 week. In Experiment 3, the same diurnally varying temperature and warm stratification were tested using multiple orchid mycorrhizal fungal isolates cultured from wild and re-introduced populations of *C. robinsonii*. Without warm stratification, germination was greatest at 20°C (21%), but only 4% of seedlings developed to the green-leaf stage, whereas fungal growth was greatest at 27°C. Stratification increased germination (79%) and development to the green-leaf stage (47%), but more so with subsequent incubation under constant 20°C than diurnal 22°C/18°C. Fungal isolate affected total germination (53–69%) and development to the green-leaf stage (26–41%); isolates from the wild population were less effective than were those from re-introductions. Warm stratification and specific seasonal temperatures significantly improved germination, both factors being typical of seeds with physiological dormancy. Mimicking in situ conditions can provide a strong basis for ex situ germination strategies and predicting future outcomes.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

OPTIMIZING ORCHID SEED VIABILITY STAINS FOR APPLIED CONSERVATION

Jason Ligon*, Emily E. D. Coffey

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Abstract

Effective protocols are necessary to evaluate how well an orchid seed accession retains viability under conventional seed bank storage conditions, but protocol optimization requires substantial investment. Since 2019, the Conservation Seed Bank of the Southeastern Center for Plant Conservation has committed to evaluating the efficacy of existing protocols to assess seed viability. Located in the United States, the Atlanta Botanical Garden's Conservation and Research Department vigorously pursues its mission: to lead innovative strategies and partnerships to conserve imperiled plants and natural communities. A significant portion of this work includes seedbanking imperiled orchid species and conserving their natural communities across the southeastern United States and the Caribbean. This talk will highlight how three decision matrices and available viability assessments inform the workflow of incoming seeds for ex situ safeguarding to support a species' survival in the wild. Outcomes include recommendations for conservation practitioners.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

FROM SINGLE FUNGI TO SYNTHETIC FUNGAL COMMUNITIESTHE IMPACTS OF EX SITU SEEDLING BAITING SEEDLING-ASSOCIATED FUNGI ON THE GROWTH AND CONSERVATION OF *DENDROBIUM OFFICINALE*

Yihua Wu*, Jiangyun Gao

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Abstract

Successful seedling recruitment is crucial for orchid propagation, and using orchid mycorrhizal fungi (OMFs) to facilitate orchid proliferation is considered an effective method of orchid conservation. However, the absence of the seedling-associated fungi is a major bottleneck in orchid conservation efforts. For endangered orchids like *Dendrobium officinale*, obtaining seedlings from natural habitats is impractical, necessitating the development of in/ex situ seedling baiting methods for mycorrhization. This study sought to capture *D. officinale*'s seedling-associated fungi through ex situ baiting using natural habitat substrates. Within 75 days, fungi successfully colonized the roots of sterile seedlings, forming numerous pelotons. A total of 251 pure fungal isolates associated with the seedlings were cultured, identifying 16 OMFs and 11 non-OMFs species. Fungal community compositions differed among habitats, yet each featured at least two dominant fungi (isolation frequency >4 in a single habitat). *Tulasnella* strains—TP-2, TP-8, TP-9, TP-11, and TP-13—identifyied as keystone OMFs, consistently establishing stable symbioses across habitat and significantly enhancing seedling growth. Specifically, Strains TP-11, TP-13, TP-8, TP-3, and TP-2 were identified as dominant growth-promoting fungi, significantly enhancing the development of leaves, roots, tillers, plant height, and stem diameter, respectively. TP-11 and TP-13 also significantly promoted seed germination, leading to advanced seedling development (with 3-4 leaves and about 3 cm roots). These keystone OMFs could mitigate growth challenges in *D. officinale* seedlings, offering substantial promise for orchid conservation and cultivation. Moreover, offset effects in synthetic fungal communities, established by growth-promoting keystone OMFs, proved more significant than synergistic effects, indicating that the influence of these communities on plant growth is not merely a cumulative function of individual fungal contributions. In these communities, the functionality of specific fungi may undergo adjustments due to interspecific interactions. This underscores the criticality of considering fungal interactions when formulating and implementing orchid conservation strategies.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

MULTIPLE ORCHID MYCORRHIZAL FUNGI CAN PROMOTE THE SEED GERMINATION IN *PLEIONE BULBOCODIOIDES* WITH DIFFERENT EFFICIENESS

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Abstract

Generally, orchids highly depend on specific fungi for seed germination and subsequent seedling development. For medicinal orchids, obtaining compatible fungi is prerequisite for imitation of wild cultivation and conservation. In this study, an important traditional Chinese medicinal orchid, *Pleione bulbocodioides*, was studied to screen out effective fungi for seed germination and seedling development. We selected two strains (GYBQ01 and Agp-1) of *Tulasnella* and two strains (LQ and PI) of Sebacinales from our laboratory fungal bank for symbiotic germination. *P. bulbocodioides* can germinate and form protocorms in all fungal and control treatments, but seedlings only developed in fungal LQ and PI treatments and MS treatment. In addition, we used ex situ seed baiting to isolate fungi, and a total of ten strains of fungi were obtained. The results of molecular identification showed that one strain (ZK) of Meruliaceae, one strain (PL01) of Tricholomataceae, six strains (PL03, PL04, PL06, LS01, PM01, and PM02) of Sebacinales and two strains (PL02 and LS02) of *Tulasnella*. From the isolated fungi, we found that all of the Sebacinales and *Tulasnella* species can promote the development of the seeds to the seedling stage. In particular, at 90 days after incubation, the percentages of seedings development of LS01 treatment (66.81±6.57%) was the highest, which was significantly higher than that of all other treatments (all $p < 0.0001$). For the strain LS01, we conducted a field seeds sowing directly, at 30 days after incubation, the percentage of seed germination was close to 50%, and the percentage of protocorm formation reached 30%. We suggested that the fungi LS01 can be used in the conservation practices or imitation of wild cultivation of *P. bulbocodioides* so as to promote the industrialization of this important medicinal orchid.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

EX SITU CONSERVATION OF NEW ZEALAND POTATO ORCHIDS (*GASTRODIA*)

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Abstract

The genus *Gastrodia* includes leafless terrestrial mycoheterotrophic plants which derive nutrition solely from mycorrhizal fungi. Most known species (~80) are found in Asia and the Pacific islands, with a further ten species in the African region. Throughout its distribution the underground tubers, which give rise to the common name Potato orchid, are valued as food and medicine. Four species (*G. cooperae*, *G. cunninghamii*, *G. minor*, *G. molloyi*) are endemic to New Zealand (NZ) while *G. sesamoides* is found in NZ and Australia (naturally) and South Africa (non-native, naturalized). Despite the relatively high number of *Gastrodia* species globally, research into seed germination and propagation is limited. The Lions Ōtari Plant Conservation Laboratory received a three year grant from Lottery Environment and Heritage to investigate conservation of five threatened NZ orchids, one of which is the critically endangered *G. cooperae*. We recorded flowering, pollination strategies, capsule production and investigated whether fungi isolated from *G. sesamoides*, which grows sympatrically with *G. cooperae* in our study area, can support in vitro seed germination and seedling development in *G. cooperae*. We optimized long-term storage of seed and fungi, and established ex situ back-up collections of three *Gastrodia* species. Our study is the first report on symbiotic seed germination of NZ *Gastrodia* species and will advance orchid conservation in NZ and globally.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

THE ORCHID GARDEN CONSERVATION NETWORK (OGCN) A WORK IN PROGRESS

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Abstract

Orchid Conservation initiatives are essential to the preservation of species in a changing world. The Anthropocene (the epoch in which mankind plays a disruptive role in all aspects of our Earth), and its many modifications and threats to natural systems, requires conservationists to take proactive steps to protect species whose habitats are vanishing or degrading beyond the in-situ and ex-situ approaches currently employed. A hybrid approach to orchid conservation needs to be more widely adopted. The Circa-situm strategy, defined as the preservation of species within their natural ranges, is an approach already adopted successfully in many gardens and reserves around the world. Such conservation initiatives provide opportunities for studies in ecological observation and experimentation, scientific and public engagement, and an economic engine for local communities. However, these efforts are often independent, and employed in isolation. We believe that networking circa-situm conservation gardens and reserves has the potential to greatly enhance their outreach and success. To this end, we have created the OGCN (Orchid Garden Conservation Network). An online platform for communication and sharing of resources, currently in development, promises to advance orchid conservation worldwide. Examples of individual Circa Situm efforts such as the one at the El Tular Reserve in Guatemala will be featured: Santuario Natural El Tular is a private nature reserve made up of humid, montane pine-oak forest in central Guatemala. Within the 36 hectares of the reserve, 98 orchid species have been found. This is especially impressive when one considers that there are between 900 and 1,000 orchid species in Guatemala: 1 out of every 10 orchid species in the country exists in the reserve. An orchid garden was set up within the sanctuary to conserve and showcase hundreds of specimens rescued from around the reserve. The garden is a prime example of circa situm conservation, where organisms are preserved in an environment that emulates their natural habitat within the species' geographical range. A 2023 Conservation Grant from the American Orchid Society is currently supporting an in-depth survey of the number and relative abundance of orchid species within the reserve and will make possible in 2024 a free workshop for anyone interested in rescuing and caring for native orchid species in the greater Guatemala City area.

3 - Ex Situ Conservation: Are we Making a Difference?

ORAL PRESENTATION

DEVELOPING AN ORCHID CONSERVATION HORTICULTURE PROGRAM AT LONGWOOD GARDENS

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Abstract

Public gardens have an important role to play in addressing the current plant extinction crisis. Recognizing this, Longwood Gardens (Kennett Square, PA) elevated its conservation focus and brought it to the foreground in its mission. Understanding that horticulture is essential for conservation, the team at Longwood Gardens developed a conservation horticulture strategic plan that focused on plants of conservation concern aligned with institutional strengths, infrastructure and expertise. The primary focus of the program began with U.S. native orchids and how ex situ conservation practices can play a role in protecting them. This multi-faceted program incorporates field work, seed and fungal banking, symbiotic and asymbiotic in vitro laboratory seed propagation, living collections development, associated original research, and engaging and activating audiences. This presentation will highlight three case studies that exemplify these efforts and highlight how public gardens are a necessary component of multi-disciplinary conservation activities world-wide. These stories include:

1) Seed banking for the future: how the team implemented a comprehensive approach to seed collection for both banking and propagation research purposes, with an increased focus on banking U.S. native orchid seeds;

2) Propagating the impossible: how the use of fungi isolated from orchid protocorms can improve the germination percentage and seedling development compared with fungi isolated from mature plants of *Platanthera*;

3) A local to global focus: how work with native orchids in Pennsylvania resulted in expansion of the program to document and research terrestrial orchids in the highlands of southern Tanzania, where they are being harvested for food production in neighboring countries.

4 - In Situ Conservation

ORAL PRESENTATION

EVALUATING PHOTOSYNTHESIS OF *HABENARIA RHODOCHEILA* HANCE IN RESPONSE TO CLIMATE CHANGE

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Abstract

Understanding plant photosynthesis is critical for effectively cultivation and conservation, particularly for species such as *Habenaria rhodocheila* Hance, which is endangered by climate change. Studying its adaptability and endurance is critical for predicting its long-term sustainability and establishing future conservation efforts.

The aim of this study is to assess *H. rhodocheila*'s response to global warming demand by examining its ability to photosynthesize under conditions that imitate global warming, including increasing light intensity (100 and 500 PPFD), carbon dioxide concentration (420 and 700 ppm), and temperature (25 and 30 °C). This study used portable Li-COR equipment (model Li-COR 6800S) to assess the plant's photosynthetic capacity and efficiency.

This experiment evaluated plants' photosynthetic responses in the vegetative and reproductive stages. Both stages showed comparable rates at low light levels (less than 200 PPFD), with no significant variation in overall photosynthetic rates. Light compensation points were slightly lower in the reproductive stage (15.28 PPFD) than in the vegetative stage (17.68 PPFD), indicating that the reproductive stage may have a greater light saturation point. Both phases reached a point of stability about 600 PPFD, indicating that light saturation occurs in this range. The reproductive stage reached its maximum photosynthetic rate at around 1000 PPFD, while the vegetative stage continued to rise at a slower rate, suggesting it may reach its maximum at higher light levels.

When comparing carbon absorption rate, stomatal conductance, and transpiration rate, plants in the reproductive stage showed higher photosynthetic capacities, although the difference was not statistically significant. Increasing light intensity and carbon dioxide concentration resulted with enhanced carbon dioxide uptake and somewhat decreased stomatal opening and transpiration. Increasing leaf temperature enhanced carbon dioxide uptake substantially but did not affect stomatal opening or transpiration significantly.

The change in photosynthetic capacity between stages is most likely related to the increased energy requirements of reproduction. This work sheds light on the photosynthetic characteristics of *H. rhodocheila*, emphasizing the importance of using correct approaches that mimic the species' natural conditions for culture and conservation.

4 - In Situ Conservation

ORAL PRESENTATION

CONSERVATION EFFORTS OF THE ENDANGERED *CYPRIPEDIUM SUBTROPICUM*: PROGRESS AND CHALLENGES

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Abstract

Cypripedium subtropicum, a unique and endangered slipper orchid, is native to the subtropical regions from Tibet, southwestern China and northern Vietnam. *C. subtropicum* possesses perennial areal shoots (long-lived plicate leaves) and natural populations are sparse and scattered in dense, subtropical jungles. Since its rediscovery in 2009 in Malipo County, Yunnan, this species has faced significant threats from illegal collection. Our studies have revealed that hoverflies are drawn to the flowers of *C. subtropicum*, which mimic aphid colonies both visually and chemically. Our findings indicate that *C. subtropicum* utilizes visual mimicry by presenting an aphid-colonized labellum with a nutrient reward, as well as chemical mimicry by emitting aphid alarm pheromones. This combined strategy successfully attracts hoverflies for pollination. To aid in the conservation of *C. subtropicum*, we investigate its seed development and germination requirements. Structural studies of embryo development from fertilization to seed maturity reveal that the optimal seed germination occurs when seeds are collected at 105 days after pollination. The addition of cytokinins improves both asymbiotic germination and protocorm survival. Currently, we are investigating the mycorrhizal fungal diversity among different populations. Preliminary results suggest a range of rhizoctonia fungi, including Tulasnellaceae and Serendipitaceae associated with this species. A comprehensive understanding of the pollination biology, seed germination, and mycorrhizal association of *C. subtropicum* will contribute to the propagation and reintroduction works of this species, thereby supporting broader orchid conservation programs.

4 - In Situ Conservation

ORAL PRESENTATION

CONSERVING RARE ORCHIDS AND RESTORING LOST LANDSCAPES IN THE CUMBERLAND PLATEAU OF KENTUCKY, USA

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Abstract

The white fringeless orchid, *Platanthera integrilabia* (Correll) Luer, is a federally threatened terrestrial species with a limited distribution along the southern Appalachians of the United States. Its preferred habitat consists of upland headwater seeps and streamside bogs mostly concentrated in the Cumberland Plateau of Kentucky and Tennessee. Human activities and land mismanagement have led to its widespread decline, now reduced to 53 populations across five states. In Kentucky, populations have dwindled from logging, fire suppression, and browsing by herbivores, primarily deer. These wetlands feature precipitation-fed soils that contain a fragipan that facilitates ponding. Canopy cover varies but is typically closed (>90%), with some exceptions (e.g., open areas beneath powerlines). Annual monitoring has revealed that fewer orchids flower under a closed canopy compared to those that frequent open wetlands. Evidence of hydrologic alterations like headwater cuts and lowered water tables exacerbate wetland degradation, compounded by increased evapotranspiration from shrub thickets. Adjacent pine barren grassland remnants, historically maintained by periodic fire and animal disturbance, highlight the need for community connectivity and holistic landscape restoration. In Kentucky, recovery efforts involve monitoring, canopy and shrub reduction, debris dam installation, and prescribed burning. Over 14 years, these efforts have led to positive outcomes, namely increased orchid population size and viability, and enhanced floristic quality and biodiversity. In two restored wetlands, the number of flowering plants increased from < 5 to > 165/75, and fruit production increased by over 4500%. Although browsing pressure persisted, burning upland habitats reduced herbivory damage overall, probably by expanding foraging habitat in adjacent woodlands. This research supports existing management practices for this and possibly other rare orchids and their habitats. Research efforts are underway to further understand management effects on other co-existing plants, and the pollinators and mycorrhizal fungi that the species relies on for survival.

4 - In Situ Conservation

ORAL PRESENTATION

CONSERVATION OF GRASSLAND ORCHIDS FROM THE TASMANIAN MIDLANDS

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Abstract

The Tasmanian Orchid Conservation and Research Program (TOCRP), a partnership between the Royal Tasmanian Botanical Gardens and the Landscape Recovery Foundation, is working to conserve a suite of threatened orchids endemic to the Tasmanian Midlands. The habitat of these orchids is highly restricted with over 80% of the grasslands that they occur in lost due to clearance and many of the remaining patches continuing to decline in condition. An extension approach is being taken to conserve these orchids, building on existing realationships with land managers and the conservation organisations that support them. Orchid sites are under threat from high browsing pressure, inappropriate mowing, grazing and burning regimes and encroachment of weeds including invasive perennial grasses. Sites that are considered to have public access are also threatened by over visitation for nature photography. Conservation actions of the TOCRP include protection of orchid sites during flowering and seeding, extension surveys to locate new sub-populations, ex situ conservation for long-term storage in the Tasmanian Seed Conservation Centre and symbiotic propagation for translocation purposes. Translocations will be undertaken on private land with conservation stewardship agreements in place that will be integrated into broader grassland restoration efforts. The TOCRP also undertakes public education focused on ethical orchid photography, including provision of alternative photography opportunities and workshops on low impact photography techniques. The conservation approaches of the TOCRP and the programs progress toward the first threatened orchid translocation in Tasmania will be discussed.

4 - In Situ Conservation

ORAL PRESENTATION

THE GHOST ORCHID, *DENDROPHYLAX LINDENII*, OF NORTH AMERICA: ONE VOICE IN A QUIETING ECOLOGICAL CHORUS

Lawrence W. Zettler*, Adam R. Herdman, Ernesto B. Mujica, Mark W. Danaher, Elaine H. Gonzalez, Michael E. Kane, Carrie Reinhardt Adams

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Abstract

Orchids epitomize the ongoing global decline of biological diversity and illustrate the challenges associated with conservation and restoration of rare species. Current estimates suggest about half of the family's 29,000+ species are now vulnerable to extinction worldwide. In Florida and Cuba, the ghost orchid, *Dendrophylax lindenii* (Lindley) Bentham ex Rolfe, is one such species at an extinction precipice. Like most orchids, *D. lindenii* relies on insects (sphingid moths) and mycorrhizal fungi (*Ceratobasidium*) for successful pollination and seed germination, respectively. It is also very limited in distribution; in Florida, *D. lindenii* is largely restricted to the Fakahatchee Strand – a narrow (2 x 35 km) unique wetland in the southwestern part of the state. This small area, dubbed the 'Amazon of North America', likely supports the highest diversity of orchids and bromeliad species in North America, many of which are also threatened with extinction. Regarded as the most familiar and sought-after of all orchids in the Western Hemisphere, the ghost orchid serves as an ecological flagship species for its unique wetland habitats where many other, lesser known co-existing species also occur. Current ghost orchid numbers in both countries have shown a marked decline. In Cuba, only 220 individuals were recorded in 2018 from the largest known population (Guanahacabibes Peninsula). In Florida, the numbers appear to have dropped to just 912 individuals as of 2023, down from 2,242 estimated previously. Although most of the orchids in both countries are restricted to habitats protected by law, poaching and habitat alteration remain a persistent threat even today. This talk will present an overview of ongoing efforts (e.g., orchid and pollinator surveys, genetic analysis, seed propagation, reintroduction) involving a collaborative network of researchers and institutions (e.g., University of Florida, Chicago Botanic Garden, Soroa Orchid Garden) all working together to conserve this iconic species and its habitat.

5 - Ecological interactions to Improve Conservation Outcomes

PLENARY LECTURE

ROLE OF FUNGAL INTERACTIONS IN ORCHID CONSERVATION: LESSONS LEARNED AND FUTURE DIRECTIONS

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Abstract

The presence of suitable mycorrhizal fungi is essential for orchid seed germination and, consequently, orchid conservation. Most orchids retain these fungi into adulthood as they provide critical advantages in nutrient-poor environments, enhance stress resilience, and support long-term survival and reproductive success. Fungi from the polyphyletic rhizoctonia group are major orchid symbionts that can be cultivated under lab conditions. While the biogeography and ontogenetic changes in orchid-fungus interactions are relatively well explored at least for some orchid groups, there is limited knowledge about the nutritional demands, functional traits, cultivability, genetic diversity, and spatial distribution of rhizoctonian fungi within and between sites. In my talk, I will summarize findings from in vitro and in situ experiments with European orchid species and their rhizoctonian fungi conducted in my lab over the past ten years, highlighting the gaps in our knowledge that need to be addressed to successfully bridge ex situ and in situ conservation..

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

THE MULTIPLE NICHES OF ORCHID MYCORRHIZAL FUNGI (OF EUROPEAN ORCHIDS)

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Abstract

Survival of all orchids in nature is strictly dependent on the association with orchid mycorrhizal (OrM) fungi, which allow seed germination, sustain the mycoheterotrophic protocorm stage and continue to provide adult plants with organic carbon and other essential nutrients even in photoautotrophic species. European green orchids mainly associate with the so-called 'rhizoctonias', a polyphyletic group encompassing members of Tulasnellaceae, Ceratobasidiaceae, Serendipitaceae and Sebacinaceae. Most of these fungi are generally regarded as unspecialized soil saprotrophs, and some of them have been found to associate with the roots of non-orchid plants. It was therefore suggested that the main occurrence of OrM rhizoctonias is outside the orchid hosts. However, their actual distribution in the environment and their nutritional strategies remain elusive.

We have found that across its distribution range in northern Italy *Anacamptis morio* associate with a varied range of OrM fungi which is mostly uncoupled from the diversity in the same fungal groups found in either rhizosphere and bulk soil. Furthermore, a manipulation experiment we performed with *Spiranthes spiralis* indicated that although removal of the surrounding soil and/or neighbouring non-orchid plants affected fungal colonization of newly-formed orchid roots, most OrM fungi were consistently associated with the host roots. Results of a metabarcoding study aimed at detecting the occurrence of OrM fungi in organs other than roots in *Spiranthes spiralis, Serapias vomeracea* and *Neottia ovata* will also be presented.

Our findings outline a complex scenario, and expand the life history traits and potential ecological roles of OrM fungi.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

WHAT CONSTRAINS THE GERMINATION OF *BLETILLA FORMOSANA*?

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Abstract

Orchids disperse numerous dust-like seeds from a capsule, potentially enabling wide distribution if germination conditions are met. Because their embryos are rudimentary and lack endosperms as seeds matured, germination and the early growth stage depend on the nutrient supply from mycorrhizal associations. *Bletilla formosana*, a terrestrial orchid native to Taiwan, is valued both ornamentally and medicinally. It has been observed that the mature embryos of *B. formosana*, which are larger and possess a cotyledon-like structure, may exhibit a reduced dependence on fungal symbiosis for germination, possibly contributing to a wider distribution. Despite these characteristics, the species remains confined to some specific regions. This study investigates the factors limiting the distribution of *B. formosana* through in situ germination experiments. From October 2022 to September 2023, approximately 2000 seed packets were buried at six sites across Taiwan, with germination rates assessed after 3, 6, and 9 months. Following retrieval, germination rates were accessed, and the germinated protocorms were collected for analysis of fungal associations and stable isotope abundance. Preliminary findings show no significant germination differences between plots with or without conspecific orchids, and a variation in germination rates across different months. It is notable that in situ germination of *B. formosana* using seed packets revealed higher germination rates compared to those of other terrestrial orchids reported in previous studies. We also recorded substantial variability in germination success across sites, with some achieving up to 45% median seed germination, and nearly all packets containing germinated seeds. Sanger sequencing of in situ germinated protocorms from some seed packets indicated associations with fungi from the genera *Ceratobasidium*, *Sebacina*, and *Fusarium*. Further studies on signatures of the stable isotope abundance in combination with fungal communities using next-generation sequencing would provide deeper insights into the distribution and conservation of *B. formosana*.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

BACK FROM THE BRINK: ECOLOGICALLY INFORMED ORCHID CONSERVATION IN WESTERN AUSTRALIA

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Abstract

Global rates of biodiversity decline are accelerating, and many species are being tipped towards the brink of extinction. Western Australia is a biodiversity hotspot and home to over 400 species of orchids, the majority of which are endemic to the state. Western Australia's terrestrial orchids are not immune to these global declines and are over-represented in the state conservation listings, with 30% of species of conservation concern. The Orchid Conservation Program at Kings Park Science (DBCA) delivers research informed, conservation outcomes for Western Australia's iconic orchid flora. Arresting decline in orchid species requires a multifaceted approach of in situ and ex situ management and orchid program is uniquely placed within government to deliver on this. Using examples from the program we highlight how successful conservation outcomes are underpinned by an ecological understanding of orchid biology, pollinator, and mycorrhizal interactions. We show how ex situ collections are the foundation for securing long-term species conservation and how pro-active collections can place the program in a 'conservation action-ready' state.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

MULTI-ELEMENT STABLE ISOTOPE NATURAL ABUNDANCE IN ORCHIDS FROM THE MEDITERRANEAN REGION OF EUROPE AND SW-AUSTRALIA

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Abstract

Within the Orchidaceae the usually mutualistic mycorrhizal symbiosis has been modified in several directions. In nature, all orchids start into their life by completely relying on their mycorrhizal fungi as nutrient source. This unique initially mycoheterotrophic nutrition provides a predisposition to remain relying on fungal partners as nutrient supplier throughout the entire life cycle. In fact, more than 200 orchid species are known to survive achlorophyllous in a fully mycoheterotrophic nutrition mode. These orchids thrive in light-limited forest-ground habitats and use fungi simultaneously forming ectomycorrhizas with trees or wood or litter decomposing fungi as mycorrhizal hosts. Initially and fully mycoheterotrophic orchids bear the isotopic composition of their host fungi and thus, are isotopically distinguished from accompanying autotrophic plants. Due to an isotopic positioning between autotrophic and mycoheterotrophic plants, a steadily increasing number of adult chlorophyllous orchids is identified as gaining carbon simultaneously from two sources, own photosynthesis and fungal hosts. This mixotrophic mode of nutrition is known as partial mycoheterotrophy. Fungal partners of partially mycoheterotrophic orchids can be either ectomycorrhizal fungi or saprotrophic fungi of the ubiquitary rhizoctonia group or both of them. Specifically, hydrogen stable isotope abundance turned out as an elegant tool to identify partial mycoheterotrophy among rhizoctonia-mycorrhizal orchids.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

PLATANTHERA POLLINATION GAPS AND INSIGHTS

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Abstract

The *Platanthera* Rich. (Orchidoideae) comprise a speciose genus of orchids primarily in the northern hemisphere, with up to 200 known species worldwide. Individual species are known to self-pollinate, but many rely on insect pollinators. Due to the likely effects of climate change and ongoing development on *Platanthera spp*. habitats, these orchids and their associated pollinators should be a focus of conservation attention and management. However, while there is a fairly substantial literature coverage of *Platanthera*–pollinator occurrence and interactions, there are still wide gaps in our understanding of the species involved in these systems. In a recent review, we assessed the pollination literature for 157 *Platanthera* species, showing that little information exists for many species. As part of ongoing efforts to increase our knowledge of *Platanthera* pollination, we include some of our recent work to identify pollinators facilitating hybridization among *P. dilatata* and *P. aquilonis*.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

UNCOVERING SYMBIOTIC PARTNERS: A TOP PRIORITY TO SAVE *CORYBAS CARSEI*, ONE OF NEW ZEALAND'S MOST THREATENED ORCHIDS

Carlos Lehnebach*, Jennifer Alderton-Moss, Tingyu Qin, Jennifer Tate, Vaughan Symonds, Alastair Robertson, Andrew Munkacsi, Karin van der Walt

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Abstract

The swamp helmet orchid *Corybas carsei* is one of New Zealand's most threatened orchids. Currently it is known from a single population in a wetland in the central North Island, where about 400 plants occur. This population has been the subject of yearly demographic surveys and controlled winter burns, after experimental burns suggested a positive effect on the orchid's vegetative growth and flowering. Two decades later the population has remained stable, however, the risk of extinction remains. As ecological interactions with mycorrhizal fungi and pollinator(s) have remained unexplored, efforts to create back-up populations either ex situ or in situ are out of reach. To fill this critical gap our team have been studying aspects of pollination biology, mycorrhizal associations, and genetic diversity. We are also investigating the effect of fire on the fungal community and mycorrhizal interactions of other sympatric orchid species. Our preliminary data suggest genetic variability within the population is extremely low. Pollination experiments have confirmed this orchid is nectarless and self-compatible, but allogamous, so pollinator visitation is required for fruit set. Video recordings and direct observations have failed to identify the orchid's pollinator, but the use of Malaise traps have confirmed the presence of fungus gnats (Mycethophilidae: Diptera) at the site. This is relevant because members of this family are known to pollinate other *Corybas* species in New Zealand. Unlike our findings in the sympatric greenhood orchid *Pterostylis paludosa*, isolation and identification of *C. carsei*'s mycorrhizal partner(s) has been challenging. More than 18 fungal associates have been isolated and identified from *C. carsei* but none of them are known orchid mycorrhizal fungi nor promoted germination under lab-conditions. Observations from ongoing asymbiotic germination experiments, on the other hand, are promising but progress is extremely slow; 18 months from seed planting to leaf primordium formation!

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

DIVERSE FUNGAL ASSOCIATIONS IN FULLY MYCOHETEROTROPHIC ORCHIDS FROM TAIWAN

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Abstract

Fully mycoheterotrophic plants parasitize fungi to obtain all their nutrition, as they lack chlorophyll and cannot photosynthesize. In the dim forest understory, these plants remain underground, connected to fungal mycelia, and only emerge above the soil and leaf litter for brief periods during flowering and fruiting. Here we investigated the physiological ecology of six fully mycoheterotrophic orchids in Taiwan: *Stereosandra javanica*, two *Yoania* species (*Y. japonica* and *Y. amagiensis* var. *squamipes*) and three *Didymoplexis* species (*D. micradenia*, *D. pallens* and *D. siamensis*). We used high-throughput DNA sequencing to analyze fungal communities associating with mycoheterotrophic orchids. Besides, natural stable isotope abundances were analyzed to confirm the mycoheterotrophy and reveal the pathways for nutrient acquisition. Diverse fungal associations were identified: *S. javanica* associated predominantly with ectomycorrhizal fungi belonging to Clavulinaceae (*Clavulina*); *Y. japonica* and *Y. amagiensis* var. *squamipes* associated mostly with *Phlebia* (Meruliaceae); *D. micradenia* and *D. pallens* associated with litter-decomposing fungi from the Marasmiaceae and Mycenaceae families. Few sequences of ectomycorrhizal fungi from the Hygrophoraceae and Russulaceae families were also detected. *D. siamensis* primarily associates with ectomycorrhizal fungi from the Sebacinaceae (Group A) and Clavulinaceae families. Isotope abundance patterns of carbon and nitrogen in *S. javanica* were found to be in the typical range of fully mycoheterotrophic orchids associated with ectomycorrhizal fungi, while the isotope signatures of the three Didymoplexis species were considerably more variable. The isotope abundance signature of the two *Yoania* species represents a unique new pattern probably related to their association with *Phlebia*, a typical white-rot fungus. In Taiwan, the warm, humid climate provides an optimal environment for the establishment and thriving of symbiotic relationships between mycoheterotrophic orchids and saprotrophic fungi. Diverse fungal associations in some of these fully mycoheterotrophic orchids enable obviously the simultaneous utilization of various nutrients from different functional types of fungi.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

THE NORTH AMERICAN ORCHID CONSERVATION CENTER: TAPPING INTO THE POWER OF COLLABORATION AND AN ECOLOGICAL APPROACH TO CONNECT THE "PUZZLE PIECES" OF ORCHID ECOLOGY AND ADVANCE ORCHID CONSERVATION IN THE U.S. & CANADA

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Abstract

Orchid ecology is analogous to a puzzle, with a myriad of pieces to link together to unlock the secrets that will ensure successful and sustainable species conservation. Since 2013, the Smithsonian's North American Orchid Conservation Center (NAOCC) and our growing network of over 60 U.S. and Canadian collaborators continue to tap into the power of collaboration to make strides in ecologically-based orchid research and conservation. We are embracing the value of partnerships, new technologies, and innovative practices to pull the pieces of the orchid ecology puzzle into place. In this presentation, we offer a brief overview of NAOCC's work, and a few snapshots of what our network has accomplished so far. We show how we are incorporating interactions with other species, including studies of how pollinator and mycorrhizal relationships differ among species and across species ranges, with genetic analyses and more to assemble the orchid ecology puzzle. We then describe how our collaborative research is guiding our studies of symbiotic germination and propagation methods as we launch a new collaborative project to test and develop best practice protocols for the sustainable establishment of native orchid ex-situ collections and in-situ restoration work. Finally, we touch on the importance of educational outreach and citizen science to support orchid conservation efforts, giving examples of some educational initiatives by NAOCC and our collaborators.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

POLLINATION SYSTEMS, FLORAL SCENT SPECIFICITY AND FLORAL TRAIT DIVERSITY IN NATURAL TROPICAL EPIPHYTIC COMMUNITIES IN CAMEROON

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Abstract

Angraecoid orchids (c.a 780 species in Africa and Madagascar) are major constituents of tropical epiphytic communities in Central African rainforests. They present diverse floral traits (color, morphology, scents, and rewards) that attract pollinators and their survival depends on their pollination which is poorly understood. Among the floral traits involved in pollinator attraction, floral scents, and rewards are neglected. The greater scent diversity of species in this group, their influence on pollinator specialization, and their pollinators remain unknown. Within this group, only sphingophily - a syndrome attributed mainly to long-spurred whitish orchids is the best studied syndrome whereas several species in diverse genera present non-sphingophilous characteristics. This study assesses floral scent specificity, pollination mechanisms, and phylogenetic relationships of Angraecoids across different environments in Cameroon. Specific objectives are to investigate: i) the use of ex-situ collections (shade houses) in floral scent chemistry; ii) pollination systems within two non-sphingophile Angraecoids; iii) floral scent compositions of about 40 Central African species in 21 (of 49 Angraecoid) genera; and iv) phylogenetic mapping of floral VOCs to evaluate their contribution to pollinator specialization and convergence. Floral scents were sampled using the dynamic headspace method and VOC identifications were carried out using gas chromatography coupled to mass spectrometry. To annotate VOCs, mass spectrums and retention indexes were compared to those on existing floral scent databases. Our results show that: i) within shade houses species produce the same VOCs as their counterparts in situ; ii) three pollination systems ensure the survival of two species; and iii) over 340 different VOCs are produced by 42 species. The phylogenetic mapping is currently ongoing. While improving on the vacancy in African orchid chemical ecology, this study equally contributes to the current knowledge of non-sphingophile orchid-pollinator interactions, highlighting pollinator species to be prioritized for conservation purposes.

5 - Ecological interactions to Improve Conservation Outcomes

ORAL PRESENTATION

CONSEQUENCES OF LOCAL ADAPTATION AMONG ORCHIDS, SOIL, AND MYCORRHIZAL FUNGI FOR SPECIES CONSERVATION

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Abstract

Local adaptation within the species is a commonly observed phenomenon which provides them greater ability to survive and develop in the local environment. Although it is generally known how the populations adapt to their local abiotic environments, biotic interactions are often neglected despite they can affect fitness and exert selective pressures. Orchids depend on particular groups of mycorrhizal fungi (OMF) for germination and subsequent growth. However, little is known about local adaptations in a tripartite system orchid-OMF-soil. We investigated whether orchid populations display local adaptations to OMF by performing in vitro symbiotic seed germination experiments to understand the orchid-fungus physiological specificity. In 2022, the roots and seeds from 4 orchid species differing in the breadth of orchid-fungus specificity were collected in the Czech Republic, Sweden, and Italy, and OMF were cultured. Seeds were germinated reciprocally with fungal isolates from home and non-home sites. Germination success was compared with geographical distances between populations. Besides results from in vitro experiment, we will also present preliminary data from a greenhouse pot experiment which includes also soil from home and non-home sites.

6 - Genetics and Molecular tools for conservation

ORAL PRESENTATION

ELUCIDATING EVOLUTIONARY RELATIONSHIPS OF RARE AND THREATENED MYCOHETEROTROPHIC ORCHIDS: A PHYLOGENOMIC STUDY IN THE AUSTRALASIAN GENUS *DIPODIUM*

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Abstract

Orchids are among the most threatened flowering plant group globally, facing multiple threats such as deforestation, urbanisation, overharvesting and climate change. Due to their often highly specialised and complex ecological interactions with pollinators and mycorrhizal fungi, orchids are facing an increased risk of extinction. Their dependence on other organisms which are also affected by multiple anthropogenic and environmental pressures, increases their vulnerability to environmental changes. In mycoheterotrophic orchids, reliance on fungal partners for nutrients is increased and fully mycoheterotrophic orchids completely rely on their fungal partner for nutrient supply. The orchid genus *Dipodium* comprises photosynthetic species as well as mycoheterotrophic species, the latter with centre of diversity in Australia. The conservation status of the Australian *Dipodium* species has six species categorised as vulnerable or endangered at the state level, while nationally, two species are classified as endangered (*D. campanulatum* and *D. pandanum*). However, evolutionary relationships in *Dipodium* remain unclear and the extent to which mycoheterotrophic *Dipodium* species rely on their fungal partners are still unknown. Here we investigated phylogenetic relationships in *Dipodium* based on 68 plastid loci and comparatively studied 24 newly assembled *Dipodium* plastomes representing 14 species and two potentially new species to shed light on the evolution of mycoheterotrophy in the genus and the temporal dynamics of plastome degradation. Section *Dipodium*, which comprises all mycoheterotrophic species in the genus and one autotrophic species (*D. ensifolium*), was found to comprise six main lineages including four species complexes. Mycoheterotrophy was found to have evolved only once in *Dipodium* and *D. ensifolium* was sister to all mycoheterotrophic species. Our divergence time estimations shed light on the climatic and environmental changes on the Australian continent over the late Cenozoic that may have influenced the transition from autotrophy to mycoheterotrophy within the genus. This study provides important insights into plastid genome degradation along the transition from autotrophy to mycoheterotrophy in a phylogenomic and temporal context. Levels of plastid gene degeneration were found to vary among species as well as within species, providing evidence of relaxed selection for retention of plastid genes. Patterns of plastome degeneration in *Dipodium* are consistent with those predicted for the early stages of plastome degeneration. This study underscores the importance of understanding the evolutionary dynamics of mycoheterotrophic orchids and emphasises the need for further study to assess species concepts in *Dipodium* crucial for conservation efforts.

6 - Genetics and Molecular tools for conservation

ORAL PRESENTATION

CONSERVATION HOLOGENOMICS – INTEGRATING HOST GENOMIC AND MICROBIONT COMMUNITY DATA

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Abstract

As the importance of the microbiome for plant health and ecology becomes increasingly evident, it seems clear that effective conservation of wild plant species needs to integrate knowledge on both the plants and their microbionts, i.e., the holobiont. Conservation of the genetic diversity in a plant species could, therefore, be extended to involve considerations for its diversity of functionally important microbionts. Population genomics offers valuable tools for designing better conservation strategies for vulnerable plant species, but does not take into account host-specific interactions with and dependence on specific microbionts. We propose that integrating population genomics with host microbiome studies into a wider plant conservation hologenomics context is needed for the conservation of the plant holobiont. This approach offers novel levels of understanding habitat requirements, landscape genomics and identification of evolutionary significant units. With their dependence on specific mycorrhizal fungi, orchids provide a great illustration of the importance of the microbiome component in conservation studies. While it is known that most orchids have specific requirements for their mycorrhizal partners and that abundance of these partners effects distribution of the orchids, there is a scarcity of knowledge on how the orchid microbiome varies with populations and across the landscape, and how it correlates with host genomic variation. We here demonstrate an orchid conservation hologenomics approach by combining population genomics with root microbiome data for two closely related orchid species (*Platanthera chlorantha* and *P. bifolia*) across populations in Denmark, and discuss the implications of our findings for conservation efforts.

6 - Genetics and Molecular tools for conservation

ORAL PRESENTATION

RESOLVING THE TAXONOMY OF THE *CALADENIA PATERSONII* AND *C. RETICULATA* (ORCHIDACEAE) SPECIES COMPLEXES IN SOUTH AUSTRALIA FOR IMPROVED CONSERVATION OUTCOMES

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Abstract

Intense taxonomic activity over the past thirty-five years within the *Caladenia patersonii* and *C. reticulata* species complexes in South Australia (~31 spp.) has not resulted in a functional taxonomic framework. The difficulty in identifying species within these groups, including those listed as endangered, has hampered conservation efforts and led to inefficient use of resources. My PhD project seeks to address this issue through a systematic approach. Initially, I have assessed the species that can be recognized and identified inconsistencies among their diagnoses, descriptions, and types. This has been followed by sample collection of these *Caladenia spp*. throughout South Australia for morphological and molecular analyses. The molecular dataset was constructed using DNA sequences derived from hybrid capture utilizing the Angiosperm353 and OzBaits kits (including nuclear and chloroplast loci) and a genome skim. A more recent additional approach is population level structure analyses through SNP array data. The collected material indicates greater morphological variation in current populations than documented in descriptions or types, with similar variation also found in historical collections predating the period of heightened taxonomic activity. Initial phylogenetic analyses reveal paraphyletic taxa and suggest frequent hybridization with the *C. dilatata* species complex. Some clades contradict our current understanding of this genus in South Australia.

6 - Genetics and Molecular tools for conservation

ORAL PRESENTATION

UTILISING GENOMIC INSIGHTS TO INFORM TAXONOMIC AND CONSERVATION ASSESSMENTS OF RARE FLYING DUCK ORCHIDS (*PARACALEANA GRACILICORDATA*/ *P. GRANITICA* SPECIES COMPLEX).

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Abstract

The genus *Paracaleana* comprises 14 species of sexually deceptive terrestrial orchids found in Australia and New Zealand, with a centre of diversity in Western Australia. Traditionally, three species were recognised within the genus until recent taxonomic revision saw the description of 11 narrow-range species endemic to Western Australia. Species delimitation was based on subtle variations in floral morphology regarded as significant for pollinators and thus hypothesised to function as reproductive barriers between these taxa. A thorough review of species boundaries based on molecular data is required to underpin conservation assessments for species of concern within the genus. Among these are *P. gracilicordata* and *P. granitica*, endemic to small distributional ranges on granitic rock outcrops in the Perth Hills area that have been identified as high priority for survey and evaluation of conservation status so that consideration can be given to their declaration as threatened flora. With increasing anthropogenic threats to their habitat, a conservation genomic assessment of their taxonomic status and genomic diversity is urgently needed. In this talk, we will share the results of a genomic analysis of 192 individuals of *P. gracilicordata*, *P. granitica* and closely related species using an informative genotyping-by-sequencing dataset including over 7,000 SNPs. Our analyses, including SplitsTree phylogenetic networks and STRUCTURE population clustering, resolved several genetic lineages, clarifying evolutionary relationships among species. The relationships revealed have enabled an assessment of species delimitation among *P. gracilicordata*, *P. granitica*, and their close relatives and indicate a revision of current species concepts is necessary. Implications for taxonomy, conservation assessments and management recommendations will be discussed.

6 - Genetics and Molecular tools for conservation

ORAL PRESENTATION

POPULATION GENETICS, MYCOBIOME METABARCODING, AND FUNGAL ENDOPHYTE ISOLATION HAVE IMPLICATIONS FOR THE CONSERVATION OF NORTH AMERICA'S NATIVE *VANILLA* SPECIES

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Abstract

Although *Vanilla* is a tropical genus, there are six species indigenous to the continental United States. Four are native: *Vanilla barbellata*, *V. dilloniana*, *V. mexicana*, & *V. phaeantha*. Unfortunately, the first two are already considered extinct in North America, whereas *V. barbellata* and *V. phaeantha*'s distributions are limited now only to a few protected areas. Their habitats face numerous environmental threats including loss of pollinators, habitat fragmentation, and rising sea levels due to climate change; conservation efforts are needed immediately. For *V. barbellata*, a total of 115 individuals from two Florida populations and three Caribbean Island populations were sampled. The *V. planifolia* draft genome was used to identify a filtered set of 12,825 single nucleotide polymorphism markers generated via Genotyping-By-Sequencing (GBS). From these data we calculated current genetic diversity, investigated evidence of gene flow, and documented low heterozygosity among disjunct populations of sampled populations. Additionally, we isolated and cultured endophytic fungi to ascertain their diversity and to acquire fungi with the potential to germinate Vanilla seeds ex situ. Furthermore, we compared and contrasted the mycobiomes of leafless *V. barbellata* and leafy *V. phaeantha* from soil and among their different biological compartments such as leaves, terrestrial roots, and aerial roots. We argue that future conservation efforts must consider relocation of *Vanilla* species via assisted migration and/or ex situ cultivation; they also should consider these documented fungal communities in such efforts.

7 - Citizen Science and School Programs

ORAL PRESENTATION

EXPLORING THE LAND STEWARDSHIP STRATEGY AS A TOOL FOR THE IN-SITU CONSERVATION AND MONITORING OF TERRESTRIAL ORCHIDS AND THEIR HABITATS IN ITALY: LESSONS LEARNED DURING THE LIFEORCHIDS PROJECT

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Abstract

LIFEorchids (LIFE17 NAT/IT/000596), a project co-financed by the European Union LIFE Programme in 2018-2023, aimed at preventing the decline of wild orchids and their natural habitats in northwestern Italy (Piedmont and Liguria). The project targeted in particular orchids of (semi)dry grasslands, a priority habitat for conservation in Europe (6210*) only if it is an important orchid site.

LIFEorchids' direct conservation interventions in the target regions were combined with the Land Stewardship approach, aimed at involving citizens to extend orchid protection beyond the boundaries of Protected Areas and of direct public management. Goals were achieved and exceeded, with the engagement of 122 Orchid Stewards (OSs) for a total of over 1000 hectares. Furthermore, this action developed unexpectedly in several directions. Initially focused on the direct intervention areas, it expanded in geographical terms (23 OSs in other Italian regions). Originally mainly aimed at private owners (30 farms, 69 non-productive land), it included several municipalities and local authorities (23 OSs).

Since 6210* grasslands are a semi-natural habitat where orchids thrive provided that the growth of shrub and tree species is restricted and coarse grasses and tall herbs are prevented from achieving dominance, continued management by OSs resulted in a steady increase of orchid populations (which have sometimes come to consist of hundreds of plants) in their lands. The involvement of OSs, which allowed access to their private areas, also made it possible to obtain data on orchid presence and distribution that would otherwise not have been possible to collect.

The enthusiastic engagement of OSs and the continuing commitment of project's beneficiaries are extending activities beyond the period of cofinancing by EU: an Orchid Stewardship Festival including dozens of events is being spontaneously organized every year in spring, whereby the general public is introduced to the importance of orchids and their habitats.

7 - Citizen Science and School Programs

ORAL PRESENTATION

THE MILLION ORCHID PROJECT: 10 YEAR UPDATE

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Abstract

Ten years ago, Fairchild established the Million Orchid Project, now the largest and most successful public outreach program focused on orchids. The program is propagating endangered native orchids and planting them in urban areas in southern Florida and beyond. To date we have successfully planted hundreds of thousands of native orchids back into public spaces throughout South Florida with the goal of re-establishing one million plants in the region. Orchids are grown from seed in the micro-propagation lab at Fairchild, with tremendous assistance from volunteers, college students, and K-12 students. Plants are also produced on The STEMLab: Fairchild's mobile micro-propagation laboratory built within the frame of a decommissioned school bus. The project is unique in its scale, its focus on public spaces, and its involvement of the local community. More than 100 schools and 75 community partners have been enlisted to grow orchids in their classrooms and surrounding neighborhoods. To date, school landscapes, parks, hospitals, and urban tree plantings have been the primary recipients of the reintroduction initiatives. At its core, The Million Orchid Project is a massive living science experiment that allows us to make important discoveries about how better to conserve orchids through propagation and develop more general strategies for rescuing rare plants within a highly developed urban environment.

7 - Citizen Science and School Programs

ORAL PRESENTATION

STEMLAB: A MOBILE CLASSROOM FOR RARE ORCHID PROPAGATION AND STUDENT ENGAGEMENT

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Abstract

STEMLab is a unique mobile orchid propagation facility operated by Fairchild Tropical Botanic Garden in Miami, Florida, USA, as part of its Million Orchid Project initiative. Designed and built by university architecture students within the frame of a school bus, STEMLab offers a practical learning environment that includes two large laminar flow hoods, an autoclave, and equipment for media preparation. The project aims to teach students aged 12-14 sterile propagation methods for rare and locally extinct native orchids. Since the program began in 2015, we have tracked and measured the number of participating schools and students, the number of orchid species and individual orchids propagated, the contamination rate of cultures, and teachers' perceptions of student learning. Each year, more than 1,500 students from an average of 30 schools participate, producing over 10,000 orchid plants annually. Students have successfully propagated six orchid species and outplanted them at their schools. The contamination rate remains below 10%, despite all participants learning sterile technique for the first time. Teachers report that students gain knowledge of species conservation, demonstrate increased engagement compared to traditional classroom activities, and show a heightened interest in STEM. This model holds promise for replication in other regions, potentially yielding significant educational and conservation benefits.

7 - Citizen Science and School Programs

ORAL PRESENTATION

THE IMPORTANCE OF COMMUNITY EDUCATION AND INVOLVEMENT FOR THE LONG-TERM PROTECTION OF THREATENED SPECIES.

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Abstract

Community engagement in conservation is a common method for achieving conservation goals, that may be otherwise unachievable, through the additional resources from volunteer hours. However, for realistic long-term protection of threatened species, including orchids, there needs to be greater focus on community education and involvement in their protection. Community groups are a perfect example of where this knowledge and stewardship can be harnessed. Bendigo field naturalists in North-central Victoria have been paramount, especially in the earlier days, for the conservation and discovery of the McIvor spider orchid (*Caladenia audasii*) and the Mandurang spider orchid (*Caladenia* sp aff *fragrantissima*). They have conducted search days, hand weeded sites, participated in re-introductions and communicated with government to protect sites in fuel reduction burns. Without this intervention, these species would likely be extinct. Another example is the Australasian Native Orchid Society - Victoria Group (ANOS Vic), which contribute anywhere between 500 and 600 hours of volunteer work each year for the management and protection of Sunshine Diurus (*Diuris fragranstissima*). Another important, but less common example, is to support specialist individuals or groups to propagate threatened orchids for re-introduction. There are many barriers to this including financial, permitting and politics within the field of orchid conservation. Although a sound knowledge of orchids is required for orchid work, especially propagation, support should be provided to individuals or groups that can demonstrate this knowledge as there are already too many threatened species to be feasibly grown by a single or even multiple growers. Having multiple growers can also spread the risk of loss from unforeseen events such as pest infestations or malfunctioning equipment.

7 - Citizen Science and School Programs

ORAL PRESENTATION

WORKING TOGETHER TO MAKE A DIFFERENCE: AUSTRALASIAN NATIVE ORCHID SOCIETY VICTORIAN GROUP INC. (ANOS VIC).

Wendy J Probert*

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Abstract

ANOS Vic has a dedicated, enthusiastic Conservation Group and Conservation Officer. Of the 381 named and ~ 66 undescribed Victorian orchid species, about 200 of these are listed as threatened under the Victorian Flora and Fauna Guarantee Act 1988 and its Amendment Act 2019. We are busy!

Our activities include: surveying for new populations of threatened orchid species, monitoring of existing populations, preparing submissions to government authorities about proposed developments on important orchid habitat, hand pollination and seed collection, pollinator baiting, seed raising, plant cultivation, rewilding (reintroduction) of plants on public and private land, caging, weeding and habitat planting. Members assist with orchid research projects, providing their personal knowledge of orchid distribution to government bodies, scientists, and students supervised by university researchers. Experienced terrestrial growers have also passed on their cultivation skills.

ANOS Vic works closely with a wide range of different groups and individuals to enhance the survival of threatened orchids. These include official organisations such as the Department of Energy, Environment and Climate Action (DEECA), Parks Victoria, the Royal Botanic Gardens Melbourne, and local Councils responsible for managing bushland reserves. We work with like-minded community groups such as Friends Groups, Landcare Groups, and Field Naturalists as well as individual environmental consultants. Numerous people from these groups are, in turn, ANOS members. Volunteers have assisted with the Research Project 'Preventing Extinction in Bushfire Affected Orchids' in the Alpine National Park and with the reintroduction of thousands of orchids into the Riverina. A healthy cross-fertilisation of knowledge, experience and labour leading to some successful reintroductions!

Some species with which ANOS Vic assists include: *Caladenia audasii*, *C. amoena*, *C. cretacea*, *C. cruciformis* and *C. flavovirens*, *Diuris fragrantissima* and *D. ochroma*, *Pterostylis chlorogramma*, *P. cucullata* and *P. smaragdyna*, *Thelymitra epipactoides* & *T. mackibbinii*.

7 - Citizen Science and School Programs

ORAL PRESENTATION

THE AOF PAST AND PRESENT…THE AOF IN THE FUTURE

Helen Richards, John Varigos

Australian Orchid Foundation

Abstract

The Australian Orchid Foundation was founded in 1976 as a voluntary organisation to support orchids in Australia, their preservation, protection, promotion and cultivation of all species and hybrids. This is done through financially supporting education and research, especially of rare Australian orchids, and assisting young people in their orchid related endeavours. It is governed by a Board of Directors and research projects are approved by a CSIRO legal endorsed Research Committee. Funding is derived from membership fees, tax-deductible donations and bequests. Funding is supplemented by the sale of books, seed via the orchid seed bank, and orchid plants. Since its establishment in 1976, close to 300 projects have been funded over a wide range of research and community projects. The AOF also publishes research in the Australian Orchid Research series. The seed bank promotes and facilitates conservation of species by making seed available to hobbyist. As an acknowledgement of people's contribution to orchids in Australia, there is an annual Award of Honour and a Memorial Register. To encourage people to write of their experiences to benefit others, there is an annual Essay Competition. In the future these activities will continue together with a focus on increasing membership, broadening the number of species available in the seedbank and exploring other sources of funding. There will be a greater emphasis on the promotion of the conservation work being supported by the AOF and to funding projects involving community groups. The AOF has recently entered a collaboration with the World Orchid Conference Trust with one objective being to work on supporting conservation projects both in Australia and globally.

7 - Citizen Science and School Programs

PLENARY LECTURE

SAVING ORCHIDS IN THE 21ST CENTURY: A REASON FOR OPTIMISM

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Abstract

Almost a quarter century has passed since the first IOCC was held here in Perth, Australia at a time before some of you reading this sentence were born. Subsequent conferences have been held all around the globe and, like the boomerang, it has returned full circle. What have we accomplished, and why should we care about orchids per se? Regarding the latter, about 8-10% of all named plant species are orchids, most of which are connected to a myriad of other organisms (e.g., insect pollinators, mycorrhizal fungi) within a complex ecological framework. We are just beginning to understand these complex interactions aided by modern techniques (e.g., camera traps, amplicon sequencing) that have catalyzed conservation efforts worldwide, even involving tropical epiphytic orchids high in the forest canopy. We are beginning to pinpoint why orchids are the first plants to disappear in the landscape allowing us to take appropriate steps to safeguard what remains and to recover species at an extinction precipice. What gives us hope is the number of young people who are eager to play an active role in these efforts. But challenges remain as the clock ticks on. For example, we still know very little about the specific insect pollinators that many specialist orchids rely on for fruit set and how to go about conserving these important biotic agents in the landscape. Conferences such as IOCC help to bridge the gap and instill optimism, but as we depart feeling good about ourselves, we must effectively communicate what we do with the greater global community and not merely 'preach to the choir'. We must also remember that to save these remarkable plants, we need to educate more young people – tomorrow's stewards – so that future generations can live in a world filled with the same orchids we grew up with.

POSTERS

1 - Conservation: Global Perspectives

POSTER

KENTUCKY ORCHID CONSERVATION PROGRAM

Tara R. Littlefield*, Chris Barton

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Abstract

Did you know there are 43 species of terrestrial orchids found in Kentucky? Thirty of those species are either federal or state listed, globally rare or declining in the state. To increase orchid conservation efforts, the Kentucky Orchid Conservation Program was created by the Office of Kentucky Nature Preserves (OKNP), in partnership with the North American Orchid Conservation Center, Atlanta Botanical Garden, Longwood Gardens, Illinois College University, University of Kentucky, Kentucky Native Plant Society, Kentucky Plant Conservation Alliance and other state and federal agencies. The goal of the program is to conserve and restore rare orchids and their habitats through in situ conservation efforts such as site protection, land acquisition, habitat management, monitoring, and research as well as ex situ conservation efforts such as seed banking, propagation, translocations, living collections, genetic banking, and associated research. OKNP monitors numerous populations of 21 state listed orchids in our natural heritage database, with over 40% of these populations occurring on protected lands. Monitoring orchids helps us focus conservation efforts where it's needed the most. Currently, there are active projects on seven rare orchids: Kentucky Ladyslipper (*Cypripedium kentuckiense*), White fringeless orchid (*Platanthera integrilabia*), Rose pogonia (*Pogonia ophioglossoides*), Appalachian Dragonhead Pogonia (*Calopogon tuberosa*), in forests and wetlands of eastern Kentucky; Small white ladyslipper (*Cypripedium candidum*) and Great Plains Ladies'-tresses (*Spiranthes magnicamporum*) in limestone prairies and glades in the interior plateau in Central Kentucky; and Southern Twayblade (*Neottia bifolia*) in upland depression ponds in the interior plateau of central/western Kentucky. While work to preserve and enhance orchid populations and habitat is underway, future work will focus on efforts to reintroduce extirpated populations back into suitable habitat on our natural areas.

3 - Ex Situ Conservation: Are we Making a Difference

POSTER

EXPLORING THE MYCORRHIZAL RELATIONSHIPS AND GERMINATION HABITS OF NEW ZEALAND *CORYBAS*

Jennifer Alderton-Moss*, Karin van der Walt, Carlos A. Lehnebach

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Abstract

With a short stature, subtle colouring, and typically scentless flowers, the orchid genus *Corybas* is often overlooked. *Corybas* are terrestrial orchids, found throughout Asia and the Pacific. Although some efforts have been made towards identifying their mycorrhizal partners and developing germination protocols success to date has been variable and there is still significant work to be done, particularly in New Zealand (NZ). There are 22 species of *Corybas* in NZ, all of which are endemic. As a joint project between Ōtari Native Botanic Garden and Te Papa Tongarewa Museum of New Zealand, funded by the Australian Orchid Foundation, we have been working towards the conservation of NZ native *Corybas*, including the Critically Endangered species *Corybas carsei* and *Corybas dienemus*, as well as the tag-named entity, *Corybas* "Tridodd", known only from a single site. To achieve this, we have been isolating the mycorrhizal fungi from *Corybas*, and identifying them using ITS barcode sequencing. With these fungi, we have then been trialling symbiotic germination on oatmeal agar, as well as asymbiotic germination with commercial media such as Knudson C and Murashige and Skoog. Here, we share our progress in these efforts and the challenges we have faced. Understanding how to grow *Corybas* from seed will have a profound impact on the conservation of some of NZ's most threatened plants and improve outcomes for the wider genus.

3 - Ex Situ Conservation: Are we Making a Difference?

POSTER

INTERACTIONS AMONG MYCORRHIZAL FUNGI ENHANCE THE EARLY DEVELOPMENT OF A MEDITERRANEAN ORCHID

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Abstract

Orchids depend on mycorrhizal fungi to germinate from seed. While multiple orchid mycorrhizal (OrM) taxa are often found associated with adult orchids, the relative contribution of particular OrM taxa to germination and early orchid development is poorly understood. We isolated 28 OrM fungi associated with the Mediterranean orchid *Anacamptis papilionacea* and tested the efficiency of five isolates on germination and early development, four belonging to the *Tulasnella calospora* species complex and one belonging to *Ceratobasidium*. Co-cultures of varying two-way and three-way combinations of OrM isolates were used in vitro to compare the simultaneous effect on seed germination rate with monocultures. We then tested whether, when given initial priority over other fungi, particular OrM taxa were more effective during the early stages of development. Seedlings germinated with different isolates were transferred to a growth chamber, and either the same or different isolate was added 45 days later. After 3 months, the number of roots, length of the longest root, and tuber area were measured. All OrM fungi resulted in seed germination; however, lower germination rates were associated with the *Ceratobasidium* isolate compared to the tulasnelloid isolates. There was significant decreased germination in co-culture experiments when the *Ceratobasidium* isolate was added. Despite being associated with reduced germination rates, the addition of the *Ceratobasidium* isolate to the seedlings germinated with tulasnelloid strains resulted in significant increased tuber size. Although *A. papilionacea* associates with many OrM taxa, these results show that OrM fungi may play different roles during orchid germination and early development. Even when given initial priority, other fungi may colonize developing orchids and interact to influence early orchid development.

3 - Ex Situ Conservation: Are we Making a Difference?

POSTER

INVESTIGATING THE CELL WALL REMODELING BEFORE AND AFTER *GASTRODIA ELATA* SYMBIOTIC CULTURE WITH *ARMILLARIA SPP*.

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Abstract

Gastrodia elata, a fully myco-heterotrophic orchid, relies entirely on a symbiotic relationship with *Armillaria* for its nutrient supply during tuber development. This species, also esteemed in Chinese herbal medicine for its potential to treat neuron diseases, is at a high risk of extinction because of the substantial demand in markets. In the symbiotic culture system, notable changes were observed in the cell structures of the basal mycorrhizal tuber. Cells differentiated into distinct types: infected cells, large cells, and inner cortical cells, with hyphae invasion being limited when they penetrate large cells. Simultaneously, the large cells enlarge significantly, with some uneven thickening on their walls. Thus, we seek to explore the changing in cell wall components during symbiosis with *Armillaria*, focusing on extensin, arabinogalactan proteins (AGPs), and proteins related to pectin modification. We use immunogold labeling to understand the distribution of the component before and after infection, as well as across different cell layers. Additionally, RNA extraction from the three cell types allows for RT-PCR analysis of specific cell wall component genes. Our preliminary findings show specific labeling of JIM 11 and JIM20 on the large cell wall. We hypothesize the presence of additional structural proteins on the large cell layer, potentially serving as a barrier against *Armillaria* invasion. Despite the use of symbiotic culture in cultivating *G. elata* for many years, the yield of *G. elata* tubers remains inconsistent. A better understanding of the changes in cell wall composition throughout symbiosis could shed light on how to more effectively cultivate this rare, fully myco-heterotrophic orchid.

4 - In Situ Conservation

POSTER

ORCHIDS IN U.S. SOUTHWEST ECOSYSTEMS: A COLLABORATION OF THE DESERT BOTANICAL GARDEN[1], THE SMITHSONIAN ENVIRONMENTAL RESEARCH CENTER[2], & NORTH AMERICAN ORCHID CONSERVATION CENTER (NAOCC)[3]

Andrew Salywon[1,3], Steve Blackwell[1,3], Dennis F. Whigham[2,3], Melissa K. McCormick[2,3], Julianne McGuinness[3]

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Abstract

The Smithsonian's North American Orchid Conservation Center (NAOCC) and the Desert Botanical Garden have a collaborative project to link native orchid ecology and conservation with existing efforts to restore or enhance water resources through watershed management in Southwest ecosystems. The project is designed to characterize and compare the environmental conditions of orchid habitats in the Sky Islands region of southern Arizona - both where native orchids occur, and at sites managed with watershed-scale installations of rock detention structures to retain water on the landscape longer - a traditional Indigenous practice in the region. A goal is to increase the water storage capacity of the landscape, reverse watershed degradation, and positively impact multiple biophysical systems, including carbon sequestration. Such water management should increase the availability of appropriate habitats for native orchids by improving hydrologic conditions that support both the orchids and their mycorrhizal fungi. Over the next two years, the research will focus on quantifying the distribution and abundance of orchid mycorrhizal fungi associated with soils in native orchid habitats and managed sites to determine if the newly created habitats could support the fungi. Microloggers are installed to monitor soil moisture and temperature continuously. Trained scent dogs have been employed to locate elusive orchids. Seed germination experiments are in progress in the field and laboratory, with the initial focus on propagation of the Endangered Canelo Hills ladies' tresses (*Spiranthes delitescens*), a species endemic to Arizona and currently found at only two of the five sites where it historically occurred. Funding for the initiative is a generous grant from the Biophilia Foundation.

5 - Ecological interactions to Improve Conservation Outcomes

POSTER

CAN ORCHID MYCORRHIZAL FUNGI IMPROVE CONSERVATION OF THE VULNERABLE *CATTLEYA CRISPA* IN BRAZIL?

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Abstract

Symbiotic orchid seed germination is a promising alternative for orchid propagation and reintroductions, as it showed to improve seedling growth and greenhouse acclimatization.

This project aims to expand populations of the threatened orchid *Cattleya crispa* in the Brazilian Atlantic Forest biome, and engage college students in science communication for orchid conservation. We isolated potentially orchid mycorrhizal fungi (OMF) from adult plants occurring at the private reserve 'Alto da Figueira' (Rio de Janeiro/Brazil), and collected seeds for storage. Seed germination tests were initiated to evaluate the effect of OMF in embryo survival and development. The seeds were inoculated with the isolated fungi on oatmeal agar medium. We are also asymbiotically propagating seeds using Murashige and Skoog medium as a control. Embryo development is being monitored weekly and described according to Zettler & Hofer (1998). The isolated fungi morphologically identified as members of the Rhizoctonia complex were submitted to DNA extraction and sequencing to identify the fungi at the genus/species level. In 2024, undergraduate and graduate students at the State University of Campinas presented three workshops, attended by 400 people, including students aged 12-18 and adults. The workshops aimed to communicate the fragility of orchids, their dependence on fungi, and empower participants to take an active role in conservation.

5 - Ecological interactions to Improve Conservation Outcomes

POSTER

MYCORRHIZAL FUNGAL ASSOCIATES OF NORTH AMERICAN NATIVE ORCHIDS: SPECIALISTS, GENERALISTS, AND GEOGRAPHIC VARIATION

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Abstract

Mycorrhizal fungi are critical for orchid seed germination and for allowing mature orchids to tolerate stressful conditions. As a result, they are essential to orchid conservation. While considerable attention has been paid to identifying the mycorrhizal fungi associated with particular orchid species in specific locations, the mycorrhizal fungi required by most orchids are still unknown. Identifying and banking these fungi is necessary for effective conservation and restoration that pairs orchids with the mycorrhizal fungi they need to successfully grow and reproduce. The North American Orchid Conservation Center's (NAOCC) network of collaborators and volunteers have amassed a collection of orchid mycorrhizal fungal associates from the United States and Canada that includes more than 2000 root samples and over 1000 sequences of mycorrhizal fungi, as well as a living collection that includes representatives of fungi from many species. We present a phylogenetic analysis of nuclear ITS sequences from fungi in our collection, metabarcode ITS sequences from root remnants in our collection, and sequences downloaded from GenBank accessions from published studies, to gain new insights into drivers of orchid distribution, geographic patterns of genetic variation, and natural hybridization, and how this informs our native orchid propagation efforts. Using species in the genus *Platanthera* as an example, we show how species with genetic intermixing also share mycorrhizal fungi, as well as how geographic patterns in genetic variation are mirrored in mycorrhizal associations.

5 - Ecological interactions to Improve Conservation Outcomes

POSTER

THE DEVIL IS IN THE DETAIL: ORCHID FLORAL DISSECTION CARDS

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Abstract

The Australian National Herbarium (ANH) holds a unique collection of orchid floral cards: careful dissections of complete orchid flowers from across Australia, pulled apart and mounted flat on a card under clear tape. This preservation method makes for a clear display of the complex internal structures of each orchid flower, and allows for direct and convenient comparison within and between species.

This active collection contains an estimated 25,000 cards, starting in the 1970s with associated collector and locality information and represents decades of taxonomic research.

We are integrating the floral card research collection into the main ANH collections. Starting alphabetically from 'A' for *Acianthus*, each card is checked against existing records in the ANH database. Any new records are added using information sourced from collector's field books in our archive or from information provided on the floral card. These additions include new floral cards, specimens that did not contain other physical items (herbarium sheet, spirit collection, live plant), or cultivated vouchers, which will also be linked to the initial collection. The accession number, a unique identifier, is written onto the floral card, and the imaging team creates a barcode matching this accession number when the floral card is imaged.

Once imaged, the digital images of each card will be made freely available online via the CSIRO Data Access Portal https://data.csiro.au/domain/nrca), facilitating access to the estimated 25,000 specimens with their valuable collection context and exposing the historical and geographical data for each species, linked to the image records. Researchers will be able to utilise these images for morphological comparison, differentiation of species and tracking species change over time. Mobilising this collection online will enable the use of AI technologies to measure, collate, and contrast differing details in flower morphology, which furthermore aims to aid researchers in taxonomic research and delimitation of species.

5 - Ecological interactions to Improve Conservation Outcomes

POSTER

NUANCES OF FLORAL MIMICRY: TARGETED MIMICRY AND GENERALIZED FOOD DECEPTION IN QUEEN OF SHEBA ORCHIDS

Daniela Scaccabarozzi*, Gavin Flematti, Mark Brundrett, Mario Vallejo-Marin, Klaus Lunau, Monica Gagliano, Emiliano Pioltelli, Terry Houston, Andrea Aromatisi, Nina Sletvold

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Abstract

Efficient conservation requires an understanding of the reproductive ecology of target species. We examined whether pollination of two deceptive orchids in the threatened *Thelymitra variegata* complex (Queen of Sheba orchids) is more consistent with generalized food deception or with specific mimicry of buzz-pollinated tinsel lilies (*Calectasia spp*.), by i) assessing overlap in floral traits and pollinator visitation between orchids, tinsel lilies, and other flowers in the rewarding community, and ii) quantifying orchid reproductive success in relation to abundance of tinsel lilies and other rewarding species. We found that *T. speciosa* is indistinguishable from its co-flowering tinsel lily *C. narragara*, regarding both flower colour and scent, while *T. porphyrosticta* differs from co-flowering *C. valida*. The orchids and tinsel lilies shared pollinators, and pollen-seeking *Anthoglossa* bees, displayed a behaviour on orchids, that was associated with buzz pollination of tinsel lilies. However, other bee species foraging nectar in the rewarding community are also likely to be important for orchid pollination. In *T. speciosa*, fruit production increased with abundance of its co-flowering tinsel lily, but also with abundance of rewarding species with similar flower colour, suggesting a combination of mimic and magnet effects. However, these relationships were entirely driven by high fruit set in a single, tiny population. We conclude that results are consistent with specific mimicry towards the co-flowering tinsel lily in *T. speciosa*, and with a more generalized deception strategy in *T. porphyrosticta*. We suggest that the *Thelymitra variegata* complex constitutes an ideal system for exploring factors governing the transition between specific mimicry and generalized deception. Our findings provide valuable insights for future orchid conservation efforts, that recognises the importance of key companion plants for their nectar- and pollen-seeking pollinators. In the case of *T. speciosa*, conservation practices should consider the presence of the tinsel lily and more abundant rewarding species such as *Melaleuca suberosa* to support the orchid reproductive success. Thus for *T. porphyrosticta*, conservation might require habitat preservation supporting a diverse vegetation assemblage with mixed floral resources.

6 - Genetics and molecular tools for conservation

POSTER

EXPLORING GENETIC VARIATION OF *HABENARIA RHODOCHEILA* HANCE IN THAILAND

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Abstract

Habenaria rhodocheila Hance is terrestrial orchid with a wide range of flower color forms, including red, yellow, orange, and pink, with each color often appearing in different areas. The aim of this research was to use inter-simple sequence repeat (ISSR) markers to analyze the genetic diversity of *H. rhodocheila* in different flower color forms across 9 population groups, including (1) two orange forms: OLEI and OCPM, (2) a yellow form: YSNK, (3) three pink forms: PSNK, PKSN, and PMDH, and (4) three red forms: R1NM, R2NMA, and RNPM. Of the 13 ISSR primers, 11 (84.61%) could amplify and give 106 polymorphic bands (91.37%) from a total of 116 generated bands ranging in size from 140 to 2,576 bp across all samples. Genetic diversity and clustering methods were analyzed using POPGENE version 1.31. The RNPM population had the highest observed number of alleles (Na), effective number of alleles (Ne), gene diversity (H), and Shannon's Information index (1.31±0.46, 1.21±0.34, 0.12±0.19, and 0.18±0.27). This finding suggests that the RNPM population has higher genetic diversity than other populations. However, there was low population diversity as evidenced by the mean H value of 0.094. The UPGMA genetic similarity dendrogram revealed five groups of *H. rhodocheila*. Group 1 comprises of OLEI and OCPM, group 2 of YSNK, group 3 of PSNK, PKSN and PMDH, group 4 of R1NMA and R2NMA and group 5 of RNPM, which corresponds to the form of each flower color. The finding of this study highlights the importance of *H. rhodocheila* that should be protected. It is beneficial for population restoration and can be used to guide future breeding efforts.

6 - Genetics and molecular tools for conservation

POSTER

NANOPORE SEQUENCING REVEALS A HISTORY OF HYBRIDISATION IN THE ORIGIN OF NEW ZEALAND SPECIES OF *THELYMITRA* (ORCHIDACEAE)

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Abstract

Thelymitra, otherwise known as the sun orchids, is a genus of orchids common throughout Australasia, known for their brightly coloured, albeit simple, flowers and tendency for self-pollinating. Historical research has identified at least six species present both in New Zealand and Australia that may have arisen through hybridisations. This conclusion was based on observations of morphological and cytological characters in suspected hybrids, and the morphological intermediacy between two different species. In this presentation we will discuss the use of nanopore sequencing, its utility in phasing nuclear amplicons, how this was applied to identify hybrid species through allelic variation, and how this was used to infer progenitor species. Amplicons of the LFY gene from twenty-one species of *Thelymitra* was sequenced on a Flongle flow cell using a MinION Mk1C, and resultant data phased. Sanger sequencing of a chloroplast marker ycf1 was also performed and used to construct a phylogeny and infer maternal progenitors in hybrids. Hybrid species had two diverged copies of the LFY amplicon, and in all but one case these copies corresponded closely to sequences from the hypothesised progenitor species. Through this approach we found support for a hybrid origin for the six species mentioned previously, as well as one species previously not suspected to be of hybrid origin. These results support the hypothesis that hybridisation has played a significant role in the evolutionary history of New Zealand sun orchids – and that phasing nuclear amplicons using nanopore is an effective method for identifying hybrid species. This result has implications in the conservation of these sun orchid species, as hybrids remain a controversial topic in this field.