



# The First International Orchid Conservation Congress

**Incorporating the 2nd International  
Orchid Population Biology Conference**

**24-28 September, 2001**

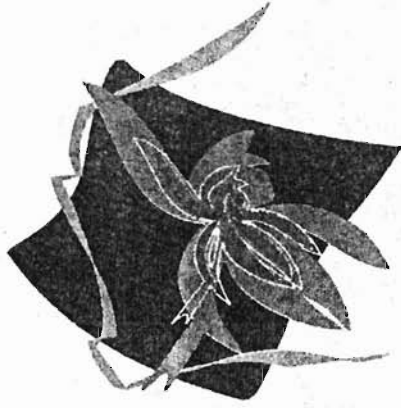
## Program and Extended Abstracts



**Herman Slade  
Foundation**







# The First International Orchid Conservation Congress

Incorporating the 2nd International  
Orchid Population Biology Conference

24-28 September, 2001

## Program and Extended Abstracts



The Botanic Gardens and Parks Authority (Kings Park and Botanic Gardens) are pleased to present the inaugural International Orchid Conservation Congress.

This important occasion, which has been over 3 years in the making, offers a unique and incredible opportunity for each of us to come together for a common purpose – to broaden and deepen our networks in orchid conservation around the world and to globalise and collaborate on the issues that we face in conserving the world's orchid flora.

Perth offers a dramatic backdrop for the Congress. Surrounded by one of the oldest, most ecologically diverse and beautiful natural landscapes in the world, and with one of the most biodiverse floras known, delegates are assured of an inspiring botanical and ecological experience.

The First International Orchid Conservation Congress offers an opportunity for us to stretch our limits and blend our perspectives. You'll meet new people and create linkages with professionals from 16 countries from around the world. An important component of the Congress is the meeting of the Orchid Specialist Group of the IUCN which will focus on national action in orchid conservation.

The workshops and sessions that are planned touch on most of the crucial areas in orchid conservation. In addition to learning opportunities, there will be plenty of time to network with colleagues old and new, relax and have fun at receptions, tours and of course, enjoy the splendour of the Kings Park Wildflower Festival. The Festival is the largest natural species flower show in Australia. Delegates will be amazed at the sheer beauty, breadth and diversity of the displays.

Our post-congress tour will offer the opportunity of a lifetime to explore this orchid rich region. The tour will traverse some of the most spectacular wilderness areas and will include the remarkable Stirling Range National Park.

Inspiring speakers, dynamic and practical sessions, exciting tours and fun activities are all part of this inaugural Congress. We invite you to participate to the fullest in this historic congress and help guide and direct orchid conservation on a global scale.

First International Orchid Conservation Congress, September 24-28, 2001, Perth, Western Australia. Book of Extended Abstracts.

Hosted by Kings Park & Botanic Garden (Botanic Gardens & Parks Authority); the Orchid Specialist Group of the Species Survival Commission of the IUCN, the World Conservation Union; Botanic Gardens Conservation International; the Australian Network for Plant Conservation; the Hermon Slade Foundation; the Australian Orchid Foundation; Rocla Quarry Products and the American Orchid Society.

Edited by Russell L. Barrett and Kingsley W. Dixon.

Published by the Plant Conservation Association Inc., Perth, Western Australia.

Printed by Image Source, Perth.

*Copyright*

© Plant Conservation Association, Inc., and individual authors 2001.

This publication is copyright. Apart from any fair dealing for the purposes of private study, research, criticism or review as permitted under the *Copyright Act* 1968, no part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, photocopying or other, without prior permission of the Copyright holder.

*Disclaimer*

The opinions expressed in this document are the views of the authors and do not necessarily reflect those of the Plant Conservation Association Inc.



## Contents

Committee and sponsors .....	4
Program – Quick reference guide .....	5
Hyatt Map .....	6
General Information .....	7
Social Functions .....	9
Field Trips .....	10
Conference Program .....	11
Presentation abstracts .....	19
Poster abstracts .....	86
Abstracts in absentia .....	110

### Special congress lectures and activities.

#### **The history of Angiosperm diversity.**

Professor Peter R. Crane

Director, The Royal Botanic Gardens, Kew

Tuesday September 25 at 6:30 pm.

Geography Lecture Theatre, University of Western Australia, Nedlands.

Over the last 15 years the discovery of diverse and exquisitely preserved Cretaceous fossil flowers has yielded a wealth of information on the structure of ancient angiosperms. Integrated with evidence from extant plants, and particularly increasingly secure hypotheses of relationships based on molecular evidence, these data constrain ideas on the pattern and timing of angiosperm diversification. Paleobotanical data provide clear evidence of a major diversification of flowering plants between about 130 and 80 myr before present, but the relatively late appearance of the most diverse angiosperm clades implies extraordinarily high rates of diversification, as well as a relatively recent and rapid origin for much of extant angiosperm diversity over the last several million years. In the coming decades this upward trend in diversity seems likely to be reversed. Increased rates of extinction will be driven in part by direct habitat destruction and degradation, but also by breakdown of geographical and ecological barriers that helped generate and maintain diversity in the past.

#### **Exploring botanic garden's around the world – from Vietnam to voodoo.**

Dr Peter Wyse Jackson

Secretary General, Botanic Gardens Conservation International

Thursday September 27 at 7:30 pm.

Constitution Centre, Corner of Parliament Place & Havelock Street, West Perth.

Cost: \$5 donation for supper.

RSVP: (08) 9480 3643 by 26 September 2001.

Dr Wyse Jackson has toured the botanic gardens of the world and his fascinating lecture will explore the way in which many of the 1800 botanic gardens create centres of horticultural excellence. He will take you on a tour from the great to the humble with exciting images of plants and people in action in the world's botanic gardens.

#### **Make sure you put time aside to visit the World Famous Kings Park Wildflower Festival – a panorama of wildflowers to celebrate springtime.**

Living masterpieces of floral design are created to enhance the beauty and diversity of Western Australian flora. Enjoy free guided bush walks, guest speakers, demonstrations, famous explorers and camels, art, gifts, craft, science techniques, growing native plants, children's activities or a chance to just sit and enjoy the diversity and beauty of the unique flora of Western Australia.

9am-5pm daily, September 21 - October 1, 2001. Free entry for Congress delegates. General enquiries (08) 9480 3600.

## International Advisory Committee

Leonid Averyanov	Komarov Botanical Institute
Phillip Cribb	Royal Botanic Gardens, Kew
Kingsley Dixon (Chair)	Kings Park & Botanic Garden
Shelagh Kell	Orchid Specialist Group, SSC, IUCN
Marilyn Light	University of Ottawa
Ned Nash	American Orchid Society
Hanne Rasmussen	Danish Forest and Landscape Institute
Gloria L.P. Siu	Kadoorie Farm and Botanic Garden
Kiat Wee Tan	Singapore Botanic Garden
Jo Willems	Utrecht University
Peter Wyse Jackson	Botanic Gardens Conservation International

## National Organising Committee

Russell Barrett	Kings Park & Botanic Garden
Andrew Brown	Department of Conservation and Land Management
Mark Brundrett	Kings Park & Botanic Garden
Kingsley Dixon	Kings Park & Botanic Garden
Kristy Harris	Kings Park & Botanic Garden
Trevor Hein	Kings Park & Botanic Garden
Stephen Hopper	Kings Park & Botanic Garden
Helen Richards	Australian Native Orchid Society
Ben Wallace	CSIRO Centre for Plant Biodiversity Research

## Student Committee

Nura Abdul Karim	University of Western Australia / Singapore Botanic Garden / Kings Park & Botanic Garden
Nika Debeljak	University of Ljubljana, Slovenia / Kings Park & Botanic Garden
Penny Hollick	Murdoch University / Kings Park & Botanic Garden
Sofi Mursidawati	University of Western Australia / Kings Park & Botanic Garden

## Acknowledgements

The Committee gratefully acknowledge the help and support of Botanic Gardens and Parks Authority staff, the Department of Conservation and Land Management Western Australia, and the staff of Congress West, particularly Elisa Marino, Sharon Boynes and Katie Clarke.

Thanks also to Thalia Economo (Theta Graphics) for designing the conference logo and Peter Hood for graphic design and layout of the numerous Congress documents.

## Sponsors

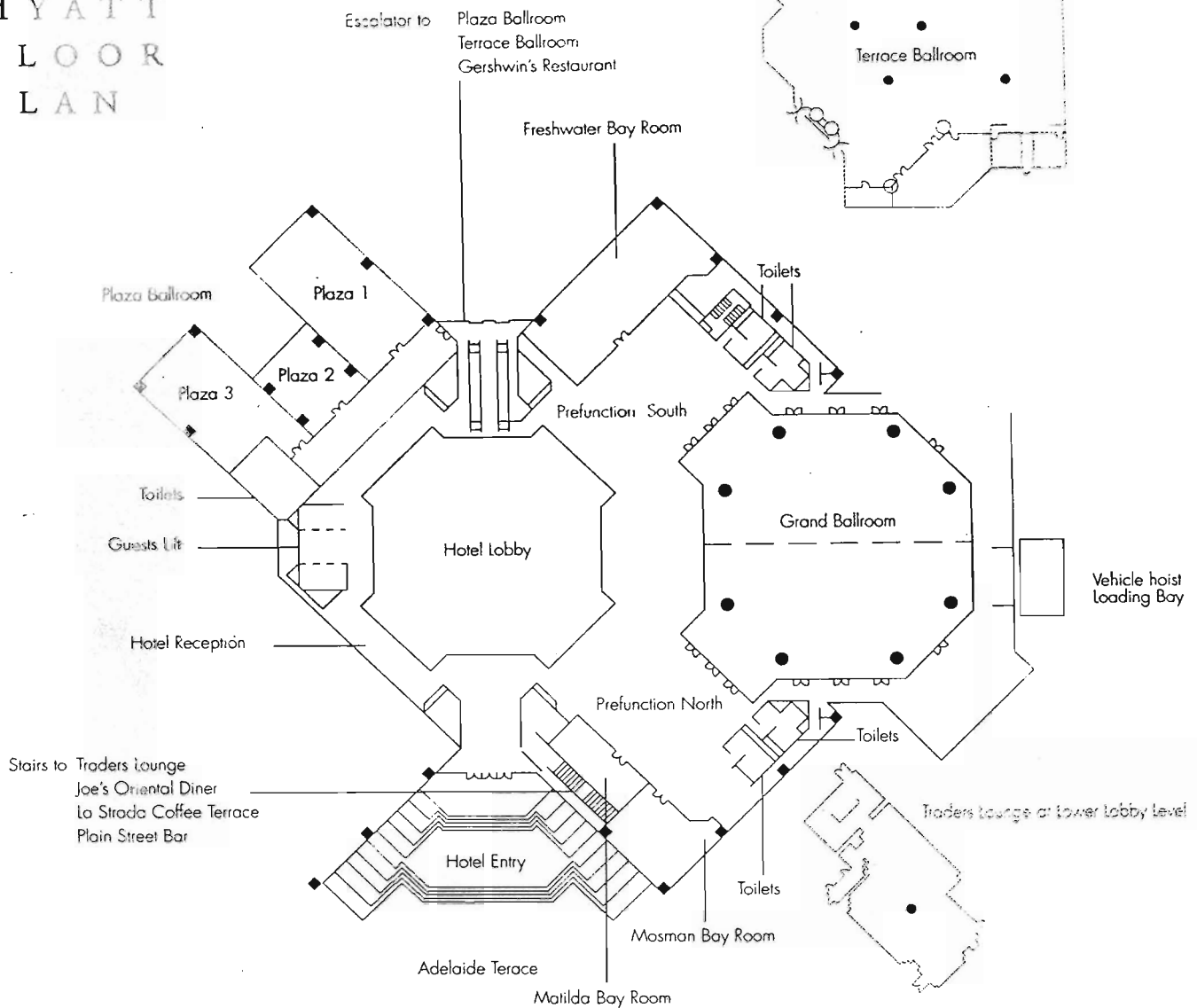
The following sponsors have provided outstanding support for the congress:

Hermion Slade Foundation  
Rocla Quarry Products  
Australian Orchid Foundation  
Perth Convention Bureau  
Australian Fine China  
Amberley Estate Wines  
Swan Brewery  
Western Power



	Sunday	Monday	Tuesday A	Tuesday B	Wednesday	Thursday	Friday A	Friday B
8:30						OSG overview: Phillip Cribb and Shelagh Kell		
9:00		Introduction and launch			Field Trip			
9:30		Conservation overview : Peter Wyse Jackson	Recovery (1): <i>Ex situ</i> studies in Orchid conservation: Margaret Ramsay	Population Biology (1): Jo Willems and Dennis Wigham		Orchid Specialist Group Regional reports: OSG Regional Chairs	Case studies in orchid conservation: Shelagh Kell and Kingsley Dixon	Pollination biology (1): Dave Roberts
10:00		Orchid Conservation overview: Phillip Cribb						
10:30		Morning Tea						
11:00		Phylogeny overview: Mark Chase						
11:30		Species concepts and conservation: Alec Pridgeon	Recovery (3): Recovery of plants to sites: Kingsley Dixon and Hanne Rasmussen	Population Biology (2): Raymond Tremblay and Michael Hutchings			Role of orchid societies and growers in orchid conservation: Henry Oakeley	Pollination biology (2): Mark Chase
12:00		Australian Orchid Conservation: Steve Hopper				OSG Regional workshops: OSG Regional Chairs		
12:30		Lunch break	Global Warming Talk	Matthew Buckels				
1:30		Case studies and assessment of conservation priorities: Shelagh Kell and Kingsley Dixon	Mycorrhiza for conservation (1): Finn Rasmussen and Lawrence Zettler	Managing the trade in wild orchids: Roddy Gabel		Conservation genetics (1): Diversity and priorities: Mike Fay, Seigy Krauss and Walter Rossi	Taxonomy for conservation: Ed de Vogel	
2:00								
2:30								
3:00		Afternoon tea	Photo Session					
3:30			Mycorrhiza for conservation (2): Finn Rasmussen, Lawrence Zettler and Mark Brundrett			Conservation genetics (2): Recovery and sampling strategies - Seigy Krauss, Mike Fay and Walter Rossi	Conclusions and recommendations: Steve Hopper	
4:00								
4:30		Threatening processes: Harold Koopowitz					NEXT CONGRESS	
5:00	Welcome function		Poster Session					
6:00			Peter Crane: 6:30					
7:00								
8:00						Peter Wyse Jackson: 7:30	Congress Dinner Speaker: Henry Oakeley	

# HYATT FLOOR PLAN



## Facilities

- Stage pieces of various heights in modules of 2400 x 1200mm
- Portable dance floor
- Audio-visual and lighting control room
- Concealed uni-struts for free hanging displays
- A comprehensive range of audio-visual equipment
- Qualified technical staff in-house
- Fibre optic link (permanently installed)
- Fully equipped business centre, telex, facsimile, photocopier, word processing and secretarial services
- Disabled facilities
- Loading hoist 5400 x 2400mm safety holding area with a capacity of 2.4 tonnes
- Ballroom floor loading of 408kg per square metre
- ISDN lines
- Vehicle access, ideal for motor vehicle launches
- Internet access

## Legend

- ▲ 10 AMP 240 volt GPO
- ▲ Telephone extension
- ▲ ISDN, TEL, FM
- Microphone input socket
- MATV socket
- House light dimmer
- ⊗ Wilco 3 Phase 20 AMP outlet
- ▲ 20 AMP ceiling outlet through patch panel
- Mic Patch Point
- Stage lighting remote control
- ▲ 15 AMP 240 volt GPO
- ▲ 15 AMP 240 volt outlet through
- Stage lighting remote control
- Curtain control
- ISO port, ISO node
- Speaker reassessed in ceiling
- Multiple line input
- Patch from control room for audio
- Aux inputs for audio equipment



## **General Information**

Perth is the capital of Western Australia, Australia's largest state. Western Australia is a place of enormous contrast — lush grape growing areas and wineries, vast deserts, rugged ranges and giant karri forests. The diversity of the scenery and attractions represents an excellent opportunity for pre- and post-conference touring during your stay in the West.

The city of Perth is set along the beautiful Swan River, just a few kilometres from the ocean. The metropolitan area extends from the hills in the east to the vibrant breezy port of Fremantle to the south. Perth is on the same time zone as Singapore and Hong Kong, and in September is two hours behind Sydney, Melbourne and Brisbane. During September, average daily temperatures are mild and pleasant and range from 10 to 20 degrees Celsius.

## **The Venue**

The Congress will be held at the Hyatt Regency in the city of Perth. The venue is five star and within easy walking distance of Perth's business, commercial and entertainment districts. Excellent public transport services are available to the door of the hotel and very regular bus services link the city with Kings Park.

## **Registration**

The Registration Desk, located in the South Foyer of the Hyatt Regency Perth, will be open at the following times:

Sunday 23 <sup>rd</sup> September	1500 - 1730
Monday 24 <sup>th</sup> September	0730 - 1700
Tuesday 25 <sup>th</sup> September	0730 - 1700
Wednesday 26 <sup>th</sup> September	CLOSED
Thursday 27 <sup>th</sup> September	0730 - 1700
Friday 28 <sup>th</sup> September	0730 - 1600

## **Admission**

Admission to all congress sessions, lunches, morning and afternoon teas and social functions is by name badge only. You are therefore requested to wear your name badge at all times.

## **Lunches, Morning and Afternoon Teas**

Lunches, morning and afternoon teas are included as specified for the different registration categories. Lunch will be served daily in Ballroom North and morning/afternoon tea will be served in the poster display area – South Foyer.

## **Congress Sessions**

All plenary congress sessions will be presented in the South Ballroom, and concurrent sessions will be held in the Freshwater Bay Room.

## **Photo Session**

**Tuesday 25<sup>th</sup>, 3:20-3:40 pm in the Hotel Lobby**

## **Poster Presentations**

Poster presentations will be displayed from Monday 24<sup>th</sup> September to Friday 27<sup>th</sup> September inclusive. All Posters may be viewed in the South Foyer during morning and afternoon tea.

**Note: All poster authors are requested to be present on Tuesday 25 September, from 5:00 – 5:45 pm.**

## **Smoking Policy**

The Congress has a No Smoking policy for the well being of all its participants. Government legislation requires all public buildings to be smoke free. Persons may smoke in their hotel room on the proviso they have specifically booked a “smoking room”.

## **Parking**

Delegates are responsible for their own parking expenses. The daily rate in the Wilson Car Park (located under the Hyatt Regency Hotel) is \$6.00 for delegates of the Orchid Congress. In order to obtain this special Congress rate please ask for a parking voucher from the Congress Registration Desk prior to leaving each day.

## **Public Transport**

The Hyatt Regency Perth is located on a main thoroughfare for public transport. There is an excellent bus service along Adelaide Terrace, and the CAT (Central Area Transport) bus which runs free of charge around the city centre.

If you would like further information on services and schedules, please call Transperth's *Infoline* on 13 62 13

## **Mobile Phone Policy**

Mobile phones **must be switched off** in the Conference rooms whilst sessions are in progress.

## **Messages**

All messages for delegates will be posted on the message board near the Registration Desk. Delegates are requested to check the board regularly. Messages for delegates may be phoned through to the secretariat on Tel: +61 8 9225 1711 or Fax: +61 8 9225 1712.



## Social Functions

### Welcome Cocktail Party –Kings Park Wildflower Festival

Come and celebrate the opening of The First International Orchid Conservation Congress, in the Orchid Pavilion. Meet old friends and new colleagues while viewing the array of orchids on display.

Date: Sunday 23<sup>rd</sup> September  
Time: 5.30pm – 7.30pm  
Cost: Included for full delegates  
Extra tickets are available at \$45.00

All delegates will need to make their own way to Kings Park Botanic Garden which is a short stroll from the city centre. On arrival please present your name badge for entry into the Festival (a map of the area is available in your congress backpack). At the conclusion of the function (7.30 pm), a coach will be available to take delegates to the Hyatt Hotel.

**About Kings Park Botanic Garden:** Located in the heart of Perth, the capital city of Western Australia, Kings Park and Botanic Garden covers an impressive 400 ha with over two thirds still bushland. For such an urbanised, natural area within five minutes of the central business district it is therefore surprising that 52 orchid species and varieties still grow naturally in many areas — with most of these species in full bloom during the congress time (spring). Within 10 minutes of the congress hotel, it is possible to be wandering through beautiful and extensive displays of wild cowslip orchids (*Caladenia flava*), donkey orchids (*Diuris magnifica*) and spider orchids (*Caladenia*).

Kings Park is also the State's botanic garden, which is free and open 24 hours a day, every day of the year. The botanic garden comprises sweeping landscapes and vistas with planted displays of some of the 10,800 species native to Western Australia. Noteworthy is the *Banksia* garden which represents the largest public collection of this iconic group of Australian species and the award-winning water garden which meanders through landscaped gardens of Western Australian wetland plants.

Kings Park also offers over 50km of walking and nature trails and extensive jogging areas — or just quiet spots for relaxation after a hard day at the congress!

### Congress Dinner – Hyatt Regency Hotel – Terrace Ballroom

Date: Friday 28<sup>th</sup> September 2001  
Time: 7.30pm  
Dress: Formal – Lounge suits  
Cost: Included for full delegates  
Extra tickets are available at \$95.00.

The dinner has some special treats in store so we hope to see you there!

## Mid Week Day Trip

Date: Wednesday 26 September 2001  
Coach Departs: 8.30am  
Departure Point: Bus zone on Adelaide Terrace just in front of the lower lobby of the Hyatt Regency Hotel.

Delegates attending the mid-week day trip will visit some of the most species-rich orchid habitats on the west coast of Western Australia. The tour will include sites of special scientific interest and a range of habitats from swamps to granite rocks.

Delegates who have booked to take this tour will need to assemble in the Hyatt Hotel foyer at 8.20am on Wednesday 26 September 2001. On boarding the coach you will receive a tour booklet outlining the days activities and points of interest.

*Tip: Wear sturdy footwear and old clothing as you will be going through some rough and burnt terrain to see the orchids. The weather is likely to be fine and warm at this time of the year, however if it looks like raining please bring a raincoat or umbrella. A water drink bottle is also advised. Lunch is provided.*

## Post-Congress Tour – W.A. Orchids

Departure Date: Saturday 29 September 2001  
Departure Time: 8.30am  
Departure Point: Bus zone on Adelaide Terrace just in front of the lower lobby of the Hyatt Regency Hotel  
Return Date: Wednesday 3 October 2001

The Post Congress Tour of the International Orchid Conservation Congress has been designed to introduce you to the unique and diverse orchid flora of the southwest of Western Australia. Some 60-100 species will be seen over 5 days in a range of habitats and geographical regions in both high and low rainfall areas. Of course, the number of species that flower in 2001 will be influenced by weather and fires. Both common and rare species will be seen and the reasons for the threatened status of our most endangered species explained first hand. Some 32 species of orchids from this region are currently listed as threatened and some are subject to recovery programs co-ordinated by the WA Department of Conservation and Land Management's Nature Conservation Division (Western Australian Threatened Species and Communities Unit) and the Division of Plant Science of the Botanic Gardens and Parks Authority.

Delegates who are booked on the Post Congress tour will need to assemble in the Hyatt Hotel's foyer on Saturday 29 September 2001 at 8:15am. All luggage will be loaded on the coach and the tour will officially depart at 8:30am. Latecomers will be left behind!

*Tip: Bring sturdy foot-ware and long pants as we may be walking through thick prickly scrub and occasional burnt swamps that may still be wet. Remember to bring a hat and sun-screen to protect yourself from the sun which will often be strong at this time of year. Spring weather in WA is variable, so be prepared for both cool and warm days. Also, come prepared for occasional showers with a raincoat or umbrella. It is especially important to bring lots of film or recording media for your camera, as you will probably take many more images than you expect due to the magnificent scenery, the spectacular wildflowers and, of course, the beautiful orchids you will see.*



## First International Orchid Conservation Congress - Session Layout

Start times are given in [square] brackets following the presentation title.

This timetable was correct as at 18 September 2001, however given the current circumstances, some changes can be anticipated. We apologies for any inconvenience this may cause.

### Monday A

1. Introduction and Launch. [9:00]
2. **Peter Wyse Jackson**: Plant conservation - an international overview. [9:30]
3. **Phillip Cribb**: Orchid conservation at the cross-roads. [10:00]

### Monday B

1. **Mark W. Chase**, John Freudenstein & Kenneth Cameron: DNA data and Orchidaceae systematics: a new phylogenetic classification. [11:00]
2. **Alec Pridgeon**: Modern species concepts and practical considerations for conservation of Orchidaceae. [11:30]
3. **Stephen Hopper**, David L. Jones & Andrew P. Brown: Australian Orchid Conservation. [12:00]

### Monday C

Case studies and assessment of conservation priorities. Chairs: **Shelagh Kell** and **Kingsley Dixon**

1. **Wendy Strahm**: Orchids and Red Lists: identifying species in need of conservation action. [1:30]
2. **Yam Tim Wing** & Aung Thame: Orchid conservation at the Singapore Botanic Gardens. [2:00]
3. **Datuk Lamri Ali**: TBA. [2:20]
4. **Weerachai Nanakorn**: Status of Thai native orchids. [2:40]

### Monday D

Threatening processes. Chair: **Harold Koopowitz**

1. **Harold Koopowitz** & P.S. Lavarack: The nature of threats to orchid conservation. [3:30]
2. **David Jones**: Threatening processes in Eastern Australia. [4:00]
3. **Carlos Lehnebach** & M. Riveros: Chilean orchids: What do we know about them? [4:20]
4. **RC Srivastava**: The depleting orchid flora of Sikkim and strategies for conservation. [4:40]

### Tuesday Stream 1: Session A

Recovery (1): *Ex situ* strategies in orchid conservation. Chair: **Margaret Ramsay**.

1. **Margaret Ramsay**: The role of *ex situ* techniques in re-establishment of terrestrial orchids in the United Kingdom. [9:00]
2. **Nika Debeljak**: Tuber production as a method for improving conservation of terrestrial orchids. [9:30]
3. Colin Night, **Elizabeth James** & S. Akiyama: Reversing the decline of *Diuris fragrantissima*. [9:55]

### Tuesday Stream 1: Session B

Recovery (2): Recovery of plants to sites Chair: **Kingsley Dixon & Hanne Rasmussen**

1. **Hanne Rasmussen**: Substrate requirements for recruitment of orchid seedlings. [11:00]
2. **Ruth Raleigh**, R.G. Cross, A.C. Lawrie, F. Coates & A.C.A. Moorrees: Research into the propagation of eastern Australian *Caladenia*. [11:30]
3. Andrew Govanstone, John Hill, **Andrew Pritchard**: Mellblom's Spider-orchid *Caladenia hastata* - Managing the *in situ* recovery of a Critically Endangered Spider Orchid. [11:50]
4. **Andrew Batty**, K.W. Dixon, M. Brundrett, K. Sivasithamparam: Improving the success of translocation of terrestrial orchids. [12:10]

**Lunch: Matthew Buckels**: The issues and impacts of global warming. [12:30]

### Tuesday Stream 1: Session C

Mycorrhiza for conservation (1). Chairs: **Finn Rasmussen** and **Lawrence Zettler**

1. **Leka Manoch** & Kanungnid Busarakam: Biodiversity and distribution of endophytic, pathogenic and rhizosphere fungi from terrestrial orchids in Thailand. [1:30]
2. **Pornpimon Athipunyakom** & Leka Manoch: Diversity of orchid mycorrhiza in Thailand. [1:50]
3. **Penelope Hollick**, J.A. McComb & K.W. Dixon: Natural hybrids as a means for investigating specificity of orchid mycorrhizal fungi. [2:10]
4. **Magali Wright**, David Guest & Rob Cross: The development of mycorrhizal infection in *Caladenia tentaculata*. [2:30]

**Delegates Photo.** [3:20]

### Tuesday Stream 1: Session D

Mycorrhiza for conservation (2). Chairs: **Finn Rasmussen**, **Lawrence Zettler** and **Mark Brundrett**

1. **Mark Brundrett**: Do orchids have true mycorrhizal fungi? [3:40]
2. **Tien Huynh**, C.B. McLean & A. Lawrie: Seasonal observations of the endangered terrestrial spider orchid, *Caladenia formosa* G. W. Carr. [4:00]
3. **Rodrigo Reinoso**, J. Becerra, N. Garido & M. Silva: Determination of new endomycorrhizal fungi from two Chilean orchids, *Chloraea crispa* and *C. gaviu* (Orchidaceae). [4:20]
4. Ann Lawrie, **T. Huynh**, R.E. Raleigh, **C.B. McLean**, R.G. Cross, F. Coates & A.C.A. Moorrees: Molecular biology of mycorrhizal fungi from Australian terrestrial orchids. [4:40]

## Tuesday Stream 2: Session A

Population biology (1) Chairs: **Jo Willems** and **Dennis Whigham**

1. **Jo Willems** & D.F. Whigham: The role of long-term, individual based demographic studies in the conservation of terrestrial orchids. [9:00]
2. **Tiiu Kull**, K. Tali & T. Tuuliki: Population dynamics of north temperate orchid species. [9:30]
3. **Fiona Coates** & I. Lunt: Patterns of appearance and transition to flowering in *Prasophyllum correctum* D.L. Jones, a threatened orchid from south eastern Australia. [9:50]
4. Jana Jersakova & **Pavel Kindlmann**: Factors affecting fruit set in *Orchis morio*. [10:10]

## Tuesday Stream 2: Session B

Population biology (2) Chairs: **Raymond Tremblay** and **Michael Hutchings**

1. **Dennis Whigham** & Jo Willems: Population studies of terrestrial orchids – habitat management and annual counts of aboveground plants are only part of the picture. [11:00]
2. **Michael Hutchings**: TBA. [11:30]
3. **Raymond Tremblay** & Michael Hutchings: Population dynamics in orchid conservation: A review of analytical methods based on the rare species *Lepanthes eltoroensis*. [11:50]

**Lunch: Matthew Buckels**: The issues and impacts of global warming. [12:30]

## Tuesday Stream 2: Session C

Managing the trade in wild orchids. Chair: **Robert R. Gabel**

1. **Robert R. Gabel**: The impact of international trade on orchids. [1:30]
2. **Geoff Stocker**: CITES – Conservation showpiece or opportunity lost. [2:00]
3. **Gloria L.P. Siu**: TBA. [2:20]
4. **Jin Xiaohua**, Qin Haining & Gloria, Siu Laiping: Traditional usage of orchids in China. [2:40]

## Thursday: Session A

Orchid Specialist Group (OSG) Meeting. Chairs: **Phillip Cribb & Shelagh Kell**

1. Introduction: **Phillip Cribb and Shelagh Kell** [8:30]
2. OSG strategic plan: **Phillip Cribb**
3. Secretariat report: **Shelagh Kell**

*Regional group reports:*

4. Afro-Madagascar: **George Mugambi**
5. Australasia: **Kingsley Dixon**
6. East Asia: **Gloria L.P. Siu**
7. Indian Subcontinent: **Udai Pradhan**
8. Meso America: **J Warner**
9. North America: **Marilyn Light**
10. South America: **Alex Hirtz**
11. Europe: **Phillip Cribb**

## Thursday: Session B

OSG Regional workshops. Chairs: **Regional Chairs**

1. *Ex situ* conservation group report: **Phil Seaton** [11:00]
2. Use of Revised Red List Criteria, RAMAS Red List and SIS: **Wendy Strahm**
3. Any other business
4. **Date and venue of next meeting:** 17<sup>th</sup> WOC, Shah Alam, Malaysia.

## Thursday: Session C

Conservation genetics (1) Diversity and priorities. Chairs: **Mike Fay, Seigy Krauss and Walter Rossi**

1. **Walter Rossi**, M. Cristina Mosco & Luciano Bullini: Genetic erosion in Italian populations of threatened orchids: *Cypripedium calceolus*, *Orchis palustris* and *Dactylorhiza incarnata*. [1:30]
2. **Faridah Quamaruz-Zaman**, Michael Fay, John Parker & Mark Chase: Conservation genetics of rare and endangered British Orchids. [2:00]
3. Peter Hollingsworth, **Richard Bateman** *et al.*: Population genetic structure of species of *Epipactis*. [2:20]
4. **Richard Bateman** & Peter Hollingsworth: Species delimitation and conservation prioritisation: operating at the boundary between phylogenetics and conservation genetics. [2:40]

## Thursday: Session D

Conservation genetics (2) Recovery and sampling strategies. Chairs: **Seigy Krauss, Mike Fay and Walter Rossi**

1. **Siegfried L. Krauss** & Robyn Taylor: Restoration genetics of *Caladenia* in urban bushland remnants in Perth, Western Australia. [3:30]
2. **Michael F. Fay**, Mark W. Chase, Robyn S. Cowan & Mikael Hedrén: Orchid conservation genetics - How many data do we need? [4:00]
3. **Rod Peakall**, D. Ebert, J. Mant & F. Schiestl: Application of genetic tools for the conservation of orchids: premise and pitfalls. [4:25]

## Friday Stream 1: Session A

Case studies in orchid conservation. Chairs: **Shelagh Kell** and **Kingsley Dixon**

1. **Denise Wilson**: Orchid conservation efforts in Costa Rica. [9:00]
2. **John Sawyer** & Peter de Lange: Biogeography and orchid conservation in New Zealand: Case studies from the Department of Conservation orchid files. [9:30]
3. **Gary Backhouse**, Fiona Coates & James Todd: An overview of the conservation status of the orchids of Victoria. [9:50]
4. **Rusea Go** & A. Julaihi: Diversity and conservation of limestone orchids in Sarawak, Malaysia. [10:10]

## Friday Stream 1: Session B

Role of orchid societies and growers in orchid conservation. Chair: **Henry Oakeley**

1. **Henry Oakeley**: The importance of the National Plant Collection schemes in orchid conservation. [11:00]
2. **Alan Dash**: The role of the Australian Native Orchid Society Inc. in orchid conservation. [11:30]
3. **Chitrapan Piluek**, Pramote Triboon, Chukiat Tapsan and Derake Tonpayom: Wild orchid conservation for ecotourism in Thailand. [11:50]
4. **Helen Richards**: The Australian Orchid Foundation and conservation. [12:10]

## Friday Stream 1: Session C

Taxonomy for conservation. Chairs: **Mark Clements** and **Ed de Vogel**

1. **Mark Clements**: Systematics and its implications for orchid conservation. [1:30]
2. **Ed de Vogel** & André Schuiteman: The project "Orchids of Southeast Asia" on CD-ROM. [1:50]
3. **Stephen Hopper**: Overcoming the taxonomic impediment: A case study in south-west Western Australia. [2:10]

## Friday: Session D

Conclusions and recommendations. Chair: **Stephen Hopper** [3:00]



## Friday Stream 2: Session A

### Pollination biology (1) Chair: **David Roberts**

1. **David Roberts** & Chris C. Wilcock: Levels of fruiting success in the Mascarene Islands orchid flora: implications for conservation. [9:00]
2. **Pati Vitt**: The effects of hand-pollination on growth, survival and reproduction in *Platanthera leucophaeta*, the eastern prairie fringed orchid. [9:30]
3. **Carlos Lehnebach**, A.W. Robertson: Pollination ecology of the genus *Earina* Lindl. in New Zealand. [9:55]

## Friday Stream 2: Session B

### Pollination biology (2) Chair: **Mark Chase**

1. **Topa Petit** & Doug Bickerton: Preliminary investigation on the pollination, ecology and conservation of the endangered *Caladenia behrii* in South Australia. [11:00]
2. Florian Schiestl, **Colin Bower** *et al.*: Chemical communication in sexually deceptive orchids – implications for evolution and conservation. [11:20]
3. **Colin Bower**: Specific pollinators and Australian sexually deceptive orchids: General principles and implications for conserving their biodiversity. [11:40]
4. **Jim Mant**: Pollination of *Chiloglottis* by sexual deception of Thynnine wasps: ecosystem interactions and implications for conservation. [12:00]

### ***Poster Presentations:***

**Nura Abdul Karim, K. Sivasithamparam & K.W. Dixon:** Pectic zymogram analysis of orchid fungal endophytes.

**Ruy José Válka Alves, Regina Braga de Moura & Luiza Carla Trindade de Gusmão:** Herbarium orchid database – Museu Nacional, Rio de Janeiro.

**Ruy José Válka Alves:** Orchids as agents of primary succession on rocks in Brazil.

**Georgia Basist, A.C. Lawrie, R.E. Raleigh & R.G. Cross:** Identification of threatened *Caladenia* species (Orchidaceae) using traditional and molecular techniques to aid in their conservation.

**M.A. Cisternas & Carlos Lehnebach:** Pollination studies on *Bipinnula fimbriata* (Poep.) Johnst. in Central Chile.

**Stephen Clarke:** Recovery planning for three endangered orchid species in SE New South Wales - Crimson Spider orchid (*Caladenia concolor*), Tarengo Leek orchid (*Prasophyllum petilum*) and Rhyolite Midge orchid (*Genoplesium rhyoliticum*)

**Alan Dash, Ruth Rudkin, Peter Eigelshoven & Russell Wales:** Australasian Native Orchids Society Inc.: The role of ANOS groups in New South Wales in orchid conservation. [X 3]

**Trevor J. Edwards, S. Piper, & D.I. Thompson:** Conservation threats and the current status of South African terrestrial orchids with respect to *in vitro* germination.

**Karina Fitzgerald & D.L. Jones:** Endangered *Prasophyllum* R.Br. in south-eastern Australia.

**Elizabeth A. Fraser:** *Thelymitra matthewsii* – A specialist orchid maintaining a precarious ‘toe-hold’ in New Zealand’s far north?

**Wesley Higgins:** Conservation through propagation.

**Jana Jersakova & Pavel Kindlmann:** Orchid population dynamics under human influence.

**Saleh B. Kadzimin & Philip Sipe:** Cryopreservation of protocorm-like bodies of *Dendrobium* by vitrification technique.

**Susana Luna-Rosales, P. Ortega-Larrocea, V. Chávez, A. Barba-Alvarez:** *In vitro* symbiotic germination of *Bletia urbana*.

**Barabara Rodríguez Marcano, Raymond Tremblay, Elvia Meléndez-Ackerman, Carla Cortés & Owen McMillan:** Paternity analysis and gene flow in a lithophytic orchid using AFLP.

**Sarat Chandra Misra:** The orchid flora of Orissa: status and strategies for conservation.

**George K. Mugambi:** Ensuring survival of Kenyan orchids: the *ex situ* conservation interventions.

**Sofi Mursidawati:** Conservation biology of an achlorophyllous orchid: A case study on the underground orchid (*Rhizanthella gardneri*).

**Leticia Quay et al.:** Is there a one-to-one specificity between *Diuris* species and mycorrhizal fungi?

**LV Semerenko & IV Shvets:** The genus *Dactylorhiza* in the flora of Belarus.

**Ish Sharma, D.L. Jones, M.A. Clements & C.J. French:** Unusually high genetic variability revealed through allozyme polymorphism of eastern (*Pterostylis gibbosa*) and Western Australian (*P. aff. picta*) endangered orchid species.

**Dave I. Thompson, Trevor J. Edwards and Johannes van Staden:** Conquering coat-imposed seed dormancy in the South African *Disa* (Orchidaceae): old ideas and new techniques.

**David Wilyams:** Micropropagation of jarrah forest plant species at Marrinup Nursery (ALCOA World Alumina-Australia).

**Jin Xiaohua and Qin Haining:** The orchids of the Northwest Yunnan, China.

***Abstracts in absentia:***

**Leonid V. Averyanov:** Analysis of orchid endemism in eastern Indochina.

**Lorena Endara:** Pollination ecology of four *Dracula* species growing sympatrically in northwestern Ecuador.

**Lorena Endara, Renato Valencia & Susana León-Yáñez:** Conservation status of endemic Ecuadorian orchids.

**Mohammed Kamrul Huda et al.:** Colonisation and diversity of epiphytic orchids on trees in disturbed forests.

**Udai Pradhan:** Achlorophyllous orchids of the Indian Region and their conservation needs.

**Irina V. Tatarenko:** Population studies in the epiphytic orchid *Sarcochilus japonicus*.

## Plant conservation – an international overview.

Peter S. Wyse Jackson

Botanic Gardens Conservation International, Descanso House, 199 Kew Road, Richmond, Surrey, TW9 3BW, U.K. Email: pwj@bgci.rbgekew.org.uk

The paper presents an overview of contemporary efforts being made throughout the world for plant conservation, including a recent initiatives to establish a Global Strategy for Plant Conservation through the Convention on Biological Diversity.

It is pointed out that over the last decade the basis for and practice of plant conservation has broadened considerably. New efforts are being made to develop measures to complement *in situ* conservation, including habitat recovery, plant introductions and restitutions, ex situ conservation, supported by new and extensive research in many aspects of plant conservation biology and complementary educational and public awareness programs. Such integrated conservation measures now form an intrinsic part of most conservation policies and programs.

Plants are universally recognised as a vital part of the world's natural heritage and an essential resource for the planet. Plant species are a key component of global sustainability, but despite this, tens of thousands of plants are currently threatened and despite best efforts, this total is likely to rise over the coming century.

Worldwide something in the region of 270,000 plants have been described, of the estimated total of 320,000 species that probably exist. Of these plants, the majority are flowering plants, as well as very significant numbers of bryophytes, ferns, conifers and other plant groups. Good information is available on plant taxonomy and distribution, but less than 20% of the world's plants have had their conservation status comprehensively assessed. The 2000 IUCN Red List of Threatened Species records 5,611 species as threatened. The previous IUCN Red List of Threatened Plants of 1997 included 33,798 threatened plants, to which the old Red List Categories had been applied. This figure represented c.12.5% of the world's vascular flora.

Plants are endangered by a combination of factors; population growth causes ever-increasing pressure on the world's remaining natural areas, with unsustainable agriculture and forestry practices contributing to considerable ongoing losses in plant diversity. Other factors include over-collecting and unsustainable exploitation of plant resources, urbanisation, pollution, agricultural intensification and land use changes, the spread of invasive alien plants, animals and pathogens all too contribute to this crisis for plants. The impact on plants of global warming, and climate change in general, still cannot be quantified adequately but must surely be increasingly significant.

Major efforts throughout the world are being undertaken by international agencies and national governments, supported by a great diversity of institutions, organizations and other groups at all levels to develop practical actions to safeguard plant diversity.

The presentation described the current efforts being made by a range of organisations to create a *Global Strategy for Plant Conservation* through the Convention on Biological Diversity, as well as complementary initiatives, such as *The International Agenda for Botanic Gardens in Conservation*, that can contribute to such as strategy. The ultimate aim of the Global Strategy will be to halt the current and continuing loss of plant diversity by providing a framework for existing initiatives and facilitate harmony between them; to identify gaps and mobilise the resources needed for the achievement of the goals of the Strategy. The strategy will also provide a framework for actions at global, regional, national and local levels. A global dimension to the strategy is important because it can facilitate the development of a global consensus of key objectives, targets and actions and enhance collaboration at all levels.

The major elements of the proposed strategy are:

- Understanding and Documenting Plant Diversity
- Conserving Plant Diversity
- Using Plant Diversity Sustainably
- Promoting Education and Awareness about Plant Diversity
- Building Capacity for the conservation of Plant Diversity

The development of targets for plant conservation has been a key part of this process. Outcome orientated targets have been proposed, to be implemented by 2010. These types of proposed targets have been increasingly used in recent years to provide a framework for actions by multiple actors. The best known example is the set of International Development Goals which has been adopted by most countries, both donors and developing countries, as well as by relevant international institutions. They are drawn from the goals adopted by a series of UN conferences in the 1990s.

The Strategy is not intended to be a “program of work” analogous to existing thematic and cross-cutting programs of work under the Convention. It does not, therefore, contain detailed activities, expected outputs etc. Rather, the strategy provides a framework by means of setting outcome-orientated targets

The Strategy, and its 14 targets, are intended to provide a framework for policy makers and public opinion and catalyse the reforms necessary to achieve plant conservation. Clear, stable, long-term targets that are adopted by the international community can help shape expectations and create the conditions where all actors, whether governments, the private sector, or civil society, have the confidence to develop solutions to address threats to plant diversity. For the targets to be widely understood, and appealing to public opinion, they need to be kept fairly simple and straightforward. They should be understood in a common sense rather than a literal way. In order that the number of targets be kept manageable, they need to focus on a set of activities that are strategic, rather than aiming to be comprehensive.

It is hoped that the CBD will endorse the Strategy in April next year and invite Parties, Governments and relevant organisations to adopt these targets, and, as appropriate, to incorporate them into relevant plans, programs and initiatives, including national biodiversity strategies and action plans, in order to promote a common effort towards halting the loss of plant diversity.



## 1. Orchid conservation at the cross-roads.

Phillip Cribb

Deputy Keeper of the Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AE, UK. Email: p.cribb@rbgkew.org.uk

Orchids are not only one of the most diverse and most widespread families of flowering plants but one of the few plant families that have a profile that can benefit plant conservation on a broad scale. In short, orchids are sexy. Orchid conservation, however, is at a crossroads. We understand more about the distribution, rarity, threats and extinction of orchids than ever before, and we have the scientific tools to address many of the problems; yet not everyone is convinced that there are problems or that, if they exist, there are solutions. Very positive and exciting progress is being made on many fronts. Some of the lessons learned by successful projects need to be more widely disseminated and can be useful on a broader front. Some of the successes will be discussed. The IUCN/SSC Orchid Specialist Group has a vital role to play in catalysing orchid conservation by promoting effective orchid conservation, in educating the orchid community and in liaison with governmental and international conservation organisations.

## **1. DNA data and Orchidaceae systematics: a new phylogenetic classification.**

Mark W. Chase<sup>1</sup>, John V. Freudenstein<sup>2</sup> and Kenneth M. Cameron<sup>3</sup>

1. Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom. Email: m.chase@rbgkew.org.uk

2. Ohio State University Herbarium, 1315 Kinnear Road, Columbus, Ohio 43212-1157, USA

3. The Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies, The New York Botanical Garden, Bronx, New York 10458-5126, USA

Orchidaceae are rapidly becoming one of the best-studied families of the angiosperms in terms of infra-familial phylogenetic relationships. These studies demonstrate that several previous ideas about phylogenetic patterns were incorrect, which make all previous classifications out of date. Therefore, in this paper we describe the emerging patterns and propose a new phylogenetic classification of Orchidaceae that accords with these newly discovered relationships. We recognise five subfamilies: Apostasioideae, Vanilloideae, Cypridioideae, Orchidoideae and Epidendroideae, the last containing the bulk of the taxa in the family. Apostasioideae are sister to all the rest, followed successively by Vanilloideae, Cypridioideae and the remainder of the monandrous orchids, Orchidoideae and Epidendroideae. Although only an interim classification, it should help to focus other areas of orchid research and stimulate the creation of new hypotheses that will direct orchid researchers to new questions.

## 2. Modern species concepts and practical considerations for conservation of Orchidaceae.

Alec M. Pridgeon

Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, U.K. Email: a.pridgeon@rbgkew.org.uk

In descriptions of new orchid species, monographs, and floras, authors rarely mention which of the 22 or so published species concepts they have applied in circumscribing taxa or compiling checklists. The philosophical underpinnings of how we think of a species – as naturally organisms interacting with their environment as well as a class, the end point of speciation, with defining characteristics – still provoke debate among biologists about what theoretical and practical criteria should be used in recognising species and determining their boundaries.

Every species concept (e.g. Biological, Morphological, Evolutionary, Hennigian, Phylogenetic, Genealogical) has its proponents and critics, but two fundamental questions can and should be asked about each: 1) Which (if any) theoretical considerations should be included in the definition of a species? and 2) How can we make the concept operational for fieldwork and conservation management without sacrificing theoretical substance and scientific rigor? Whether we should admit information about processes such as reproductive isolation into our definition depends on how readily we can assess their effects, a difficult if not impossible proposition in allopatric populations or in the tropical canopy. Whether we insist that species themselves be monophyletic depends on whether we consider the population or the individual organism the unit of study. Finally, if our species concept is character-based, must the diagnostic characters be autapomorphies or can they simply be unique combinations of character states?

The exigencies created by habitat destruction, global warming, mass collecting, and ultimately human overpopulation compel us to get into the field with a phylogenetic but character-based concept, train others to join us, and thereby document biological diversity while it still exists. Only after we have a full inventory of life can we afford the luxury of debating the more theoretical considerations of what a given species is – or was.

### 3. Australian orchid conservation.

Stephen D. Hopper<sup>1</sup>, David L. Jones<sup>2</sup> and Andrew P. Brown<sup>3</sup>

<sup>1</sup>Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, West Perth WA 6005, AUSTRALIA.  
Email: steveh@kpbg.wa.gov.au

<sup>2</sup>Centre for Plant Biodiversity Research, PO Box 1600 Canberra ACT 2601, AUSTRALIA

<sup>3</sup>Department of Conservation and Land Management, Western Australian Threatened Species and Communities Unit, PO Box 51 Wanneroo WA 6065, AUSTRALIA

Because of their wide public appeal, Australian orchids have been among the first native plants to receive conservation attention. For example, in the early 1900s, prohibition of picking orchids close to Perth and on main road verges was specified in the Native Flora Protection Act. However, it would be many decades before State legislation was introduced protecting the habitat of orchids through establishing conservation reserves, and only over the past decade has Federal legislation been proclaimed to back up that in most States and Territories protecting threatened species.

Effective conservation of Australian orchids is critically dependent on a sound taxonomic understanding of the composition of the flora, of orchid distribution and biology, and then local, regional and national conservation organisations and individuals committed to conserve this remarkable heritage.

A significant taxonomic impediment has existed until recently, when a growing body of enthusiasts and botanists have pooled resources to document the orchid flora using the latest technologies and approaches, including molecular systematics and modern field travel and search methodologies. Currently about 1300 taxa are recognised in Australia, of which one sixth are epiphytes and the remaining five-sixths terrestrial. At the national level in 2001, ca. 170 are listed as threatened, including six species presumed extinct, 100 endangered and 60 vulnerable. Many of these taxa occur outside existing national parks and nature reserves. Some have been secured in new conservation reserves. Others will remain on land used for different purposes, and require broad community attention and support to avert extinction.

Recovery programs for a number of these threatened species have been developed, providing fine examples of cooperation among interested individual landowners, community groups, scientists and government agencies. In a few cases, such cooperation has yet to materialise, and urgent off site conservation action is needed as an interim insurance measure. We review major issues and trends in orchid conservation for Australia, and offer suggestions for future strategic action.

## **1. Orchids and Red Lists: identifying species in need of conservation action.**

Wendy Strahm

IUCN Plants Officer, Species Survival Programme, Rue Mauverney, 28, CH-1196 Gland, Switzerland, Email: was@iucn.org

For more than three decades IUCN Red Lists and Red Data Books have been produced by the Species Survival Commission (SSC) of IUCN-the World Conservation Union. These documents serve to identify species in need of conservation action, and give an evaluation of the global conservation status of biodiversity. In recent years an ambitious Red List Programme has been developed in order to ensure that the IUCN Red List is scientifically rigorous, and provides an objective list of threatened species. New IUCN Red List Categories and Criteria, based on extinction risks, were prepared in 1994 and revised in 2000 to ensure that conservation assessments are made in an accurate, transparent and defensible manner. This paper gives an overview of the Red List Programme and outlines the revisions that have been made to the IUCN Red List Categories and Criteria. In addition, two tools to help researchers assess species (an expert system called "RAMAS® Red List", and the Species Information Service), are discussed. Although orchids are the largest family of flowering plants and many species are at risk of extinction, they are under-represented in Red Data books and lists for a variety of reasons. This paper demonstrates how the Red List Programme as well as the Species Information Service will help in prioritising species in need of conservation action, as well as facilitate the exchange of information to support natural resource management and policy formulation.



## 2. Orchid conservation at the Singapore Botanic Gardens.

Yam Tim Wing\* & Aung Thame

Singapore Botanic Gardens, 1 Cluny Road, Singapore 259569. Email: yam\_tim\_wing@nparks.gov.sg

Singapore is centrally located in South East Asia, 1° north of the equator. The Republic of Singapore consists of one main island and 49 small islands. The total land area is 647.5 km<sup>2</sup>. It has a typical equatorial climate. The average maximum temperature is about 31°C and the average minimum is 24°C. Average daily temperature is around 27°C. Relative humidity varies from around 70% in the day to about 100% at night, but the average is 85%. The Island also benefits from having a high rainfall of about 2400 mm per year. This equatorial climate provides ideal conditions for many orchids. More than 180 orchid species represented by some 60 genera have been recorded in Singapore. This is considered quite a large number for such a small area.

About 75% of the country's orchids are epiphytes and the rest are terrestrials. We have many interesting species among the native orchids, including, one of the longest growing ones (the climber, *Vanilla griffithii*); the largest orchid in the world (*Grammatophyllum speciosum*); one "leafless" genus (*Taeniophyllum*) and several "Jewels" (*Anoectochilus*, *Nephelaphyllum*, etc).

Owing to habitat destruction and over-collection, orchid species are seriously endangered in Singapore. In view of this, a program was started to conserve the native orchids of Singapore and those of nearby regions. This paper reports some of the achievements of the conservation program. These include 1) the artificial propagation and reintroduction of *Grammatophyllum speciosum*, the tiger orchid, to its natural habitat, and 2) the production of superior, horticulturally desirable species through breeding.

### **3. TBA.**

**Datuk Lamri Ali**

The Board Of Trustees Of The Sabah Parks, PO Box 10626, Kota Kinabalu, Sabah, 88806, MALAYSIA.

Email: [sabahparks@sabah.gov.my](mailto:sabahparks@sabah.gov.my)

[Abstract not received]

#### 4. Status of Thai native orchids.

Weerachai Nanakorn

Queen Sirikit Botanic Garden, The Prime Minister Office, Maerim, Chiang Mai, 50180, THAILAND. Email: weerach@chmaiz.loxinfo.co.th

In Thailand, Orchids are found in all different habitats ranging from hill evergreen forest at 2,565 m. in the north to sea level in the peninsular. The Orchidaceae is the largest plant family represented by 170 genera and 1,230 species and among these 150 species are considered endemic to the country. Major genera are *Dendrobium* (153), *Bulbophyllum* (145), *Eria* (63), *Habenaria* (37), *Oberonia* (36), *Liparis* (31), *Coelogyne* (27) and *Cleisostoma* (25) etc. For their habit 65% are epiphytic, 35% are terrestrial and only few species in the genera *Aphyllorchis* and *Galeola* are Holomycotrophic orchids.

Important progress in the study of the Thai orchids and flora were initiated in 1955 under the Thai-Danish Flora of Thailand project. Field expeditions were undertaken intensively during 1957-1960 and the work of Professor Gunnar Seidenfaden (1908-2001) and Dr. Tem Smitinand (1920-1995) have contributed altogether approximately 15,000 herbarium specimens, preserved specimens in spirit and living material. A series of botanical papers and books were published in the Nat. Hist. Bull. Siam Soc., Dansk Bot. Arkiv., Opera Bot., Nordic. J. Bot., and Orchids of Thailand. Although quite a large number of the native Thai orchids were recorded, their information however, is still insufficient for the taxonomic revision.

Queen Sirikit Botanic Garden (QSBG) established in 1993 aiming to serve as a centre for academic studies, research and render services concerning botanical knowledge as well as to provide a place of beauty and valuable Thai flora. One of the activities is to strengthen ex-situ conservation of valuable Thai species and orchids is one of QSBG's most active programs. The living collection deposited at QSBG is approximately 70 genera and 360 species and is now serving as living library and an important source to improve knowledge on Orchid taxonomy, Orchid biology, Orchid breeding and relevant academic research. Moreover to conserve the rare and endangered species, to propagate and to prevent them from becoming extinct.

## 1. The nature of threats to orchid conservation.

Harold Koopowitz<sup>1</sup> & P. (Bill) Lavarack<sup>2</sup>

1. Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA.

Email: hkoopowi@uci.edu

2. 37 Bay St., Pallarenda, Queensland, 4810, Australia.

There is almost universal recognition that over the past several centuries that nearly all biodiversity has come under increasing threat. The orchids represent a substantial part of the planet's plant biodiversity, accounting for some 8-10% of all named plants and possibly an even higher percentage of the tropical floras. There are two fates available for ecosystems. They may either be totally converted into other systems or else they may be partially degraded. The types of threat that orchids face can be divided into two main categories, threats that result in ecosystem conversion and threats that result from commercial exploitation. Of these the first is of major importance and it can be subdivided into three main categories. There are threats that result in total conversion of ecosystems. These result from clearing forests for lumber, cutting or drowning forests for dams; converting savannahs and forests into farm lands; strip mining; large fires; urbanisation and desertification. Partial degradation of ecosystems occurs when selective logging; slash and burn agriculture; forest fragmentation occurs. Other potential problems occur with pollinator losses; keystone species are changed or lost; genetic erosion takes place; aggressive weeds are introduced; animal introductions perturb the ecosystem; fuelwood gathering takes place; small fires and changing fire regimes happen and when systems are subjected to localised pollution. The third category encompasses more widespread events such as global disruptive patterns either as a direct or indirect result of human population expansion. These effects include global warming and increased CO<sub>2</sub> emissions; rising sea levels; acid rain; large-scale pollution and ozone depletion. Threats work at many different levels and scales. Some of these such as increasing average world temperatures and rising sea levels are global in scale and may already be beyond our abilities to ameliorate them. But we need to be aware of the existence of those threats, as well as, the more mundane problems such as pollinator losses, poaching and urban encroachment, etc., that work at the small-scale local level and are solvable. In this paper we attempt to assess the nature and types of threats facing orchids and their habitats, as well as the quality of data available for making realistic estimations of the severity of these problems.

## 2. Threatening processes in Eastern Australia.

David L. Jones

Australian National Herbarium, GPO Box 1600, Canberra, ACT 2601. Email: david.jones@pi.csiro.au

Of the surviving orchids in eastern Australia, about 200 species of terrestrials and about 60 species of epiphyte are categorised as rare, vulnerable or endangered. This represents about 30% of the terrestrial orchids of eastern Australia and about 27% of the epiphytes. Also five species of terrestrial orchid and possibly two epiphytic orchids are extinct. In my opinion, based on modern studies of relict habitats, this is a huge underestimation and the real level of extinction is much higher. Orchid-rich habitats parallel the parameters required for successful agricultural pursuits (fertile soils, reliable rainfall) and as a result have suffered disproportionately from the wholesale clearing that has occurred since European settlement. Urbanisation, particularly in coastal regions, has also had drastic effects on orchid species and populations. Soil erosion and salinisation, especially of bushland adjacent to agricultural sites, results in a dramatic loss of plant diversity. Feral animals, particularly rabbits, pigs and goats, continue to have debilitating effects on orchid populations, even in reserves such as National Parks. More insidious are the deleterious effects of spreading populations of feral garden snails and slugs from urbanised areas. Recently the perils of naturalised aphid populations as potential vectors of exotic plant viruses have become apparent. With some ten percent of the Australian flora being naturalised, the competitive effects of certain weeds, such as *Asparagus asparagoides* and *Oxalis pes-caprae*, become paramount in the survival of some orchid taxa. Inappropriate burning regimes in forests, National Parks and relict patches of bushland contribute significantly to fluctuations in orchid numbers. Illegal collection of plants for cultivation can have a major impact on rare taxa. Local issues, such as rubbish dumping, firewood collection, roadworks, changes in land status and ownership and the use of herbicides can all have significant effects on orchid populations surviving in patches of remnant bushland. Largely unknown is the effects of all these factors on populations of associated pollinating insects. Unforseen are the unknown factors associated with climate change.



### 3. Chilean orchids: What do we know about them?

Carlos A. Lehnebach\*<sup>1</sup> & M. Riveros<sup>2</sup>

1. Ecology Group, Institute of Natural Resources, Massey University. Palmerston North, New Zealand. Email: C.Lehnebach@massey.ac.nz.

2. Instituto de Botanica, Universidad Austral de Chile, Valdivia, Chile.

Orchids are one of the least studied groups of flowering plants in Chile and practically all the current information dates from the late 1800's. Excluding a few studies (e.g. Reiche 1910; Gumprecht 1980; Riveros 1991; Niewenhuizen 1993 and Pridgeon 1997), orchids have been solely recorded in vegetation studies. To date, there is no national legislation to protect Chilean orchids. Hoffmann (1989) pointed out that "they are a rather numerous group that still requires more study", therefore orchids were not considered in the Conservation Status Assessment of the Chilean Monocotyledonous Geophytes.

During the last couple of years, studies about distribution, abundance and reproductive biology have been conducted in orchid species from central-south Chile. In addition to these studies, the current threatening processes have been identified in both protected and unprotected areas. Habitat destruction caused by urbanisation activities i.e. road construction or development of recreational centres in coastal biotopes is considered the principal cause of the extirpation and decline of populations. Forestry and agricultural activities and grazing pressure by livestock have reduced species richness in some areas of the country. Ironically, numerous healthy orchid populations have been recorded under the canopy of exotic tree plantations (*Pinus* sp. and *Eucalyptus* sp.). The introduction of exotic plants and animals is also considered a menace but to a minor extent. Lastly, habitat fragmentation has become an issue following studies into the reproductive biology of Chilean orchids reproductive biology. Low effective pollination, low fruit-set in the wild and the widespread occurrence of pollinator dependent taxa in both genera are features that may dramatically decrease the reproductive success of a species when in isolated populations.

#### References

- Gumprecht, R. (1980) Blossom structure and pollinating mechanisms in endemic orchids of South America. *Medio Ambiente* 4(2):99-103.
- Hoffmann, A. (1989) Chilean monocotyledonous geophytes; taxonomic considerations and their state of conservation. *Herbertia* 45(1&2): 15-28.
- Pridgeon, A. (1997) *Chloraea*: the pride of Patagonia. *The New Plantsman* 240 – 243.
- Reiche, C. (1910) Orchidaceae Chilenses. *Anal. Mus. Nac. Santiago de Chile, secc. 2, Bot., entrega* 18: 1-88.
- Riveros, M. (1991) Biología reproductiva en especies vegetales de dos comunidades de la zona templada del sur de Chile, 40° S. Tesis para optar al grado de Doctor en Ciencias mención Biología. Fac. De Cs. Universidad de Chile, 301 pp. Santiago.
- van Niewenhuizen, G. (1993) Resumen del conocimiento de las Orquidaceas Chilenas. (Unpublished).

#### 4. The depleting orchid flora of Sikkim and strategies for conservation.

R.C. Srivastava

Botanical Survey of India, Sikkim Himalayan Circle, Gangtok-737 101. Present address: Indian Botanic Garden Howrah – 711 103, India.

Sikkim Himalaya is considered to be one of the richest regions of the country in Orchid species. Out of ca. 1250 species known from India ca. 650 species occur in the North Eastern region (Old Assam) while ca. 451 species are known from this tiny Himalayan State only. But as a result of developmental activities occurring rapidly in even the remote corners of Sikkim, these remaining elements are depleting at an alarming rate. A recent study by the author revealed that out of ca. 451 species recorded from Sikkim a large number of them are known only from solitary or a few collections dating back to almost a century. Several orchids have been lost from the wild due to biotic influences and/or natural disasters like frequent land-slips etc. For example *Paphiopedilum venustum* is now extinct in the wild due to destruction of its habitat for cardamom cultivation. *Cypripedium macranthum*, *C. himalaicum* and to some extent *Pleione hookeriana* are on the verge of extinction due to demand by traders. *Diplomeris hirsuta* is restricted to a solitary locality. *Coelogyne treutleri* may already be extinct. There are many more species for which even the localities have not been known for a century.

This paper explains various aspects of this problem and recommends remedial measures for improving orchid conservation. Taxa which have not been collected recently and/or whose exact localities are not known have been enumerated in this paper.

## 1. The role of *ex situ* techniques in re-establishment of terrestrial orchids in the United Kingdom.

Margaret M. Ramsay

Micropropagation Unit, Aiton House, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB UK.

Email: m.ramsay@rbgkew.org.uk

Plant species action plans often include the use of *ex situ* conservation techniques to complement habitat protection. These techniques include seed storage, conventional propagation, micropropagation and cryopreservation.

Methods have been developed at the Royal Botanic Gardens, Kew for the *in vitro* propagation of endangered orchids and for the development and growth of seedlings in the glasshouse, garden and natural sites. The role of these methods in the recovery and re-establishment of orchid species is outlined.

Large numbers of seedlings of *Cypripedium calceolus*, a flagship species for conservation in the UK, have been grown in the laboratory using immature seed and novel asymbiotic media (Ramsay and Stewart, 1998). This is a collaborative program between Kew and English Nature's Species Recovery Program to save a plant reduced to a single site. Of these seedlings, over 1500 have been planted out at 16 different locations including a public viewing area. A seedling re-established in the wild flowered in 2000, 11 years after planting, with widespread interest in the media. This marks a significant step towards the goal of restoring self-sustaining populations. The European Action Plan (Terschuran, 1998) commends the success of this collaborative project and many countries are now initiating similar projects.

Mature seed of *Cypripedium calceolus* is now being collected for storage in the Millennium Seed Bank but is difficult to germinate routinely, while immature seed is not easily stored. A pilot cryopreservation trial using protocorms germinated from immature seed and methods developed by Wilkinson (1998) was carried out with encouraging preliminary results.

Using symbiotic culture with mycorrhizal fungi, several European terrestrial species have been propagated from seeds and re-introduced to managed sites in England. These include *Dactylorhizas*, *Ophrys apifera*, *Anacamptis laxiflora* and *Liparis loeselii*, a threatened species throughout Europe. A collection of mycorrhizal fungi is maintained and stored in liquid nitrogen.

The techniques described can be used to implement the *ex situ* conservation and re-establishment of threatened orchids in collaborative programs. They also have the potential to make artificially propagated plants widely available and thus help to reduce wild-collecting.

### References

- Ramsay, M.M. and Stewart, J. 1998. Re-establishment of the lady's slipper orchid (*Cypripedium calceolus* L.) in Britain. *Botanical Journal of the Linnean Society*, 126:173-181
- Terschuran, J.(1998) Action Plan for *Cypripedium calceolus* in Europe. Council of Europe Report: T-PVS9(98) 20 revised.
- Wilkinson, T., Wetten, A. & Fay, M. F. (1998). Cryopreservation of *Cosmos atrosanguineus* shoot tips by a modified encapsulation/dehydration method. *Cryo-Letters* 19: 293-302.

### Acknowledgements

The Royal Botanic Gardens, Kew wishes to acknowledge the financial support of English Nature (the Nature Conservancy Council for England) and to thank the Royal Horticultural Society for a bursary from the Coke Trust Awards.

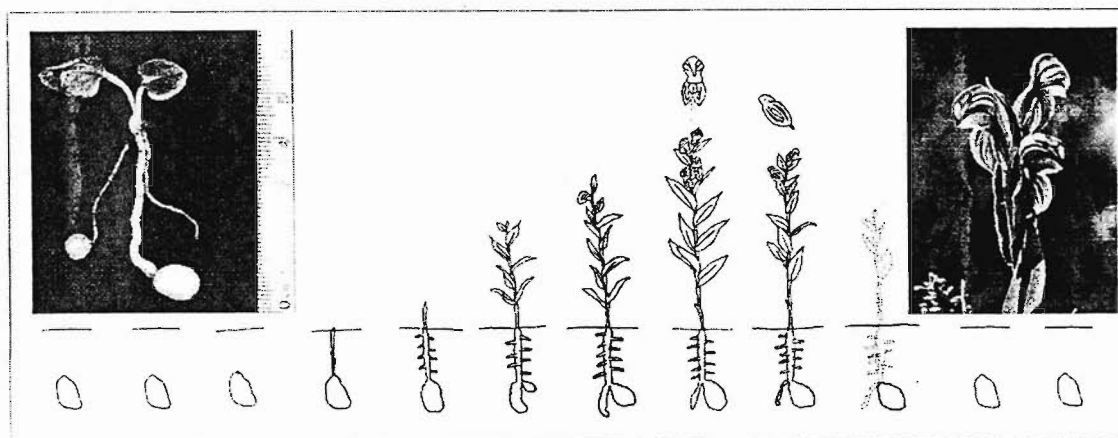
## 2. Tuber production as a method for improving conservation of terrestrial orchids.

Nika Debeljak<sup>\*1,2</sup>, M. Regvar<sup>1</sup> and K.W. Dixon<sup>2</sup>

1. University of Ljubljana, Biotechnical Faculty, Department of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia. Email: nika.debeljak@k2.net

2. Kings Park & Botanic Garden, West Perth 6005, Western Australia.

Due to human impact on the environment, orchids are regarded as one of the most vulnerable plant groups. Consequently, there is an urgent need to develop effective propagation and reintroduction methods for threatened orchid species. A number of orchids can be propagated successfully *in vitro* using asymbiotic or symbiotic (fungus assisted) seed germination methods. *In vitro* symbiotic orchid seed germination provides a reliable means to propagate orchids from seeds using naturally occurring fungi, resulting in quick development of seedlings. Mycorrhizal fungi should be included in propagation efforts to ensure that orchids persist in natural conditions where the mycorrhizal fungus is considered crucial to plant development. While symbiotic germination *in vitro* can result in a large number of healthy seedlings, these are often fragile and the survival of plants transferred to soil is low. Dormant storage organs, such as tubers, are a more ideal form to translocate orchids as tubers are robust and are better able to survive the transfer to soil. For most herbaceous terrestrial orchids, tubers are produced towards the end of the growing season, thus *in vitro* systems need to provide for seedlings to progress towards a dormancy phase to encourage adequate tuberisation. A technique to stimulate early tuberization of terrestrial orchids could serve as an effective method for proliferation of orchid plants in conservation programs. *In vitro* tuber formation of a terrestrial orchid *Pterostylis sanguinea* can be improved by the simultaneous addition of appropriate levels of jasmonic acid and sucrose to Oat agar medium. This new technique may provide benefits for rapid *in vitro* tuberization of terrestrial orchid species as an aid to improving the survival of plants generated by *in vitro* means.



Jan. Feb. Mar. Apr. May Jun. Jul. Aug. Sep. Oct. Nov. Dec.

*Pterostylis* growth cycle with plant developing tubers *ex situ*.

### 3. Reversing the decline of *Diuris fragrantissima*.

Colin Knight<sup>1</sup>, Elizabeth A. James<sup>2\*</sup>, S. Akiyama<sup>3</sup>

1. Melbourne Zoological Parks and Gardens, Parkville, Victoria, Australia. Email: cknight@zoo.org.au

2. Royal Botanic Gardens Melbourne, Birdwood Avenue, South Yarra, Victoria 3141, Australia.

Email: ejames@rbgmelb.org.au

3. Melbourne Zoological Parks and Gardens, Parkville, Victoria, Australia

Email: shiroa@myriad.its.unimelb.edu.au

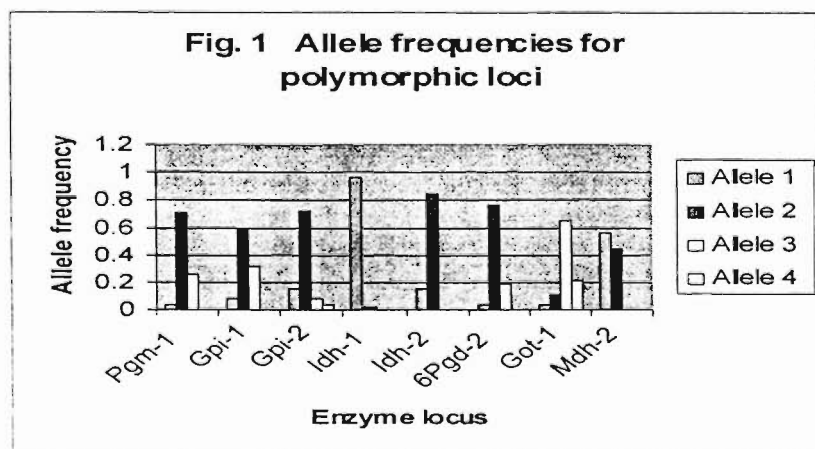
*Diuris fragrantissima* is an Australian terrestrial orchid species that is critically endangered because of changed land-use patterns. It was once a common species dotted through the basalt plains west of Melbourne. Now, only three plants remain in the wild.

A co-ordinated conservation program is underway to re-introduce the species to its former range. Ex-situ plants derived from plants collected in the wild during the 1970s and 1980s form the basis of the re-introduction material. Previously, propagation had been ad hoc with no records kept of individual crosses. Morphological measurements of ex-situ collections and genetic analyses using allozymes and AFLPs have identified a reasonable level of genetic diversity. Potential clones resulting from propagation by tuber removal have also been identified. Allozyme data (Fig. 1) has been used to devise a breeding program to increase plant numbers while minimising inbreeding. When completed, AFLP data also will be used to modify the breeding program.

In 2000, pollination trials were conducted to study the breeding system. Plant selection was based on the allozyme genotypes to ensure that cross pollinations were not carried out on plants with the same genotype. There was no difference in the level of fruit set between selfed and outcrossed flowers but no fruit set was recorded if pollen was not transferred to the stigma indicating the requirement for a pollen vector.

The first record of translocation in the species dates back to 1950 and concerted efforts in both monitoring and reintroduction were undertaken by members of the Botany Department at La Trobe University during the 1980s. All reintroduction attempts were thought to have failed but a flowering plant was found in October 2000, 15 years after reintroduction and highlights the importance of long-term monitoring. Seeds resulting from hand pollinations in spring 2000 have been propagated *in vitro* and seedlings are being acclimatised under nursery conditions. Some of these will be introduced into secure sites starting in 2002.

It is hoped that the genetic diversity, given the limitations of the starting material, will be sufficient to ensure that a number of individuals are able to thrive and develop into sustainable populations in perpetuity.



#### Acknowledgements

The generous support of the Hermon Slade Foundation in the conservation of *Diuris fragrantissima* is gratefully acknowledged.

## 1. Substrate requirements for recruitment of orchid seedlings.

Hanne N. Rasmussen

Danish Forest & Landscape Research Institute, 11 Hoersholm Kongevej, 2970 Hoersholm, Denmark. Email: hnr@fsl.dk

Conditions which promote the natural recruitment of seedlings are of paramount importance for long term success of a plant population and hence for any conservation or restoration program. The great number of seeds characteristically produced in orchids suggests that germination and early seedling development is a bottleneck phase in their life history. Many orchids are long lived plants and the populations we observe in many cases may be senile communities; they may, or may not, produce seeds whose chances of success depend on dispersal to other areas. Since mycorrhizal orchid seedlings are heterotrophic, a substrate is needed to sustain the fungi and hence the seedling. While we know a lot about substrate requirements for seedling propagation *in vitro*, very little is known about the natural substrates and their impact on natural seedling recruitment. Certain deductions can be made from the life form of the mycobionts. At least two fungal life forms are represented, i.e., saprotrophs and ectomycorrhizal fungi, which means that seedling recruitment relies either on certain kinds of organic debris or on the presence of a suitable ectomycorrhizal host tree. Experimental sowings *in situ* usually give very patchy germination results. Recent studies show that in some symbiotic terrestrial orchids, the kind and age of wood debris have a direct effect on seeds, before a symbiosis is established; after germination and mycorrhization, seedling growth is of course also affected by the substrate, probably indirectly via the mycobiont. Some epiphytic orchids are associated with a particular species of phorophyte, which probably means that the tree is a carbohydrate source, either through mycorrhizal connections between the orchid root system and the mycobiont or through fungal breakdown of its litter. The complexity of the relationship between orchids and their substrate is heightened by the diversity of mycobionts for each orchid species. Within the life of the single individual this may either be simultaneous infection by several mycobionts or a sequence of mycobiont changes.

## 2. Research into the propagation of eastern Australian *Caladenia*

Ruth E. Raleigh<sup>1,2,3\*</sup>, R.G. Cross<sup>1</sup>, A.C. Lawrie<sup>2</sup>, F. Coates<sup>3</sup> and A.C.A. Moorrees<sup>4</sup>

1. Research & Herbarium Division, Royal Botanic Gardens, Birdwood Avenue, South Yarra, Victoria 3141 Australia. Email: [rralei@rbgmelb.org.au](mailto:rralei@rbgmelb.org.au), [rcross@rbgmelb.org.au](mailto:rcross@rbgmelb.org.au)

2. Dept of Biotechnology & Environmental Biology, RMIT University, GPO Box 2476V, Melbourne, Victoria 3001, Australia. Email: [aclawrie@rmit.edu.au](mailto:aclawrie@rmit.edu.au)

3. National Parks Policy and Strategy Division, Level 9, 535 Bourke Street, Melbourne, Victoria 3000 Australia. Email: [F.Coates@parks.vic.gov.au](mailto:F.Coates@parks.vic.gov.au)

4. Threatened Species & Communities Section, Flora & Fauna, 240 Victoria Parade, Melbourne, Victoria 3001, Australia. Email: [Adrian.Moorrees@nre.vic.gov.au](mailto:Adrian.Moorrees@nre.vic.gov.au)

Six species of the terrestrial genus *Caladenia*, three common and three endangered, were studied, emphasising their propagation from seed for re-introduction to wild habitat. Common species were used to test techniques and one of these, *C. tentaculata*, was used in a detailed study of symbiotic development. For this, *C. tentaculata* seed was assayed against compatible (defined as producing orchid seedlings able to survive after deflasking into soil) and incompatible fungi (defined as seeds or protocorms being parasitised). Compatible fungi for *C. tentaculata* were also compared with fungi extracted from the other five species under study. Morphology and intracellular detail were used to determine groupings within and between compatible and incompatible fungi.

In *Caladenia*, infection by endophytic fungi occurs in the collar region, 10 – 30 mm below ground level. Individual pelotons were extracted from the collar and, although cultures were uniform and consistent in appearance, germination was highly variable for endangered species. In common species (*C. tentaculata* and *C. phaeoclavia*) germination and development of seedlings with a green leaf was always high from certain collar isolations. If one peloton cultured from a collar germinated seeds well, all pelotons from that collar did. Cultures producing high numbers of seedlings were slow-growing, creamy-white, with clusters of white, fluffy, moniloid cells breaking the surface of the agar. Hyphae were hyaline, of between 1-5 µm in diameter. No fruiting structures were ever observed.

Cultures from two endangered species (*C. fulva* and *C. venusta*) contained fungi that grew more quickly and produced hard masses of yellowish, moniloid cells within the agar and free of the surface. Germination of seeds was generally poor, although a few very robust seedlings were often produced. Fungi extracted from *C. robinsonii* were completely different. Original cultures produced creamy white cultures composed of many growth rings and were exceptionally slow-growing. Only two protocorms developed and the fungus from these protocorms grew more rapidly than the original culture and lost the characteristic growth rings. Using these second generation fungi in another sample of fresh seeds resulted in much higher germination.

In general, each fungus only produced healthy seedlings with its host species of orchid. The two common closely related orchids *C. tentaculata* and *C. phaeoclavia* gave a slightly different result. Each fungus from *C. phaeoclavia* germinated seeds of *C. tentaculata*, but germination was lower and the rhizoids were stubby structures with diminished function. Cultures obtained from adult orchids of certain species may be composed of several slow-growing fungi. The specific fungus for germination can be isolated eventually, providing a seed is germinated and grows into a protocorm with a green leaf. Isolation of several fungi from a single peloton may explain why germination is often highly variable and why certain cultures lose the ability to germinate seeds after time in storage. The germinating fungus in these species of orchid is slow-growing and appears to be a poor competitor when other fungi are present. In storage, the stronger, non-germinating fungi would eventually dominate the culture.

### 3. Mellblom's Spider-orchid *Caladenia hastata* - Managing the *in situ* recovery of a Critically Endangered Spider Orchid

Andrew Govanstone<sup>1</sup>, John Hill<sup>2</sup>, Andrew Pritchard<sup>3\*</sup>

1. Flora and Fauna Management Officer, 8-12 Julia St. Portland, Victoria 3305, Australia. Email: Andrew.Govanstone@nre.vic.gov.au

2. Portland Aluminium, Private Mail Bag 1, Portland, Victoria 3305, Australia. Email: John.hill@alcoa.com.au

3. Flora and Fauna Management Officer, 78 Henna St. Warrnambool, Victoria 3280, Australia. Email: Andrew.Pritchard@nre.vic.gov.au

Mellblom's Spider-orchid was once common in south-west Victoria in the 1940's. Since then the plant has varied from being plentiful to near extinction on a number of occasions. This orchid is spectacular, easily recognised by its white or cream flowers with long, thick, dark red or black clubs on the tips of its petals and sepals. Hot fires the previous summer promote the orchid flowering. It reaches a height of 300 mm and the flower is up to 80 mm across.

It was considered extinct until after a wildfire at a site south of Portland in the summer of 1976. The following spring, large numbers were observed flowering. In the 1980's industry was developed on the orchid site and a number of the plants were relocated to other locations. Unfortunately most of these plants did not survive. A small population remained on the industrial site.

In 1996 there were 10 remaining plants. With the assistance of Portland Aluminium, the manager of the site, *ex situ* measures were undertaken to assist in the long-term survival of the species. Symbiotic germination was done at Kings Park in Western Australia and small seedlings of the plant were reintroduced back to site, however no seedlings survived the transfer to soil.

During the flowering season of 1998 more plants were located. There were seven flowering plants with four developing seedpods. The seedpods were harvested but unknown to us some seed was accidentally spilled around one of the orchids. That following summer on-site watering of the plants was undertaken to provide some additional moisture over a long hot summer period. The following season we noticed that seedlings had started to grow around the base of a plant where seed had been spilt. In 1998 we had a total of 7 flowering plants and 27 seedlings.

In 1999 the young plants were once again artificially watered and some habitat manipulation was started to provide suitable conditions for plant growth. Seedling numbers and flowering plants increased significantly.

In Autumn 2000, seed from pods that had been harvested the previous summer was distributed around a number of adult plants. The sites around the adult orchids were manipulated with the tiny seeds being sown and a fine layer of mulch being applied to protect them. The area was watered in and during August of that year small seedlings were noticed in the areas that we had sown. The results of this resulted in a marked increase in the number of seedlings and flowering plants.

From 1996 in which there were 10 plants, to the end of 2000 when there were 19 flowering plants and 357 seedlings, there has been a significant positive change in the health of the population.

This is encouraging for the orchid's future *in situ* conservation. Further work must continue to be done to bring the numbers up to a level that they are self-sustaining in the wild.



#### 4. Improving the success of translocation of terrestrial orchids.

Andrew L. Batty<sup>1,2,3</sup>, K. W. Dixon<sup>1</sup>, M. Brundrett<sup>1,2</sup> and K. Sivasithamparam<sup>2</sup>

1. Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, West Perth, Western Australia 6005.

2. Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Western Australia 6907.

3. Corresponding author; Email: albatty@cyllene.uwa.edu.au

Many endangered plant species in Western Australia require active conservation if there is to be no further loss of species diversity. Terrestrial orchids represent the most significant family of endangered plant species in the south-west of Western Australia with 34 terrestrial taxa listed as endangered. A greater understanding of the biology of these orchids will enable more effective conservation efforts. A study was instigated to research aspects of the biology of temperate terrestrial orchids and associated fungi, including soil seed bank dynamics, symbiotic propagation, establishment to soil, and the detection of orchid fungi habitat and translocation of rare orchid taxa to field sites relative to conservation.

##### *Seed Banks*

The study found that orchid seed banks are short-lived, lasting for less than 12 months. Seeds imbibed at the onset of the wet season were unable to revert to a dormant state when conditions became dry. The lack of a persistent soil-based seed bank results in recruitment being influenced by the number of seeds entering the seed-bank the previous season.

##### *Fungal Distribution*

The distribution of mycorrhizal fungi capable of germinating terrestrial orchid seed was investigated under natural field conditions. Seed baits were located at 808 sample points to detect fungal isolates associated with a common bushland species (*Caladenia arenicola*) in the Perth region of Western Australia. Less than 1% of seed germinated to a stage where tuber formation would be capable of ensuring survival over the subsequent dry season. Germination was most likely in the vicinity of adult *C. arenicola* plants. The measurement of the spatial variability in germination events within an orchid habitat demonstrated that new recruitment sites were available.

##### *Tuberisation*

An intermediate stage between the petri dish, where seedlings were symbiotically germinated, and soil where seedlings were grown in an axenic environment with controlled humidity was found to be the key to seedling survival upon transfer to soil. There was no apparent benefit in establishing fungi in soil prior to the transfer of symbiotic seedlings to soil under glasshouse conditions. Initial survival of seedlings to soil was high, however, the failure of some seedlings to produce tubers capable of surviving summer dormancy resulted in a decline in survival of 40-60% one year after transfer to soil. The methods developed enabled the efficient production of both actively growing symbiotic seedlings and dormant tubers for translocation of Western Australian temperate terrestrial orchids to field sites.

The re-establishment of four terrestrial orchid taxa using dormant tubers or actively growing seedlings produced from *ex situ* propagation methods was more successful than *in situ* seed germination. Further field-based research is required to clarify and refine findings presented here and to make the outcomes more broadly applicable to conservation of other threatened temperate terrestrial orchids.

##### *Acknowledgments*

We thank Western Power for their support of the Endangered Species Rescue Program which this program of research.

## 1. Biodiversity and distribution of endophytic, pathogenic and rhizosphere fungi from terrestrial orchids in Thailand.

Leka Manoch\* and Kanungnid Busarakam

Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900 Thailand. Email: agrlkm@ku.ac.th

Healthy leaves of terrestrial orchids, namely *Eria albidotomentosa*, *Ludisia discolor* and *Spathoglottis plicata*, were collected from Lopburi, Chiangmai and Tak Provinces. They were used for isolation of endophytic fungi by employing surface disinfection with 70% alcohol for 1 minute and 10% sodium hypochlorite for 2 minutes, washed with sterile distilled water and placed on cornmeal agar (CMA) in a Petri dish. Endophytic fungi resulting from this isolation method were *Colletotrichum*, *Nodulosporium*, *Pestalotiopsis* and *Xylaria*.

In another investigation, terrestrial orchids, *Eria albidotomentosa*, *Spathoglottis affinis* and *S. plicata*, showing leaf spot symptoms were collected from Lopburi and Tak Provinces for isolation of plant pathogenic fungi. A tissue transplanting method was conducted by immersing diseased tissue in 10% sodium hypochlorite for 2-3 minutes, washing in sterile distilled water and tissues placed on potato dextrose agar (PDA) and Gochenaux's glucose ammonium nitrate agar (GAN). Pathogenic fungi found in this study were *Colletotrichum* sp., *Curvularia eraglostidis*, *C. sp.*, *Fusarium oxysporum* and *Phyllosticta* sp.

Rhizosphere soils of ten terrestrial orchids, *Ludisia discolor*, *Spathoglottis plicata*, *Calanthe rosea*, *Cymbidium sinense*, *C. ensifolium*, *Goodyera procera* and *Paphiopedilum* sp. were collected from Chiangmai, Nakhonratchima and Petchabun Provinces. Various isolating methods, such as the soil plate, soil dilution plate, alcohol and heat treatments and rice baiting on CMA and GAN were employed. Rhizosphere fungi of terrestrial orchids included *Absidia cylindrospora*, *Aspergillus deflexus*, *Aspergillus flavus*, *A. fumigatus*, *A. japonicus*, *A. niger*, *A. terreus*, *A. vesicolor*, *Chaetomium globosum*, *Chaetomium crispatum*, *Cunninghamella elegans*, *C. echinulata*, *Corynascus seponium*, *Cylindrocladium pavum*, *Emericella nidulans*, *Eupenicillium lapidosum*, *Eupenicillium shearii*, *Eurotium amstelodami*, *Fusarium oxysporum*, *F. solani*, *Gelasinospora* sp., *Geotrichum candidum*, *Gliocladium penicilloides*, *Gongronella butleri*, *Hamigera avellania*, *Humicola fuscoatra*, *H. grisea*, *Monodictys putredinis*, *Mucor hiemalis*, *Myrothecium verrucaria*, *Neosartorya fischeri*, *Neurospora* sp., *Paecilomyces lilacinus*, *Papulospora immersa*, *P. irregularis*, *Penicillium restrictum*, *P. rubrum*, *Phoma* spp., *Phomopsis* sp., *Pythium vexans*, *Rhizopus oryzae*, *R. stolonifer*, *Scytalidium ligincicola*, *S. sp.*, *Sordaria* sp., *Syncephalastrum racemosum*, *Talaromyces flavus*, *T. sp.*, *Thielaviopsis* sp., *Trichoderma hamatum*, *T. harzianum*, *T. virens* and *T. viride*.

Morphological characteristics as observed under stereo-and light microscopes were used for identification. Photomicrographs and camera lucida drawing was employed. Scanning electron microscopy was used for the identification of some noteworthy fungal species. Biodiversity and distribution of endophytic, pathogenic and rhizosphere fungi of terrestrial orchids are discussed.

## 2. Diversity of orchid mycorrhiza in Thailand.

Pornpimon Athipunyakom<sup>1</sup>, Leka Manoch<sup>2</sup> and M.Tanticharoen<sup>3</sup>

1. Plant pathology and Microbiology Division, Department of Agriculture, Bangkok 10900 Thailand. Email: pornpi@doa.go.th

2. Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

3. National Center for Genetic Engineering and Biotechnology (BIOTEC), Bangkok 10400, Thailand.

In the present study, mycorrhizal fungi were isolated from four terrestrial orchid species: *Goodyera procera*, *Spathoglottis plicata*, *Calanthe rubens* and *Ludisia discolor*. Information from this study could be used to aid orchid conservation in nature. The orchid hosts were collected from various parts of the country, i.e. Chiangmai, Mae-Hongson, Chanthaburi and Lopburi. A modification of the Masuhara and Katsuya method was used. Root pieces were washed with tap water and soaked in 10% sodium hypochlorite for 5 minutes and rinsed twice with sterile distilled water. The pieces were then cut into longitudinal sections and observed for the presence of hyphal coils (pelotons) under a stereo-microscope under sterile conditions. A fine needle was used to isolate a peloton and place it on Masuhara and Katsuya NDY medium. After 3 to 7 days incubation, hyphal tips were transferred onto a potato dextrose agar slant. Pure cultures were maintained for identification. Macroscopic features were investigated on colony growth pattern, color, etc. and under the compound microscope for microscopic features. Photomicrographs and camera lucida drawings were used.

Forty-four isolates of mycorrhizal fungi were found, including 2 genera and 4 species including: *Rhizoctonia cerealis*, *R. ramicola*, *Ceratorhiza goodyerae-repentis* and an unidentified *Rhizoctonia* sp. 1. *Ceratorhiza goodyerae-repentis* was abundant in *Goodyera procera* and *Ludisia discolor* collected from regions with a cooler climate such as Chiangmai and Mae-Hongson. *Rhizoctonia cerealis* was associated with *Goodyera procera* in Chiangmai, whereas *Rhizoctonia ramicola* was found in *Calanthe rubens* from Chanthaburi, in the eastern part of Thailand. *Rhizoctonia* sp. 1 was isolated from various orchid hosts from different regions in northern, central and eastern Thailand. Orchid hosts included *Spathoglottis plicata*, *Goodyera procera*, and *Calanthe rubens*. The fungus produced thin white aerial and submerged mycelium; binucleate, erect hyphae staining with safranin-o Bandoni's technique, globose to subglobose or doliform-shaped monilioid cells, 8.5-(12.1)-15.5 x 7.1-(9.3)-11.5  $\mu$ m, aggregated into microsclerotia. This fungus is very similar to Andersen's *Rhizoctonia* strain D145-4 (monilioid shape: subglobose-doliform, size: 9.6-(12.4)-15.2 x 8.8-(10.4)-12.0  $\mu$ m). In addition, three unidentified fungi producing spore and hypha coils in one isolate were found on *Spathoglottis plicata*.

Isolation - cerealis - 12 min 2700' 75% - 10 min

- Richey treatment method
- Richey treatment technique
- gel plate
- media plate

### 3. Natural hybrids as a means for investigating specificity of orchid mycorrhizal fungi.

Penelope S. Hollick\*<sup>1,2</sup>, Jen A. McComb<sup>2</sup>, Kingsley W. Dixon<sup>1</sup>

1. Science Division, Botanic Garden and Parks Authority, Kings Park and Botanic Gardens, West Perth, Western Australia, 6005. Email: hollickp@central.murdoch.edu.au

2. School of Biological Sciences, Murdoch University, South Street, Murdoch, Western Australia, 6150.

The genus *Caladenia* (spider orchids) is one of the most diverse in southwestern Western Australia, with more than 150 species occurring in this region of mediterranean climate. *Caladenia* species readily form hybrid combinations in the wild, and therefore make excellent subjects for investigating mycorrhizal specificity. The objective of this study was to investigate the mycorrhizal relationships of some *Caladenia* species and their natural hybrids, and whether *Caladenia* hybrids use the same mycorrhizal fungus as the parental species. Mycorrhizal fungi were isolated from several naturally occurring *Caladenia* hybrids and from individuals of both parental species from the same location as the hybrid. Hand pollination was carried out to produce parental and hybrid seed. The seeds were sown on oat agar previously inoculated with mycorrhizal fungi. All possible combinations of parental and hybrid seed and fungi were tested in a symbiotic germination matrix. Table 1 shows the effectiveness of the fungal isolates in promoting seed germination and advanced protocorm development at 12 weeks after sowing. The percent of seedlings that developed to or beyond the stage of trichome development varied considerably between seed/fungal combinations. The hybrid seeds germinated on all fungal isolates tested, while some parental seeds did not germinate on fungal isolates from the hybrid and/or the other parental species. This study provides excellent opportunities for investigating the molecular basis for specificity in these intriguing mycorrhizal relationships.

**Table 1: Germination of *Caladenia* species and hybrids**

The number of fungal isolates which stimulated protocorm growth to or beyond the stage of trichome development. Total number of isolates tested is shown in parentheses.

Fungal source		Seed (Brookton Hwy)					
Species	Location	<i>C. eminens</i>		<i>C. eminens x falcata</i>		<i>C. falcata</i>	
<i>C. eminens</i>	Brookton Hwy	3	(3)	3	(3)	0	(3)
<i>C. eminens x falcata</i>	Brookton Hwy	3	(3)	3	(3)	0	(3)
<i>C. falcata</i>	Brookton Hwy	2	(2)	2	(2)	2	(2)

Fungal source		Seed (Medina)					
Species	Location	<i>C. flava</i>		<i>C. flava x latifolia</i>		<i>C. latifolia</i>	
<i>C. flava</i>	Medina	3	(3)	3	(3)	1	(3)
<i>C. flava</i>	Margaret River	3	(3)	3	(3)	3	(3)
<i>C. flava x latifolia</i>	Margaret River	2	(3)	3	(3)	0	(3)
<i>C. latifolia</i>	Medina	0	(3)	3	(3)	3	(3)
<i>C. latifolia</i>	Margaret River	2	(2)	2	(2)	1	(2)

#### 4. The development of mycorrhizal infection in *Caladenia tentaculata*.

Magali Wright<sup>1\*</sup>, David Guest<sup>1</sup> and Rob Cross<sup>2</sup>

1. School of Botany, University of Melbourne, Parkville, Victoria 3010, Australia. Email: m.wright2@ugrad.unimelb.edu.au, D.Guest@botany.unimelb.edu.au

2. Royal Botanic Gardens Melbourne, Birdwood Avenue, South Yarra, Victoria 3141 Australia. Email: rob.cross@rbg.vic.gov.au

A greater understanding of the mycorrhizal infection process in the Australian terrestrial orchid, *Caladenia tentaculata*, was gained using two different mycorrhizal fungi that were isolated as single peletons from the collar region of adult plants of the same species. One fungal isolate was compatible, promoting rapid germination of *Caladenia tentaculata* seed and strong subsequent growth, and the other isolate was incompatible, being largely incapable of promoting germination.

Mycorrhizal infection during germination and protocorm development was observed microscopically, and all the stages critical to successful protocorm development were identified by comparing the effects of the two fungal isolates. Orchid embryos were sampled daily for 21 days, and weekly for 10 weeks; and were fixed for light microscopy and scanning electron microscopy. Unfixed embryos were examined under UV light for autofluorescence, which indicates the presence of phenolics compounds. A picture of the infection process and protocorm colonization by fungi was constructed from these comparisons.

The compatible isolate infected the seed through the suspensor cells. Autofluorescence compounds that contracted to the suspensor cells of the seed during imbibition were implicated in control of this infection pathway. Compatible infection was restricted to the basal cells of the protocorm, with the meristematic, storage and vascular tissue remaining uninfected. Two morphologically distinct peloton types were identified and found in two discrete layers in the cortical cells beneath the epidermis. Only one of the peloton types was subject to the cycle of digestion and re-infection.

The incompatible isolate either failed to infect the seed, or penetrated via the epidermal cells or the developing rhizoids and resulted in unrestricted colonization. *Caladenia tentaculata* seed challenged with this isolate responded with a local accumulation of autofluorescence compounds, indicating an incompatible interaction. Rarely infection occurred through the suspensor cells, and when it did the infection was not restricted to the basal cells as it was with the compatible infection. The distribution of the developing rhizoids was random unlike that during compatible infection.

## 1. Do orchids have true mycorrhizal fungi?

Mark Brundrett

Orchid Research, Kings Park and Botanic Garden, Botanic Gardens & Parks Authority, West Perth, 6005, Western Australia. Soil Science & Plant Nutrition, The University of Western Australia, Nedlands, 6907, Western Australia. mbrundrett@kpbg.wa.gov.au

The purpose of this presentation is to contrast orchid fungi with other types of mycorrhizal fungi by considering definitions and evolutionary trends. Most endophytes from orchid roots are assigned to the form genus *Rhizoctonia*, a group of soil fungi that also includes plant pathogens and saprophytes. It is not clear if orchid fungi from different geographic regions are more closely related to local saprophytic or parasitic groups of *Rhizoctonia* species, or widespread orchid fungus groups. However, it seems most likely that the orchid fungi have many separate origins and their recruitment continues today. This lack of host-fungus co-evolution would be a fundamental difference from ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi which have much older associations with plants. Orchid mycorrhizal associations contrast with other types of mycorrhizas where long-term host-fungus co-evolution has resulted in a host-fungus interface with highly specialised hyphae.

It is difficult to resolve our contrasting knowledge of orchid fungi as so little is known about the ecological role of the fungi. Some orchid fungi are pathogens in roots of other plants, some may be parasites of other fungi, while others seem primarily be mycorrhizal. Most orchids have fairly specific associates, but other orchids associate with a broad range of fungi that vary with habitats. Extremely narrow host fungus specificity of orchids would result in highly specific habitat requirements, as they can only grow in soil patches where a particular fungus is thriving. Knowledge of orchid fungus diversity and habitat specificity is essential for us to understand the biology and ecology of these beautiful and fascinating plants.

It has been assumed that orchids with chlorophyll provide their fungi with energy in exchange for soil mineral nutrients, as is the case with most other types of mycorrhizas. However, there is no real evidence that fungi receive substantial benefits from any of their associations with orchids and they generally seem to grow as well without their hosts as they do with them. It is likely that mycorrhizal associations of adult green orchids are partially or fully exploitative with much more benefit provided to the host than to the fungus. This is the reverse of parasitic plant-fungus relationships. The most important advantage provided by exploitative mycorrhizas is the capacity for the fungus to provide energy to plants so they can grow in deep shade. Many orchids seem to have a greater capacity for shade tolerance than other plants. Orchids are also renowned for producing showy flowers that are disproportionately large relative to their leaves, and their growth may occur partially at the expense of other plants (by way of mycorrhizal fungi). Achlorophyllous orchids have extremely specific associations with fungi, including agaricoid wood-rotting or ectomycorrhizal fungi. The associations between some achlorophyllous orchids and wood-rotting fungi are amongst the most bizarre symbiotic associations known, as these "untamed" fungi lack adaptations for non-detrimental colonisation of plants. The trend for increased host dominance of mycorrhizas that culminates in fully exploitative achlorophyllous plants may be an evolutionary dead end, as these seem to include a disproportionate number of rare species.

*Brundrett 2002. Nature. Mycotologist Timothy Brundrett in 1992*

## 2. Seasonal observations of the endangered terrestrial spider orchid, *Caladenia formosa* G. W. Carr

Tien Huynh<sup>1\*</sup>, C.B. McLean<sup>1</sup> and A.C. Lawrie<sup>2</sup>

1. Institute of Land & Food Resources, University of Melbourne, Burnley College, 500 Yarra Boulevard, Richmond, Vic. 3121m, Australia. Email: prasophyllum@postgrad.unimelb.edu.au, C.Mclean@landfood.unimelb.edu.au

2. Dept of Biotechnology & Environmental Biology, RMIT University, GPO Box 2476V, Melbourne, Vic. 3001, Australia. Email: aclawrie@rmit.edu.au

Whole plants of the Elegant Spider orchid (*Caladenia formosa*) were collected at five stages across the growing cycle: leafing, budding, flowering, capsule formation and senescence. Plants were divided into up to 10 sections and each section was viewed with a scanning electron microscope. Fungal pelotons were found in all plants across all stages but were only found in the epidermis and cortex of the collar region (a swollen underground region between the green leaf and the tuber). Hyphal morphology varied according to different plant growth stages. In the initial stage of growth (leafing), only very fine (1  $\mu\text{m}$  diameter) hyphae were observed, but in the later stages (budding and flowering), both fine and coarse (5  $\mu\text{m}$  diameter) hyphae were observed. At capsule formation, pelotons of fine hyphae had lost integrity and were intertwined with large (5  $\mu\text{m}$  diameter) hyphae. Fungi isolated from pelotons at different stages of growth were used in germination trials with seeds of *C. formosa*. Only fungi isolated at the leafing, budding and flowering stages enhanced germination.

leafing

### 3. Determination of new endomycorrhizal fungi from two Chilean orchids, *Chloraea crispa* and *Chloraea gaviu* (Orchidaceae).

Rodrigo Reinoso,\* J. Becerra, N. Garrido & M. Silva

Laboratory of Natural Product, Department of Botany, Faculty of Natural and Oceanographic Sciences, University of Concepción. Email: rreinoso@udec.cl

The genus *Chloraea* belongs to the family Orchidaceae, the largest group among monocotyledons.

Like most orchid species, in a part of their life cycle, they develop a specific symbiotic association with mycorrhizal fungi. Seedling germination often depends upon this symbiotic association. In this work, *Chloraea gaviu* and *Chloraea crispa* seedlings were studied. Vegetative material was obtained from Yumbel and Escuadron VIII regions of Chile, during December-February period, (year 2000). The endomycorrhizal samples were isolated from sterilised roots to use in later studies of seed inoculation assays.

In this work, endomycorrhizal symbioses of *Chloraea gaviu* and *Chloraea crispa* were described and characterised using illustration of habit and microphotography of the mycelia using optical and scanning electronic microscopes. One these endomycorrhizal strains was identified as *Rhizoctonia* sp. Future work will focus on comparative analyses between Chilean orchids and European and Australian species.

#### Acknowledgements

Project FIA C-98-1-a- 022. Faculty of Cs. Natural and Oceanographic, University of Concepción.



#### 4. Molecular biology of mycorrhizal fungi from Australian terrestrial orchids.

A.C. Lawrie<sup>1</sup>, T. Huynh<sup>1,2\*</sup>, R.E. Raleigh<sup>1,3,4</sup>, C.B. McLean<sup>2\*</sup>, R.G. Cross<sup>3</sup>, F. Coates<sup>4</sup> and A.C.A. Moorrees<sup>5</sup>

1. Dept of Biotechnology & Environmental Biology, RMIT University, Bundoora Campus, PO Box 71, Bundoora, Victoria 3083, Australia. Email: aclawrie@rmit.edu.au, prasophyllum@postgrad.unimelb.edu.au, rrarei@rbgmelb.org.au

2. Institute of Land & Food Resources, University of Melbourne, Burnley College, 500 Yarra Boulevard, Richmond, Victoria 3121, Australia. Email: C.Mclean@landfood.unimelb.edu.au

3. Plant Sciences & Biodiversity, Royal Botanic Gardens, Birdwood Avenue, South Yarra, Victoria 3141, Australia. Email: rcross@rbgmelb.org.au

4. National Parks Policy and Strategy Division, Level 9, 535 Bourke Street, Melbourne, Victoria 3000, Australia. Email: FCoates@parks.vic.gov.au

5. Threatened Species & Communities Section, Flora & Fauna, 240 Victoria Parade, Melbourne, Victoria 3001, Australia. Email: Adrian.Moorrees@nre.vic.gov.au

Warcup (1981) examined 145 species of Australian orchids in 36 genera in 3 tribes. Fungal endophytes were isolated from pelotons, some tested for efficacy in germination and all identified by casing with Urrbrae clay, producing sexual states that were identified. He suggested that Australian orchids were relatively non-specific, with genera and even parts of tribes sharing the same endophytic fungal species.

This contrasts markedly with more recent experience, which is that fungi isolated from orchids are highly specific and even closely related orchids will not germinate one another's seeds, e.g. *C. tentaculata* fungi will not germinate the seeds of *C. phaeoclavia*, yet they are so closely related that they were conspecific until recently. Also, there has been difficulty in reproducing Warcup's success in inducing sexual reproduction, leading to unnamed isolates of high known specificity.

Modern techniques in molecular biology have been used to characterise and identify some of these endophytic fungi. Fungi were isolated from single pelotons immediately after collection and assayed for effectiveness against freshly collected and aged seed of the same species. DNA was extracted and the nuclear rDNA ITS (internal transcribed spacer) and mitochondrial rDNA regions amplified as appropriate and tested for homogeneity by RFLP before sequencing and alignment.

In *Caladenia* section *Calonema* (spider orchids), isolates from five species were examined. Isolates from different species had different RFLP banding patterns and all effective isolates, which appeared pure culturally, were a mixture of two-three genomes. Warcup (1971) identified *Sebacina vermifera* as almost the only fungus isolated from *Caladenia*. The degree of dissimilarity in PCR RFLP does not support this and such heterogeneity was not expected in what appeared to be uniform cultures.

Warcup (1981) identified *Ceratobasidium cornigerum* as almost the only species of endophyte from both *Prasophyllum* and *Pterostylis* species. In *Prasophyllum correctum*, problems were encountered again with dual cultures from single pelotons producing more than one ITS product.

Three isolates from *Pterostylis* species (pn from *P. nutans* and f2 from *P. concinna*) were 95% similar, suggesting a close relationship but probably not the same species. Both were only 81-84% similar to an isolate from *P. spathulata* identified as *Ceratobasidium cornigerum*.

Mitochondrial rDNA proved useful to compare 7 isolates from *Prasophyllum* and *Pterostylis*. All were >90% similar, with over half >98% similar, suggesting close relationships. Although cross-functionality was not tested symbiotically, this supports Warcup's (1981) suggestion that the fungal endophytes from *Prasophyllum* and *Pterostylis* are closely related.

## **1. The role of long-term, individual based demographic studies in the conservation of terrestrial orchids.**

Jo H. Willems & Dennis F. Whigham

1. Utrecht University, Sorbonnelaan 16, PO Box 800.24, Utrecht, NL-3508TB, The Netherlands. Email: j.h.willems@bio.uu.nl

2. Smithsonian Environmental Research Center, Edgewater, MD 21037, USA.

By the time it becomes clear to nature conservation organisations that a given orchid population is threatened with extinction, population processes causing decline in fitness had previously started. In order to take appropriate conservation measure, it is necessary to unravel the causes of decline. Demographic studies provide an indication at an early stage of population decline, e.g. death rate versus recruitment.

Knowledge of the life history traits and changes over time can make clear what sort of habitat conditions are required for the survival of the species and, moreover, what kind of management has to be carried out to create or maintain optimal habitat conditions for the different life phases of the species involved.

Life history strategies of terrestrial orchid species may differ enormously from each other even among species occupying the same habitat. Since life histories and habitat demands have been studied in very few species and, moreover, mostly for above-ground parts of the plants, it is clear that studies of orchid population ecology have only had a modest start. However, long-term studies of orchid populations are important today as part of present-day conservation efforts attempt to maintain diversity also among terrestrial orchids.

## 2. Population dynamics of north temperate orchid species.

Tiiu Kull\*, Kadri Tali and Taavi Tuulik

1. Institute of Zoology and Botany, Riia 181 Tartu, Estonia. Email: tiiu@zbi.ee

2. University of Tartu, Lai 38, Tartu, Estonia. Email: katal@lai.ut.ee

3. Suuremõisa College, Hiiumaa, Estonia. Email: taavituulik@hotmail.ee

During the last half a century population dynamics of nearly 70 species and natural hybrids of Northern hemisphere temperate orchids have been studied and reported in over 120 papers. We examine features like flowering, fruit set, recruitment, and impact of below-ground structure and dormancy on population structure on the basis of these papers. In more detail we analyse our demographic data recorded in Estonian populations of *Cypripedium calceolus*, *Cephalanthera rubra* and *Orchis ustulata* collected over periods of seven to twenty years. **Flowering** irregularity is a common feature in most orchid populations as regular cycles of flowering have been reported in only a few species. Flowering peaks are caused by newly recruited plants, or of plants that were vegetative or dormant during the previous years. All possible transitions between flowering and other life stages are noted throughout the study periods. Weather conditions have been often hypothesised to be the reason why performance and flowering are so irregular. However, there are few examples which support this principle. **Fruit set** is mostly dependent on the availability of pollinators. However, poor recruitment is often more microsite limited. **Below-ground** structure of plants and the type of clonal growth have a very strong effect population behaviour aboveground. Tuberous species and those having pseudobulbs are more dynamic, with changes occurring quickly even throughout the vegetation period. The populations of these species may disappear even after one year of unfavourable conditions. Species with a perennial rhizome are much more stable, since clonal growth can compensate to some extent for genet extinction. **Dormancy** of adult individuals has been reported in 23 orchid species with the average maximum duration of 3 years. In *Epipactis albensis* even 11 years of dormancy has been recorded. This data set sheds light on the possible causes of rarity in terms of population parameters.

### 3. Patterns of appearance and transition to flowering in *Prasophyllum correctum* D.L. Jones, a threatened orchid from south eastern Australia.

Fiona Coates\*<sup>1</sup> and Ian Lunt<sup>2</sup>

1. Arthur Rylah Institute, Department of Natural Resources and Environment, 123 Brown Street, Heidelberg, Victoria, Australia 3084. E-mail Fiona.Coates@nre.vic.gov.au.

2. The Johnstone Centre, Charles Sturt University, Albury, NSW, Australia 2640. E-mail ilunt@csu.edu.au

All 120 individuals of the Critically Endangered orchid *Prasophyllum correctum* D.L. Jones (Gaping Leek orchid) in the largest mainland Australian population were marked and censused between 1992 and 2000. Above ground life state (sterile or flowering), non-appearance and new appearances of plants were recorded. An intensive study was conducted between 1996 and 1998 using caged and uncaged plants, when monthly or fortnightly measurements were made of leaf length and width, number of flowers and herbivory. Additional site variables recorded were time since fire (months) and biomass accumulation. Annual rainfall was obtained from Bureau of Meteorology records. The purpose of the study was to quantify patterns of appearance of life states, and to identify environmental or previous life history states that might explain annual changes in flowering within the population. Specifically we asked: under what conditions is there the highest shift to flowering states?

Flowering was erratic and there was no regular pattern of transition between states among years. At the beginning of the study, only flowering plants were observed, and between 4% and 42% of plants flowered annually in subsequent years. However, there was an overall decline in flowering over the census period. Dormant plants comprised between 29% and 71% of the observed population annually, and sterile plants ranged between 5% and 67% annually. Total annual emergence varied from 25% to 75%. Periods of dormancy occurred for 0 to 5 years, with some plants showing multiple dormant periods. Nine of the original 54 plants were still not seen after eight years and were considered dead. Only one plant emerged consecutively each year. The total known population was 111 at the end of the census period.

Large, flowering plants were most likely to emerge and flower in the following year, while increasing dormancy was likely to lead to a reduction in emergence the following year. The population is most vulnerable to grazing in a sterile or fruiting state, although flowering plants are not affected.

Flowering increased with decreased levels of biomass, however there was no relationship between overall emergence and biomass. Rainfall in the year of flowering appeared to have little direct influence over flowering, and higher numbers of flowering plants in drier years probably relates to slower biomass accumulation and/or increased grazing pressure.

Time since fire was a predictor of flowering but not of emergence, with the latter possibly more strongly dependent on previous life history state. The data available showed that fire intervals of 3 years are likely to promote flowering, a fire frequency previously demonstrated as beneficial to other botanical values at the site. There is a risk of heavy grazing pressure in the first season after fire. Indications so far are that competition from associated vegetation strongly influences flowering in the population.

Further research is needed to identify optimum conditions for recruitment.

#### 4. Factors affecting fruit set in *Orchis morio*.

Jana Jersakova & Pavel Kindlmann\*

Faculty of Biological Sciences, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic. Email: pavel@entu.cas.cz

Reproductive success is one of the main determinants of fitness in perennial plants. Large sexual reproductive success in allogamous orchids is mainly associated with nectar reward for pollinators. Fruit set in nectarless orchids is negatively affected by the lack of nectar, which results in pollinator limitation within season. This is often cited as the main cause of low fruit production, given that hand pollination normally increased fruit set in comparison with control plants. Interest of pollinators in nectarless flowers may be associated with the size of the floral display, colour polymorphism, interspecific competition or facilitation for pollen vectors with other co-flowering species, and with density dependent intraspecific competition for pollinators. Flower production and fruit set can also be limited by resources. Resources available for seed development may be restrictive both within and between seasons. Elevated fruit set within one season may come at the expense of future growth, probability of flowering, reproduction or survival. However, certain species, like *Cypripedium acaule*, have to be highly pollinated for consecutive years to express symptoms of resource limitation. Some orchid species exhibit not only cost of fruiting, but also cost of flowering. Here we investigate factors that influence fruit set in the perennial orchid *Orchis morio*. Deception in this species causes pollinator limitation within a year - the average percentage of individuals, which did not set any fruits, was 17.6%. Hand pollination increased reproductive success to 100% fruit set without any fruit abortion. However, capsule size and weight and seed weight decreased toward the top of inflorescence and fruits on less pollinated plants were larger and heavier than those on fully pollinated ones. This indicates that plants have enough resources to develop capsules from all pollinated flowers, but competition for resources within the spike lowers the amount of assimilates available for each fruit. Thus resource limitation within a season is expressed in capsule size and weight, which is proportional to the number of seeds. Resource limitation between seasons had no effect on plant performance but it adjusted the number of flowers in the subsequent year, when pollination success in the preceding year had been very low (0-10%) or very high (90-100%). Flowering regime in *Orchis morio* was not affected by cost associated with high reproductive success or by flowering itself in the preceding two years. General understanding of the cost of fruiting derived from repeated hand-pollination experiments in former studies is extended here by critical assessment, whether it is realistic to assume that such costs may occur in orchid populations under natural conditions. Fruit set was also strongly affected by year and type of habitat. Population dynamics of *Orchis morio* is not as irregular as in other orchid species, as transitions from flowering to sterile or dormant stage were caused by other factors like local disturbance, vegetative reproduction, age of plant and type of management.

# 1. Population studies of terrestrial orchids – habitat management and annual counts of aboveground plants are only part of the picture.

Dennis F. Whigham<sup>1</sup> and Jo H. Willems<sup>2</sup>

1. Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037 USA. Email: whigham@serc.si.edu

2. Plant Ecology Group, Utrecht University, PO Box 800.84, 3508 CA, Utrecht, The Netherlands. Email: J.H.Willems@bio.uu.nl

Terrestrial orchids are threatened and endangered species on all continents but the global patterns are highly variable. Thirteen species of terrestrial orchids have been listed as endangered and threatened at the national level in the U.S. and Canada. Australia and New Zealand have more than 35 species of terrestrial orchids that have been listed as endangered, vulnerable, or extinct. In sharp contrast, only a single species (*Ophrys kotschii*) has been listed as endangered in Europe and only two species (*Liparis elliptica*, *Cypripedium macranthum* var. *speciosum*) are protected at the national level in Japan. In almost all documented situations, species declines have been primarily attributed to habitat modification or removal of plants from native habitats. In recent years, efforts have been made to conserve or restore populations of terrestrial orchids, mostly through habitat management. Some efforts are international (e.g., Orchid Specialist Group of the Species Survival Commission of IUCN) but most are national (e.g., Endangered Species Act enforced by the U.S. Fish and Wildlife Service, Red Data books in Europe). Few efforts have been made to develop management plans based on either long-term studies of populations or restoration of populations using plants grown *in vitro* or *in situ*. In this paper we consider efforts that have been made to conserve or restore species of terrestrial orchids. Most efforts involve habitat conservation as the keystone management tool. Habitat restoration and management are also important elements of efforts to conserve threatened species of terrestrial orchids. While conservation, restoration and management are likely to be successful in many instances, it presumes that all of the species requirements will be met within the conserved area. We suggest that more ecological information is required to increase the probability of success of conservation and restoration efforts, especially if rehabilitation of species is of special concern. In particular, we recommend that additional long-term population studies are needed in order to understand basic ecological processes in the range of species representing different life history types. These studies should have a continental focus and include species representative of all life history types. To date, population dynamics have been examined in detail for relatively few species representing only a small number of life history types. It seems especially important to expand research into aspects of seed germination and the ecology of protocorms, particularly interactions with mycorrhiza. Almost all long-term studies of terrestrial orchid populations have focused on above-ground plants. Several authors have shown, however, that population parameters are influenced by individuals that may remain dormant for one or more years. Early life history stages have received little attention because seeds and protocorms are very difficult to observe and manipulate experimentally. Recent advances in techniques to study seed germination *in vivo* have been useful in studying early life history stages, including protocorm development. It seems especially important to study interactions between protocorms and the obligate interactions that they have with mycorrhiza. We present data from recent studies of early life history stages of terrestrial orchids, including *in situ* and *in vitro* studies of mycorrhiza. We will demonstrate that some species of terrestrial orchids growing in the same general habitat develop associations with several different mycorrhiza whereas other orchid species utilise a single mycorrhizal type. Some orchid mycorrhiza are ectomycorrhiza while others utilise a variety of organic substrates. Data from field and laboratory studies will be used to examine the relationships between protocorm growth and mycorrhiza.

## 2. TBA

Michael Hutchings  
University Of Sussex, Falmer, Brighton, Sussex, BN1 9QG, UNITED KINGDOM, Email:  
M.J.Hutchings@sussex.ac.uk

[Abstract not received]

### 3. Population dynamics in orchid conservation: A review of analytical methods based on the rare species *Lepanthes eltoroensis*.

Raymond Tremblay<sup>1</sup> & Michael Hutchings<sup>2</sup>

<sup>1</sup> Department of Biology, University of Puerto Rico - Humacao Campus, Humacao, Puerto Rico, 00791, USA.  
Email: raymondtr Tremblay@hotmail.com

<sup>2</sup> School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, BN1 9QG, United Kingdom.  
Email: M.J.Hutchings@sussex.ac.uk

Predicting population persistence, and consequently population and species extinction, depends on our ability to gather and evaluate data on population growth rate and the variables that may influence growth rate.

1. The total variation in growth rate is the sum and interaction of individual, demographic, and environmental variation.
2. Environmental variation can be further broken down into temporal, spatial and catastrophic variation.
3. If we know the effect of each of these parameters on growth rate then we can predict the probability of population growth rate and extinction.
4. Of all the different variables that can cause errors we need to know which of these are most important for conservation of orchids.
5. Using the method described in this paper, we can start evaluating the variation caused by each of these life cycle parameters and the effect of time and space.
6. In this paper, we will describe how to measure the basic growth rate of populations (Lefkovitch model), and derive a 95% confidence interval on growth rate from inclusion of each of these parameters.
7. To evaluate the probability of extinction, we must look at elasticities and the demographic analysis to understand which of the matrix elements are most likely to be in need of manipulation within the population for improving population growth rate. This system is extremely powerful and gives us the opportunity to establish experimental design and test for significant growth rate differences among treatments using the Monte Carlo simulation in time and space.
8. We will use *Lepanthes eltoroensis* as an example to show the value of the above method.



# 1. The Impact of International Trade on Orchids

Robert R. Gabel

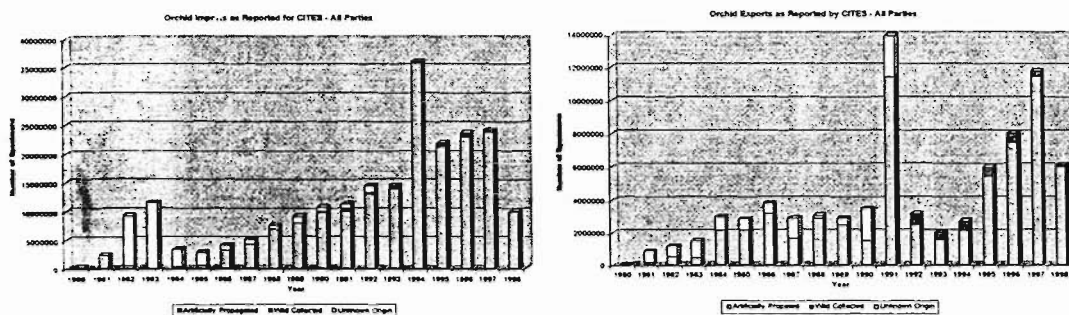
Division of Scientific Authority, U.S. Fish and Wildlife Service, 4401 N. Fairfax Drive, Room 750, Arlington, Virginia, 22203, USA. Email: rodgy\_gabel@fws.gov

The fact that orchids are traded internationally in significant numbers and the potential for trade to affect the conservation status of orchid species has long been recognised. Because trade levels were significant, all orchids have been included in the appendices to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since its inception in 1975. Although the vast majority of species are included in CITES Appendix II, which allows for regulated commercial trade, relatively few species (*Cattleya trianae*, *Dendrobium cruentum*, *Laelia jongheana*, *L. lobata*, *Paphiopedilum* spp., *Phragmipedium* spp., *Renanthera imschootiana*, and *Vanda coerulea*) which are believed to be threatened with extinction in the wild, and for which trade is a particular threat, are included in Appendix I. The IUCN/SSC Orchid Specialist Group, in *Orchids—Status Survey and Conservation Action Plan* (1996) recognised that over-collection of showy and/or rare orchids can be a major contributor to their decline in the wild.

A few species, such as *Phalaenopsis javanica*, are known to have become extinct or rare in the wild due to over-collection, and others, such as *Paphiopedilum rothschildianum*, are extremely vulnerable. Recently, newly discovered plants in the genus *Paphiopedilum* have been initially described from specimens in trade, before their actual wild localities were known. When their wild habitats have been finally documented, the plants have already been collected to near-extinction.

Artificial propagation is recognised as a means of providing large numbers of high-quality orchids for the horticultural market, which should coincidentally reduce pressure on wild populations. However, while CITES data indicate that the vast majority of orchid plants in trade are artificially propagated (>97% in recent years), trade in wild orchids still involves hundreds of thousands of specimens annually.

The CITES Plants Committee is undertaking a review of the listing of the Orchidaceae in the CITES Appendices, to determine if there is some alternative approach to the listing that will focus trade controls on species for which trade is an actual potential threat. However, this process will require the committee to address problems with data errors or gaps in trade data and a lack of information on the actual biological status of individual genera or species (distribution, population size and trends, security of habitats).



## 2. CITES – Conservation showpiece or opportunity lost.

Geoff Stocker

Stocker's Nursery, PO Box 188, Malanda 4885, Queensland, Australia. Email: [gstocker@austarnet.com.au](mailto:gstocker@austarnet.com.au)

The effectiveness of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) for the conservation of orchids is examined from the perspective of the author, a domestic and international trader in propagated orchid plants and an ecologist who has worked extensively on the management of the world's greatest orchid habitats, the tropical forests. Although CITES has been a very important factor in reducing trade in wild collected plants, it does not have a good image among many in the orchid growing community. The reasons for this situation are reviewed and although some of the criticisms can be easily dismissed, others appear worthy of closer examination or explanation. Broader questions on the CITES approach to orchid conservation, including the lack of quantitative data on the population biology of most orchid species, the problems associated with plant salvage from forests during timber harvesting and conversion to alternative agricultural use and the injustices created in some areas where local forests owners are no longer able to sell plants growing in their forests, are also canvassed. From a nurseryman's viewpoint, the most serious immediate problem with CITES appears to be that it is unnecessarily restricting trade in propagated plants. While CITES may be well intended, in some countries the related regulations are administered in such a way that many seriously question the technical competence of the enforcement authorities. The ensuing lack of confidence has promoted an underground trade which to some degree has undermined attempts by propagators to take the pressure off wild populations under threat by commercial collectors. It is concluded that a reworking of CITES in relation to orchid conservation appears necessary if this significant convention is to have greater support among orchid growing hobbyists world-wide. It is these people who will ultimately determine whether CITES will be successful or prove to be an opportunity lost.

### **3. TBA**

Gloria L. P. Siu

Kadoorie Farm and Botanic Garden, Lam Kam Road, Tai Po, New Territories Hong Kong, CHINA. Email: lpsiucon@kfbg.org.hk

[Abstract not received]

#### 4. Traditional usage of orchids in China.

Jin Xiaohua, Qin Haining and Gloria, Siu Laiping

1. Institute of Botany, Chinese Academy of Sciences, 100093 Beijing, China.

2. Kadoorie Farm & Botanical Garden, Hong Kong.

Under an investigation financially supported by the Hong Kong Kadoorie Botanical Garden and farm, we found many previously unknown but interesting uses of orchids by the Chinese people.

The Miao and Buyi, two minority people groups, live in the Southwest Guizhou province of southwest China and use *Goodyerinae* as a rare medicine for bruising and broken bones. The local market price for these small plants is very high. But the people in the Guangxi Province treat them as horticultural specimens, as do the Taiwanese people, where the golden veins of the plants represent happiness.

The Chinese cultivate *Paphiopedilum concolor* as a horticultural subject, but the people of Nonggan Natural Reserve in Guangxi province use it as medicine. They soak the leaves in wine and named it "blue sky and purple ground solution", which may be from the *P. concolor* leaves as a cure for back-ache.

In China, all the species of the genus of *Bletilla* are exploited medicinally with *B. striata* the most commonly used species. During the survey we found another genus used in the same way as *Bletilla*. The tuber of *Anthogonium gracile* Lindl. is very similar to that of *Bletilla*, especially when the plant is without flowers. Farmers of Guizhou province collect the tuber and dry them. To our surprise, the dealers purchase them and distribute them throughout the Chinese medicine market. As with *Bletilla* itself, it not only used as a medicine, but also has many other uses. The people of PU-an county consider them as a delicious food. They stew *Bletilla* with meat, and utilise it as a tonic.

As in many other countries, the tubers of Orchidinae, especially those of *Habenaria*, are used as aphrodisiacs in some villages. They collect the tuber from the wild, and cook them with pork. Many infuse the tubers in rice wine and drink the wine as an aphrodisiac.

The beautiful *Dendrobium loddigesii* is much sought after in China for its medical value (the medicinal name is Small Huangcao). But the people of Jinxi county (Guangxi Province), also use the species as an insecticide. They pound the plant and mix it with sugar and when flies are attracted to the sugar mixture, it kills them.

Also in Guangxi, the people use the plants of *Flickingeria*, (Yaoguanshihu (Legs of Locust)), as a substitution of *Dendrobium*. People in Yunnan Province also use *Flickingeria* for lung and throat ailments.

The medicinal name which is uniform in most places is Qintianqui, the dry leaves of *Nervilia*. There are 7 species of *Nervilia* in China, all are used as medicine and almost always under the same name. Usually, in one bag, Qintianqui, there can be up to 3 to 4 species mixed together. But the most used species is the widely distributed *Nervilia aragoana*. Peasants collect the plant (usually without the tuber, which remains in the soil) and dehydrates them to treat lung and internal injuries.

"Shixiantao" is a complex medicine which, according to our work, includes no less than 5 genera: *Bulbophyllum*, *Pholidota*, *Coelogyne*, *Calanthe*, and *Liparis*. The most commonly used genera are *Pholidota* and *Coelogyne*. The pseudobulbs and stems are collected and sold to local people fresh at local markets or dried and sold to dealers, who distribute them to other markets. The usage varies widely. In Yinchou district of Guangxi Province, "shixiantao" is used to cure coughs and asthma, while in Liuzhou district, the medicine is used to treat stomach pains.

#### Acknowledgments

The program is financially supported by the Kadoorie Farm & Botanical Garden team in Hong Kong and is also supported by the Forest Department of China.

### Thursday: Session A

Orchid Specialist Group Meeting. Chairs: **Phillip Cribb & Shelagh Kell**

1. Introduction: **Phillip Cribb** and **Shelagh Kell**
2. OSG strategic plan: **Phillip Cribb**
3. Secretariat report: **Shelagh Kell**
4. *Regional group reports:*
5. Afro-Madagascar: **George Mugambi**
6. Australasia: **Kingsley Dixon**
7. East Asia: **Gloria Siu**
8. Indian Subcontinent: **Udai Pradhan**
9. Meso America: **J Warner**
10. North America: **Marilyn Light**
11. South America: **Alex Hirtz**
12. Europe: **Phillip Cribb**

### Thursday: Session B

OSG Regional workshops. Chairs: **Regional Chairs**

1. *Ex situ* conservation group report: **Phil Seaton**
2. Use of Revised Red List Criteria, RAMAS Red List and SIS: **Wendy Strahm**
3. Any other business
4. **Date and venue of next meeting:** 17<sup>th</sup> WOC, Shah Alam, Malaysia.

## 1. Genetic erosion in Italian populations of threatened orchids: *Cypripedium calceolus*, *Orchis palustris* and *Dactylorhiza incarnata*.

Walter Rossi<sup>1\*</sup>, M. Cristina Mosco<sup>2</sup> and Luciano Bullini<sup>2</sup>

1. Dept. Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy. Email: vrossi@univaq.it

2. Dept. Genetics and Molecular Biology, University of Rome "La Sapienza", Via dei Sardi, 70, 00185 Rome, Italy. Email: cristina.mosco@uniroma1.it; luciano.bullini@uniroma1.it

It is often difficult to ascertain if the low genetic variation found in endangered populations is the outcome or the cause of their size reduction. However, genetically depauperate populations appear on average to be more endangered than others: they often show reduced fertility, are more affected by parasites and pathogens, are less competitive than variable populations having similar ecological requirements, etc. For conservation purposes, it is important to monitor genetic variation of threatened populations and species and to restore variation when depauperate. In this study, genetic variation in Italian populations of the threatened Lady's Slipper Orchid, *Cypripedium calceolus*, the Bog Orchid, *Orchis palustris*, and the Early Marsh Orchid, *Dactylorhiza incarnata*, has been evaluated at 15-30 enzyme loci. The observed values have been compared with those found in populations of common, phylogenetically close species. *C. calceolus* populations from the Alps show an average  $H_e = 0.09$ . This value strongly decreases in the central Apennines, reaching  $H_e = 0.03$  in the Maiella Mountain. The related *C. parviflorum* from Canada shows  $H_e = 0.16$ . Low values were observed in all Italian populations tested of *O. palustris* ( $H_e = 0.02-0.04$ ), whereas in related *Orchis* species  $H_e$  reaches 0.17. In *D. incarnata*, it was found to be monomorphic for all the loci studied ( $H_e = 0$ ) throughout Italy. In the related *D. fuchsii*,  $H_e$  ranges from 0.11 to 0.17. In order to restore genetic variability in the Apennine race of *C. calceolus*, transplantation experiments of samples from the Alps could be carried out, the two populations being not significantly differentiated from each other at genetic level. This should be done in connection with habitat restoration measures, uncontrolled beech-wood expansion having relictual favourable sites. In *O. palustris*, the differential loss of alleles found in populations respectively from northern and southern Apennines would suggest transplantation experiments to restore polymorphism. Also in this case, however, the protection of its natural habitat (marshy grassland) is a priority. To restore genetic variation of *D. incarnata* is more problematic; so far, one population from Ireland has shown some degree of genetic polymorphism; moreover, this species is often out-competed by its hybrid derivatives, the allotetraploid marsh orchids *D. majalis* s.l., *D. elata* s.l., etc.

## 2. Conservation genetics of rare and endangered British Orchids.

Faridah, Quamaruz-Zaman<sup>1\*</sup>, Michael F. Fay<sup>2</sup>, John S. Parker<sup>3</sup>, M. W. Chase<sup>2</sup>

1. Genome Centre, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia. Email: fqz@ibs.upm.edu.my

2. Jodrell Laboratory, Royal Botanic Gardens, Kew, Surrey, TW9 3AB, UK. Email: m.fay@rbgkew.org.uk; m.chase@rbgkew.org.uk

3. Cory Laboratory, University Botanic Garden, University of Cambridge, Cambridge CB2 1JF, UK. Email: jsp256@cam.ac.uk/jsp30@cam.ac.uk

An understanding of the level, structure and origin of genetic variation within and among populations of rare and endangered species is essential for devising optimum management strategies for their conservation. Recently a wide range of molecular techniques has been devised to assess genetic diversity. For this research on rare British orchids, amplified fragment length polymorphisms and plastid microsatellite markers were chosen. Amplified fragment length polymorphisms were used to partition genetic variation within and among all populations of the rare British orchids *Orchis simia*, *Orchis militaris* and *Liparis loeselii*. Where available, samples from outside United Kingdom were included to compare the level of genetic variation. The genetic variation in *Orchis mascula* was investigated to study patterns in a common relative of *Orchis*.

It has been hypothesised that the present distribution of disjunct populations of *Orchis simia*, *Orchis militaris*, and *Liparis loeselii* is the result of events occurring during the last glaciation. Among the relatively limited number of plant phylogeographic studies, the majority have relied on tree species to reveal glacial refugia, postglacial migration routes and loss of genetic diversity. Relatively little effort has been spent on herbaceous species to date. This type of plant group may provide the best model systems for future studies aimed at illuminating the role of Quaternary climate changes in driving diversification and speciation. Short-lived herbs go through a higher number of life cycles within a given time period than long-lived trees, and may have responded more quickly to environmental change in terms of Quaternary time scales. In this respect these orchid studies would be of interest to fill the current gap. With this in mind, plastid microsatellites were utilised to elucidate postglacial recolonisation events of some of the orchid species.

The results showed that for *Orchis*, amplified fragment length polymorphisms differentiated the United Kingdom and non-United Kingdom populations. Among the United Kingdom populations, there are distinct genepools. The plastid microsatellites confirmed these findings. The results from amplified fragment length polymorphisms of *Liparis loeselii* allowed the distinction between United Kingdom and non-United Kingdom populations. *Liparis loeselii* var. *loeselii* and var. *ovata* were not genetically distinct, although samples of var. *loeselii* showed much greater variation. This has implications for conservation where the preservation of natural levels of genetic variation within a species is a priority as fenland populations are more variable than dune slack populations.

### Acknowledgements

The first author would like to acknowledge Universiti Putra Malaysia, the Malaysian Government and the Overseas Research Students' Award Scheme (United Kingdom) for their funding and support in this project.

### 3. Population genetic structure of species of *Epipactis*.

Peter M. Hollingsworth<sup>1</sup>, Richard M. Bateman<sup>\*2</sup>, James H. Dickson<sup>3</sup>, Jane Squirrell<sup>4</sup> and Mark Tebbitt<sup>5</sup>

1. Royal Botanic Garden, 20A Inverleith Row, Edinburgh, EH3 5LR, UK. Email: P.Hollingsworth@rbge.org.uk

2. Botany Department, Natural History Museum, Cromwell Road, London SW7 5BD, UK. Email: R.Bateman@nhm.ac.uk

3. Division of Environmental and Evolutionary Biology, University of Glasgow, Glasgow, G12 8QQ, UK. Email: gbt08@udcf.gla.ac.uk

4. Department of Agricultural and Environmental Sciences, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, NE1 7RU, UK. Email: Jane.Squirrell@newcastle.ac.uk

5. Brooklyn Botanic Garden, 1000 Washington Avenue, Brooklyn, New York, 11225-1099, USA. Email: marktebbitt@bbg.org

Deviations from random mating are an important determinant of plant species evolution, and taxonomic complexity is often associated with departures from the classic Hardy-Weinberg equilibrium mode of population genetic structure. This can result in difficulties in assessing conservation priorities in such groups, if the taxonomic status of rare 'entities' is controversial. In *Epipactis*, this is a pertinent issue as breeding system transitions are considered to be an important mode of speciation; some of the species are considered to be outcrossers, whereas others have floral morphology consistent with self pollination. We have used molecular markers to investigate the amounts and partitioning of genetic variability in 26 species of *Epipactis* with different floral morphologies, distributions and propensities for vegetative spread. The correlations between these factors, and population genetic structure will be discussed, as will the implications for their conservation. These results will then be set in the context of a literature review of published studies of population genetic structure in orchids. Finally we will address some issues regarding the suitability of different molecular marker systems for population/conservation genetic studies. Recent developments in molecular genetic technologies have provided a suite of high resolution marker systems which have opened up a wealth of opportunities for insights into the behaviour of natural populations. We will briefly discuss some empirical and theoretical studies highlighting the potential of these techniques to inform, but also to show where caution is required in their interpretation.



#### 4. Species delimitation and conservation prioritisation: operating at the boundary between phylogenetics and conservation genetics.

Richard M. Bateman\*<sup>1</sup> and Peter M. Hollingsworth<sup>2</sup>

1. Department of Botany, Natural History Museum, Cromwell Road, London SW7 5BD, U.K. Email: r.bateman@nhm.ac.uk

2. Royal Botanic Garden Edinburgh, 20A Inverleith Row, Edinburgh, EH3 5LR, U.K. Email: p.hollingsworth@rbge.org.uk

Although species will always be the fundamental unit of conservation, in practice the delimitation of orchid species currently often falls through the rarely discussed conceptual gap between phylogenetic and population genetic studies. Phylogenetic approaches typically view evolution from the side, along a time axis, and are usually strongly typological, being based on comparison of data from few individuals of many species. Many population-level studies view evolution from above, thereby lacking an explicit time axis but allowing species delimitation by quantifying a greater number of individuals representing far fewer putative species. Both categories of analysis can be based on morphological data, molecular data, or both. Molecular characters are in most cases more numerous, more easily compared among species, and immune to convergence reflecting natural selection. Morphological characters remain essential for identifying species in the field and interpreting the processes underlying their evolution, as well as giving resolution to inconclusive molecular phylogenies that reflect rapid evolutionary radiations. In two long-term projects, we and our collaborators have investigated the molecular phylogenetics of the Tribes Orchideae (Orchidoideae, 2–3 genes) and Neottieae (Epidendriodeae, 4 genes), before applying a range of population-level techniques (notably allozymes, AFLPs and morphometrics) to chronic species-delimitation challenges in “critical” (and potentially over-split) terrestrial genera such as *Dactylorhiza*, *Gymnadenia*–*Nigritella*, *Epipactis* and, to a lesser degree, *Ophrys*. Taken together, these studies suggest that contrasting constraints on the reliability and applicability of specific types of data within these two broad categories offer renewed challenges to conceptualising and delimiting species. An idealised method of species delimitation, termed “demographic systematics”, requires large-scale sampling of individual plants within populations distributed across the full geographical and ecological range of the presumed species. Both morphological (morphometric) and molecular (population genetic) characters are quantified and then combined in a computerised ordination analysis, in search of the phenotypic and genotypic discontinuities that offer the most practical boundaries separating species. Although resource-intensive, this approach brings the longer term conservation benefits of removing from consideration the many orchid species that have been formally named but have no biological reality, while also revealing previously unrecognised but *bona fide* cryptic species.

## 1. Restoration genetics of *Caladenia* in urban bushland remnants in Perth, Western Australia.

Siegfried L. Krauss and Robyn Taylor

Kings Park and Botanic Garden, Botanic Gardens and Parks Authority, Fraser Ave, West Perth, Western Australia, 6005, Australia. Email: skrauss@kpbg.wa.gov.au

A knowledge of the genetic structure of populations, and the factors influencing these patterns, may be critical for the long-term success of species and community restoration and reestablishment projects. Important issues in which genetic markers can contribute in a practical way include the delineation of provenance (where can we collect seed from if targeting local genotypes?), the identification of levels of genetic variation within and among populations (how much genetic variation do we re-establish?), patterns of mating and gene flow (is it important that populations are connected?) and the specificity of relationships with endophytic mycorrhiza (do populations of an orchid species share the same mycorrhizal genotype associations?). For example, the introduction of non-local genotypes may compromise the success of re-establishment efforts if poorly adapted to the local environment. Alternatively, the introduction of non-local genotypes may swamp a locally significant variety. We are applying the DNA fingerprinting technique Amplified Fragment Length Polymorphism (AFLP) to address these issues for the restoration of spider orchid species (*Caladenia arenicola*, *C. georgei* and *C. huegelii*) in urban bushland remnants in Perth and rare or threatened taxa elsewhere in Western Australia (*C. winfieldii*, *C. radiata*, and *C. serotina*). We have found high levels of polymorphism within populations and a partitioning of variation within (80%) and among (20%) populations that is typical of outcrossers. However, we have also found complex provenance relationships that indicate a simple linear relationship between genetic and geographic distance does not apply and suggests subtle ecological differentiation over small spatial scales. For example, one population of *C. arenicola* in Kings Park was not differentiated genetically from a population in Bold Park (8 km away), but was significantly differentiated from three other populations less than 1 km away within Kings Park. We have found typically small conversions of flowers to fruits (<5%), and AFLP is being used to assign paternity to offspring to assess variation in male mating success, pollen flow and gene flow. These mating patterns indicate that effective population size is substantially smaller than the standing population, which can significantly influence future levels of genetic variation in the absence of gene flow. We are building on these genetic results for *Caladenia* spp. to assess genetic variation within and among populations of their endophytic fungi, again with AFLP, to identify specificity to their hosts. Results elsewhere (e.g. Taylor and Bruns, 1999, Mol. Ecol 8:1719-1732) have shown striking specificity between orchids and their fungi, and that this information is critical for the growth and reintroduction of orchids.

## 2. Orchid conservation genetics - How many data do we need?

Michael F. Fay<sup>1</sup>, Mark W. Chase<sup>1</sup>, Robyn S. Cowan<sup>1</sup> & Mikael Hedrén<sup>2</sup>

1. Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3DS, UK. Email: m.fay@rbgkew.org.uk

2. Dept. of Systematic Botany, University of Lund, 223 61 Lund, Sweden.

A wide range of techniques is now available for assessing levels and patterns of genetic diversity, and a various methods have now been applied to questions relating to conservation genetics, including isoenzymes, AFLPs and nuclear and plastid microsatellites. In this talk we will illustrate the use of some of these techniques, showing advantages and disadvantages. *Cypripedium* and *Dactylorhiza* will be used as the main examples, and pitfalls resulting from using data sets derived with only a single marker type will be discussed and possible reasons identified for these problems.

### 3. Application of genetic tools for the conservation of orchids: premise and pitfalls.

Rod Peakall\*, Daniel Ebert, Jim Mant and Florian Schiestl

Division of Botany and Zoology, Australian National University, Canberra ACT 0200 Australia. Email: rod.peakall@anu.edu.au

Microsatellites have become widely recognised as powerful and informative genetic markers in both plants and animal. The utility of microsatellites results from their inherent variability, typically far exceeding that of other kinds of genetic marker. As a consequence, microsatellites are becoming the marker of choice for the investigation of a wide range of questions in evolutionary biology, molecular ecology and conservation genetics. The one drawback of this method is the cost of developing the markers. As part of an ongoing multidisciplinary investigation of the ecology and evolution of pollination by sexual deception within Australia terrestrial orchids, we are developing a set of more than 30 microsatellites to investigate clonal structure, pollen flow, hybridisation and species relationships within the genus *Chiloglottis*. In this paper we will briefly describe the process of marker development before illustrating the application of the markers to the assessment of clonality and paternity in *Chiloglottis valida*. We will also compare the intra-generic relationships revealed by microsatellites with those revealed by other methods. Finally we will evaluate the advantages and disadvantages of using microsatellites for orchid conservation related investigations.

## 1. Orchid conservation efforts in Costa Rica.

Denise C. Wilson

Orchid Specialist Group, Denver & Boulder Orchid Societies, 34316 Gap Road, Golden, Colorado, USA 80403. E-mail: DeniseWil@aol.com

Denise Wilson is a grower, writer, photographer and educator who has spent the past decade travelling the world studying and protecting jungle orchids. She worked in all aspects of a commercial orchid greenhouse start-up for five years. She has served on the Board of Directors for both Denver and Boulder Orchid Societies, and is presently touring a program on orchid conservation to American Orchid Society chapters around the United States. Her article on growing orchids high in the Rocky Mountains was recently published in the AOS magazine, ORCHIDS.

Get an insider's look at ongoing efforts to protect orchids and other species in their natural environments in Costa Rica's national and private preserves. This visual adventure takes you from the private reserve at Hacienda Baru on the sweltering southern Pacific coast, to the cool, high-plateau cloud forests of Monte Verde. Featured are Lankester Botanical Gardens displays, miniature orchids from Gabriel Barboza's Orchid Garden, and the 2<sup>nd</sup> Meso American Seminar on Orchidology and Conservation.

You'll see how the management of these preserves are using ecotourism, *ex situ* collections, habitat protection, public education, reintroduction, and commercial flasking to rescue doomed orchids. Participants will also receive a primer on the Convention on International Trade in Endangered Species (CITES) and how you can contribute to this vital effort.

## 2. Biogeography and orchid conservation in New Zealand: Case studies from the Department of Conservation orchid files.

John Sawyer\* and Peter de Lange

Department of Conservation, Wellington Conservancy, P.O. Box 5086, Wellington, New Zealand. Email: jsawyer@doc.govt.nz, pdelange@doc.govt.nz

An overview of New Zealand native orchids and their conservation management is presented. There are thought to be 120 orchid species in New Zealand; a new system for classification of species conservation status has led to the listing of 10 nationally threatened species, 8 naturally uncommon species and 3 species in decline. Various attempts have been made at orchid conservation, including the translocation of orchids and research on fire disturbance in temperate communities. A brief overview of those attempts is described. Orchid conservation in New Zealand is in its infancy as shown by: taxonomic uncertainty in several genera; poor orchid distribution information; and a poor understanding of orchid ecology. A regional approach at orchid conservation in the lower North Island demonstrates how those problems are starting to be overcome.

We estimate at least 60 orchid species occur in the lower North Island (3 nationally threatened species, 3 naturally uncommon, 1 in decline, and 9 regionally threatened). However, the taxonomy of genera such as *Caladenia*, *Corybas* and *Pterostylis* is uncertain so the total number of species cannot be known exactly. The Department of Conservation has collated information about those species onto a regional plant database since 1993. More recently a national plant database has been established (BIOWEB) upon which all information about the biology, ecology and distribution of New Zealand's native orchids is stored.

Orchid species distributions in the lower North Island have been mapped and an assessment made of the extent to which they occur in protected natural areas. The extent to which species have declined has also been determined, as measured by species range contractions or loss, or contraction in size, of orchid populations. Orchid biogeography is now being applied in the development of regional plant conservation strategies.

Case studies demonstrate the various approaches used to conserve orchid populations in the lower North Island. Conservation management activities include: habitat protection (fencing, weeding and wild animal control); legal protection of land; *ex-situ* practices; habitat restoration (including translocation); survey and monitoring; research and public awareness.

### 3. An overview of the conservation status of the orchids of Victoria.

Gary Backhouse<sup>\*1</sup>, Fiona Coates<sup>2</sup> and James Todd<sup>1</sup>

1. Flora and Fauna Directorate, Department of Natural Resources and Environment

14/8 Nicholson Street, East Melbourne, Victoria, 3002, AUSTRALIA. Email:

Gary.Backhouse@nre.vic.gov.au, James.Todd@nre.vic.gov.au

2. Biodiversity Research, Arthur Rylah Institute, Department of Natural Resources and Environment, 123

Brown Street, Heidelberg Victoria 3084, AUSTRALIA. Email: Fiona.Coates@nre.vic.gov.au

The Australian State of Victoria lies on the south-east corner of the Australian mainland, and covers an area of about 227 000 km<sup>2</sup>, which is less than 3% of the total land area of the Australian continent. Despite this small area, Victoria supports a wider range of broad ecosystem types than any area of comparable size in Australia. Habitats include coastal scrubs, heaths, mallee, dry woodlands, wet forests including rainforest, alpine woodlands and herbfields and wetlands. Orchids can be found in virtually all of these habitats, from coastal sandunes to the tops of the highest mountains, from dry mallee 'deserts' to permanent wetlands.

Reflecting this diversity of habitats, Victoria has a relatively rich orchid flora, with about 330 species in 31 genera recorded for the State (about 25% of the total orchid flora for Australia). About 135 species (40%) are either endemic or confined almost entirely to Victoria. As the prevailing climate is temperate, with hot dry summers and cold wet winters, only five orchids are evergreen epiphytic or lithophytic species, the remainder being deciduous geophytes.

Natural habitats in Victoria have been impacted heavily by almost two centuries of European settlement. About 60% of Victoria is private land, much of which is substantially cleared for agricultural, industrial and urban development. Some habitats, such as native grasslands, grassy woodlands and shallow freshwater marshes have been almost mostly destroyed. Even on the public land estate, habitat changes have been substantial in many areas, through the impact of altered fire regimes, weed invasion, rabbit and kangaroo grazing, mineral and timber production, agricultural and recreational activities. The extent of this impact is reflected in the list of rare or threatened orchids, with an estimated 180 (55%) of Victoria's orchids considered rare or potentially threatened, including at least seven species presumed extinct, 53 species considered endangered and 41 species considered vulnerable. About 55 species (17% of Victoria's orchid flora) are considered rare or potentially threatened at the national level.

Conservation programs are currently underway for about 42 threatened orchid species. Recovery actions include preparing multi-species recovery plans, site protection, habitat management, intensive intervention such as hand-pollination, seed sowing and reintroduction, and research on germination, cultivation and population dynamics. While governments have a lead role in these conservation programs, the development of partnerships with community groups, industry, botanic gardens, universities and zoos is considered essential. These partnerships bring together a wide range of complementary skills, expertise and resources to maximise chances for successful recovery. Substantial challenges to recovery remain, including poorly defined taxonomy of many species, difficulties of identification in the field, the complex ecological relationships many orchids have, such as with their mycorrhizal fungi and insect pollinators, overcoming climatic and environmental variability, and the development of laboratory techniques for application in the field.

#### 4. Diversity and conservation of limestone orchids in Sarawak, Malaysia.

Rusea, Go\*<sup>1</sup> and A. Julaihi<sup>2</sup>

1. Department of Biology, Faculty of Science & Environmental Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

2. Herbarium, Forest Research Centre, Sarawak Forest Department, Kuching, Sarawak, Malaysia.

Limestone is of wide occurrence in Sarawak. There are six massive areas that are precipitous striking features of the landscape with altitudes up to 700 m above sea level yet these represent less than 5% of the total land area of Sarawak. The limestone flora is exceptionally rich, home for over six hundred species (Anderson, 1921) and is extremely high in endemism (Kiew, 1991). The high flora diversity is supported by the varied topography, ecological parameters and unique microhabitats of limestone areas. Limestone vegetation is well known for its richness with endemic plant species, which includes many orchids. Most of these orchids are of high commercial value as far as wild orchid collectors and enthusiasts are concerned. In Sarawak, there are more than 90 species of orchids confined to limestone habitats. Almost 50% of the 350 specimens of limestone orchids deposited in Sarawak Herbarium are from Bau area and 30% from Mulu National Park area. There are at least 10 known species endemic to Sarawak or to one locality. *Paphiopedilum sanderianum* and *Cymbidium borneensis* are reported only from Mulu National Park, Miri and *Paphiopedilum stonei* and *Habenaria marmorophylla* are endemic to the Bau limestone area. Limestone areas represent fragile habitats and are being threatened by quarrying concessions and over-collecting, resulting in extinction of endemic plants in many of these areas. Due to this pressure on orchid habitats, *ex-situ* conservation is often the only alternative for species survival. In addition, limestone vegetation is poorly studied and collections of herbarium specimens are few, therefore the possibility of disappearance of unnamed species is a risk.



## **1. The importance of the National Plant Collection schemes in orchid conservation.**

Henry Oakeley

Royal Horticultural Society, 77 Copers Cope Road, Beckenham, Kent, BR3 1NR, UNITED KINGDOM. Email: henry.oakley@virgin.net

This paper outlines how the recording within a National Collection of not only the structure but also the function, pollination, habitats, distribution, commercialism, cultivation, flowering patterns, fragrance, DNA, chromosome counts, evolution, discovery, History, their art and literature, along with breeding and conservation programs gives the botanist a wealth of data for use in taxonomy, and makes a National Collection more than just a number of plants at the bottom of the garden.

## **2. The role of the Australian Native Orchid Society Inc. in orchid conservation.**

Alan Dash

Conservation Officer, The Australasian Native Orchid Society Inc., 23 Yeramba Crescent, Terrigal 2260, New South Wales, AUSTRALIA.

### **1. THE SOCIETY:**

The Australasian Native Orchid Society (ANOS) consists of a loose association of 23 individual Groups, 20 in Australia, and 3 in New Zealand. There is a number of unattached members also, who generally reside in areas remote from Group centres or live overseas.

The objectives of A.N.O.S. include promotion of the conservation of native orchids in their natural habitat as well as to increase the scientific and cultural knowledge of Australasian orchids.

The Society publishes a high quality journal, "The Orchadian", which is used as a vehicle for the publication of descriptions of new Australasian species and regularly includes articles detailing research into various aspects of native orchid biology. A recent copy of this journal is to be distributed to all congress registrants. There is also a popular Website at [www.anos.org.au](http://www.anos.org.au)

Membership is relatively small (about 1000), and many members also belong to other orchid societies. For the majority of ANOS members, the primary activity is the cultivation and exhibition of native orchid species and hybrids, but there is a significant (and growing) number who are also very involved in observing, photographing, drawing and recording various facets of wild orchid populations. A keen interest in conservation of native orchids and native orchid habitat is a natural extension of these interests.

### **2. CONSERVATION ACTIVITIES**

Many of Australia's approximately 1200 native orchid species are now at critical levels.

Some of the many roles of ANOS in orchid conservation are: i) Assisting and cooperating with research and conservation agencies in projects to help in the survival of some of the most endangered species and include recording, mapping, fencing, weeding, preparing submissions for nomination as threatened species and cultivating of near-extinct species for future re-introduction. ii). In conjunction with others, introducing a national exhibition award for native species to encourage 'line-breeding' species plants from selected clones, aiming to produce 'superior' species orchid plants making wild collected plants, uncompetitive. iii) Acting independently on inappropriate land-use zoning and development, such action ranging from submissions, to more controversial 'direct action', usually in conjunction with other conservation and community groups.

### 3. Wild orchid conservation for ecotourism in Thailand

Chitrapan Piluek\*, Pramote Triboon, Chukiat Tapsan and Derake Tonpayom

Horticulture Department, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand. Email: chitrapanp@yahoo.com

Maehongson, the beautiful province of hills, natural streams and waterfalls with an extraordinary diversity of plants, especially orchids, is situated near the Myanmar border northwest of Chiangmai. It is a haven for biological conservation and observation of wild orchids. The promotion of research work on wild orchid conservation would benefit the local villagers in earning income from ecotourism. Such a project has created co-operative undertakings between researchers and villagers at Banhuayhi, Banhuaysuatao in Muang district as well as Bantamlod in Pangmapa district. A number of biological surveys were on wild orchids in the fertile tropical rainforests in collaboration with local villagers and hill tribes. 150 species in 54 genera of orchids were collected and identified. Most species are epiphytic with peak flowering recorded from January-May.

For sustainable conservation, the project began with the formulation of an easy-to-do aseptic medium for seed germination. The medium contains readily available orchid fertiliser, vitamins, table sugar, banana and agar and gave good results for germinating seeds of *Dendrobium chrysotoxum*. A transflask medium was prepared by adding potato into the germination medium. Both media gave good seed germination and seedling development as compared to the modified Vacin-Went medium. This technical know-how was introduced to the villagers for multiplying their orchids.

This followed practical training in seedling care for the villagers. Six month old seedlings of *Rhynchostylis gigantea* from flask, were grown in the homes of co-workers in the villages. After training, ten thousand seedlings of *Dendrobium chrysotoxum*, grown from seeds collected from Maehongson forests, were grown-on by the villagers. The seedlings thrived when restored to their natural habitat. The most vigorous seedlings were grown on tree species adjacent to tourist trails to demonstrate the benefits of the reintroduction program.

#### **4. The Australian Orchid Foundation and conservation.**

Helen Richards

Australian Native Orchid Society, PO Box 440, Yarra Glen 3775, Victoria, AUSTRALIA. Email:  
r\_angus@optusnet.com.au

[Abstract not received]

## 1. Systematics and its implications for orchid conservation.

Mark A. Clements

Centre for Plant Biodiversity Research, PO. Box 1600 Canberra ACT 2601, AUSTRALIA. Email: mark.clements@pi.csiro.au

Over that past 20 years considerable research has been undertaken on orchids resulting in a doubling in number of the species recognised in Australia. This has primarily been achieved through broad based studies of living plants, development of techniques for preservation and comparative analyses of these collections, coupled with finding and studying the types of all described relevant species. Associated with this has been the collection of material during fieldwork in New Zealand, New Caledonia, Vanuatu and Papua New Guinea for direct comparison against Australian collections. Recently, molecular techniques have also been employed as a means of determining the phylogeny of taxa and in some incidences providing support for taxonomic decisions at specific level. One consequence of our improved understanding of the systematics of the Orchidaceae in the region is that it also provides an objective means of determining the conservation status of the Australian species.

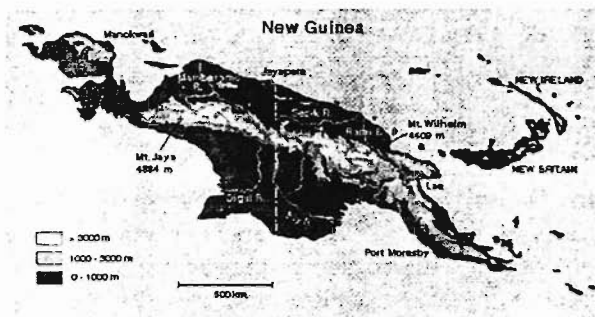
Phylog. Club Southern Ocean  
Region. *Phlogothrix*  
20/10/01

## 2. The project "Orchids of Southeast Asia" on CD-ROM.

Ed de Vogel\* and André Schuiteman

Nationaal Herbarium Nederland, Leiden branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands. Email: devogel@nhn.leidenuniv.nl

A short introduction is given on the project which aims at publishing all c. 8000 orchid species of Southeast Asia on CD-ROM.



*Orchid Genera of New Guinea Vol. I. Illustrated Checklist and Genera, February 2001.*

The huge island of New Guinea is a naturalists' wonderland. New Guinea harbours a tremendous collection of orchids, certainly in excess of 2000 species. They can be found almost anywhere, from the warm mangrove swamps and beach forests to the chilly grasslands above the timberline on the highest mountains. In the misty upland forests their abundance and diversity can be staggering.

Our knowledge of most of these orchids is very poor. Most species were described before 1940 and have never been revised. Many synonyms will undoubtedly come to light. Hundreds of species still await discovery. An estimate of the real number of species occurring in New Guinea is between 2200 and 2800 species. Thus, ten to thirteen percent of the world's orchids are to be found in New Guinea.

Rudolf Schlechter (1872-1925), J.J. Smith (1867-1947), R.A. Rolfe, H.N. Ridley and F. Kraenzlin described the large majority of New Guinean orchids. They contain species descriptions in Latin, most of which were never translated. A modern and well-illustrated overview, with the means to identify all New Guinea orchid genera is long overdue.

This CD-ROM contains 132 generic descriptions in the module *Higher Taxa, a Checklist* covering some 5400 names relating to almost 3000 accepted species, and in the module *Species* a small number of individual species are treated in detail, as a sample of what future CD-ROMs in this series will provide. Tools for identification of the genera consist of an illustrated dichotomous *Text Key* and a multi-entry multimedia key *IdentifyIt*. A hyperlinked *Glossary* is present with over 500 terms, not counting many synonymous terms. About 2000 images, c. 1000 colour photographs and 1000 pencil drawings by J.J. Smith and Indonesian draughtsmen like Natadipoera and Darmosoediro illustrate the CD-ROM. Orchid growers will be pleased to find in the checklist brief indications of the cultural requirements of almost all species; in addition the *Introduction* module contains a chapter on cultivation. A list of important publications is given in the *Literature* module, while there are many additional references in the respective fields of the *Higher Taxa* and *Species* modules.

Undoubtedly the main attraction of this CD-ROM lies in its illustrations, about 2000 in all. A dozen excellent photographers agreed to publish their often unique images on this CD-ROM. A list of institutes and individuals who have contributed to this CD-ROM can be found under the *Navigator* tab labelled *Contributors*.

The authors have freely tapped from the J.J. Smith archive which contains many unpublished drawings, and as a result have been able to illustrate in this CD-ROM almost all of the numerous species described by J.J. Smith from New Guinea. It is planned in future CD-ROMs to include all the published drawings of Schlechter as well. The second, *Orchid Genera of New Guinea Vol. II. Dendrobium and allied genera* containing c. 450 fully treated taxa is scheduled for publication at the end of 2001.

This CD-ROM is the first modern work on all orchids of New Guinea. It will prove to be an enormously useful and enjoyable source of information for botanists, orchid amateurs, professional orchid growers, conservationists, and students.

### 3. Overcoming the taxonomic impediment: a case study in south-west Western Australia.

Stephen D. Hopper

Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, West Perth WA 6005, AUSTRALIA.

Email: [steveh@kpbg.wa.gov.au](mailto:steveh@kpbg.wa.gov.au)

Taxonomic knowledge of orchids remains imperfect, particularly in the southern hemisphere where great diversity coincides with relatively low numbers of botanists trained in western systematics. Yet sound taxonomy underpins conservation. Scarce resources for recovery programs of threatened species, for example, can be wasted or not used to the best possible advantage if an imperfect taxonomic knowledge exists. Taxa most in need of conservation action can be entirely overlooked if unnamed. Conversely, poorly discriminated taxa can draw unnecessary attention and divert resources from where they are most needed. But traditional monographic taxonomy is unable to keep pace with habitat destruction and loss of threatened orchid populations.

In this paper, using the genus *Caladenia* R.Br. in south-western Australia as a case study, I illustrate how the pace of taxonomic work can be accelerated through collaboration with teams of orchid enthusiasts and botanists. Strong community support not only has assisted locating and describing new taxa, many of which are threatened, but provides an ongoing springboard of knowledgeable local people best able to undertake ongoing monitoring and management for conservation. Such collaboration runs some risk of exposing rare plants to unscrupulous collectors. However, the evidence in the south-west, overwhelmingly, is that far more positive outcomes for conservation through community partnerships have been achieved than the odd negative from rogue collectors.

## 1. Levels of fruiting success in the Mascarene Islands orchid flora: implications for conservation.

David L. Roberts\* & Chris C. Wilcock

Department of Plant and Soil Science, University of Aberdeen, Cruickshank Building, St. Machar Drive, Aberdeen, AB24 3UU, Scotland, UK. Email: dlroberts\_atm@hotmail.com

Islands, and in particular, oceanic island archipelagos are invaluable ecosystems in which to study evolutionary patterns and processes. Their isolation restricts the number of possible hypotheses that can explain evolutionary events. The three major islands of the Mascarene archipelago, Mauritius, Rodrigues and La Réunion, situated in the western Indian Ocean, are of volcanic origin and have never been connected to one another or any other landmass. The flora of the Mascarene Islands was not scientifically collected and described until long after the islands had been significantly modified by human activity. This, therefore, presents a problem to conservation biologists in determining natural levels of pollination or fruiting success. Here we present results from the analysis of over 27,500 flowers from over 60 species of orchids; in particular we focus on four species of conservation interest.

Levels of fruiting success in the Mascarene orchid flora were found to be extremely variable, ranging from 0 to 100%. The highest levels of fruiting success occurred mainly in the numerous auto-pollinating species, reflecting the survival advantage of reproductive assurance on oceanic islands (e.g. Baker's Law). *Aeranthus arachnites* var. *balfourii*, *Angraecum* sp. nr. *caulescens* and *Oeonilla aphrodite* from Rodrigues all have levels of fruiting success significantly below closely related species on Mauritius and, in the case of *O. aphrodite*, also below that of La Réunion. This is due to reduced pollinator activity, probably as a result of habitat destruction and degradation since Rodrigues now has one of the most devastated oceanic island floras in the world. In the case of *Aeranthus arachnites* var. *balfourii*, this may be due, in part, to nectar robbery by invasive ant species. However, in this species and *Angraecum* sp. nr. *caulescens* the populations are strongly positively skewed suggesting recruitment from seed. This level of population skewness is not seen in *O. aphrodite*. A similar situation to that seen on Rodrigues is found on Mauritius where less than 5% of the original vegetation still exists. The most serious case of habitat destruction in recent years began in 1967. Unique *Pandanus* marsh, *Philippia/Phyllica* heath and *Sideroxylon* thicket was cleared for softwood forestry. This area, Les Mare, was an important location for a number of now endangered species including *Beclardia macrostachya*. No fruiting success has been recorded in the last five years for *B. macrostachya*, but the species is reproductively successful (56% fruiting success) on La Réunion. In addition, the level of skewness is low compared with other angraecoid species suggesting lack of recruitment from seed. For these reasons, *B. macrostachya* and *O. aphrodite* in particular require urgent attention in order to conserve these species.

### Acknowledgements

DLR wishes to acknowledge the financial support of the Guy Harvais Studentship and Bentham-Moxon Trust while conducting this research, and from the Royal Botanic Gardens, Kew, for attendance at this congress. The authors wish to thank the logistical support of the Mauritian Wildlife Foundation, National Parks and Conservation Service and Drs. Pailler and Strasberg from the Laboratoire de Biologie Végétale, La Réunion.



## 2. The effects of hand-pollination on growth, survival and reproduction in *Platanthera leucophaea*, the eastern prairie fringed orchid.

Pati Vitt

Conservation Scientist, Chicago Botanic Garden, 1000 Lake Cook Road, Glencoe, Illinois, 60022 USA. 847-835-8268. Email: pvitt@chicagobotanic.org

*Platanthera leucophaea* is a Federally threatened orchid of grasslands in the United States. Today only 22 known populations remain in the state of Illinois, the heart of its historic range. Most of the extant populations throughout its range are quite small, with fewer than 20 individuals. While the primary threat to *P. leucophaea* is loss of habitat, other threats are over-collection and poor pollinator service caused by the use of pesticides that kill the hawk moth, the species only pollinator. In Illinois, recovery efforts for this species include hand pollination by trained volunteer stewards to increase seed set. The current project examines this practice.

Plants at an Illinois population with greater than 350 reproductive individuals (perhaps the largest currently known population of this species) were randomly assigned either to a control group, that was not hand pollinated, or to one of three treatment groups. The first treatment required hand pollination of 70% of flowers produced. The second treatment required hand pollination of 30% of flowers, while plants in the third treatment had no flowers pollinated at all. Treatments were designed to determine the effect of increased seed set via hand pollination on subsequent size, reproductive status and mortality.

Preliminary analysis has shown that the assignment of individual plants to the 4 treatment groups was accomplished in a random manner, and there are currently no detectable differences among the treatments in any of the size variables measured (plant height, length of the second leaf, width of the second leaf and basal stem diameter). Therefore we can be reasonably assured that any differences detected among the treatments in the subsequent years of the study will be due to treatment effects alone. Plant size has a significant effect on the number of flowers a plant produces, while, as expected, treatment effects alone determine the number of fruits in each treatment. The 30% hand-pollination level was set to mimic levels of natural pollination and treatments 2 (30% hand pollination) and 4 (natural pollination) had similar levels of fruit set. The variance for natural pollinations was not unexpectedly higher.

Total seed weight per capsule is significantly related to plant size (measured as the width of the second leaf), and a multiple regression reveals a significant relationship of leaf width, treatment and number of fruits produced. Results from the 2001 field season (June-August) will be presented, including the effect of the treatment on current size and reproductive status of all study plants. A LICOR data logger placed at the sites will measure climactic variables to determine their correlation with reproductive trends. This research is intended to support the development of scientific management plans for *P. leucophaea* and inform the efforts of the volunteer stewards who are working to ensure the survival of this rare and beautiful species.

**Table 1.** Number of fruits produced per treatment. Treatment 1 was 0% hand pollination, Treatment 2 was 30% hand pollination, Treatment 3 was 70% hand pollination and Treatment 4 was natural pollination.

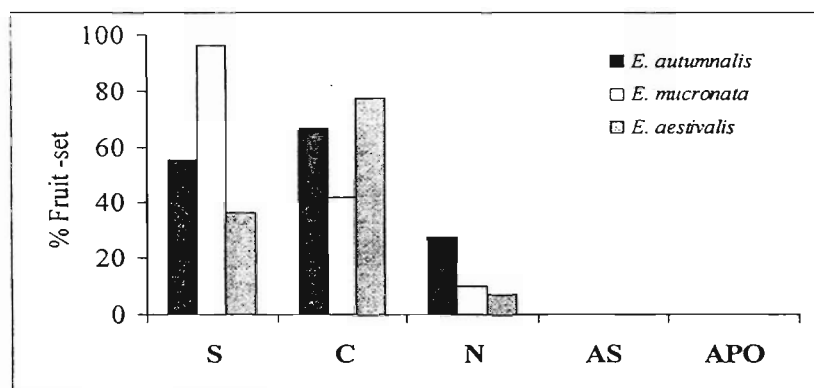
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Mean	0.0968	4.2000	7.1034	4.5909
Standard Error	0.0968	0.4282	0.5816	1.0079

### 3. Pollination ecology of the genus *Earina* Lindl. In New Zealand.

Carlos A. Lehnebach,\* & A. W. Robertson

Ecology Group, Institute of Natural Resources, Massey University, Palmerston North, New Zealand. Email: C.lehnebach@massey.ac.nz

Epiphytic orchids in New Zealand are represented by four genera, basically all of tropical origin. Currently, the genus *Earina* Lindl. comprises three species in New Zealand, two widespread species (*E. autumnalis* and *E. mucronata*) and one restricted to coastal biotopes of the North Island (*E. aestivalis*). Their flowering period shows two peaks, early autumn to late winter for *E. autumnalis* and early spring to late summer in the last two. Hand-pollination treatments have showed that the three species are self-compatible. Neither autogamy nor agamospermy is involved in seed set, thus the species entirely depend on pollinating agents for their reproduction. Although the three orchids are scented and nectariferous, it has been observed that fruit-set is low in the wild (see Figure 1). Pollinia removal rates are considerably higher than deposition in the three species, 91.5% of the flowers of *E. autumnalis* observed had their pollinia removed while deposition was only 11.9%. These values were even lower in *E. mucronata* (25.4% and 0.9% of pollinia removal and deposition, respectively) and *E. aestivalis* (44.8% and 1.2% of pollinia removal and deposition, respectively). The insects visiting *Earina* spp. belong to 8 families: Diptera- Tipulidae, Bibionidae, Empidae, Syrphidae, Tachinidae; Coleoptera- Curculionidae and Hymenoptera- Formicidae and Apidae. Of the 12 insect species observed, only 3 may be considered as probable pollinators; i.e. *Eristalis tenax* (Syrphidae) for *E. autumnalis*, *Dilophus nigrostigmus* (Bibionidae) for *E. mucronata* and an unidentified weevil (Curculionidae) for *E. aestivalis*. All the remaining insects are unlikely to achieve pollination because of their size or their behaviour while visiting the flowers (nectar robbing).



**Figure 1:** Percentage of fruit-set in three species of *Earina* Lindl after pollination treatments (S: hand self-pollination; C: hand cross-pollination; N: natural pollination; AS: bagged to test automatic self-pollination; APO: emasculated to test apomixis).

## 1. Preliminary investigation on the pollination, ecology and conservation of the Endangered *Caladenia behrii* in South Australia

Topa Petit\*<sup>1</sup> and Doug Bickerton<sup>2</sup>

1. School of Environmental and Recreation Management, University of South Australia, Mawson Lakes 5095, South Australia. Email: [sophie.petit@unisa.edu.au](mailto:sophie.petit@unisa.edu.au)

2. Lofty Block Threatened Orchid Recovery Project, Department of Environment and Heritage, P.O. Box 1047, Adelaide 5051, South Australia. Email: [bickerton.doug@sa.gov.au](mailto:bickerton.doug@sa.gov.au)

The nationally endangered orchid *Caladenia behrii* is limited to about 4,500 mature plants in South Australia. It deceives its pollinator, a male wasp, by secreting a pheromone similar to that of the female wasp. The orchid's abundance may be limited by pollination, but also predation, the availability of mycorrhiza, and ecosystem health. At the time this abstract was written, we have just started our second field season, investigating the pollination of, and predation on, this pink-lipped spider orchid.

In 2000 we tagged 337 orchids (as buds) at two sites: Para Wirra Recreation Park (PW) and Warren Conservation Park (WCP). We have added a site at Kersbrook Forestry Reserve (K) in 2001, with 200 orchids. Our preliminary findings on the natural pollination rate of *Caladenia behrii* indicate that at PW in 2000, the natural rate of pollination was 8%, whereas it was 28% at WCP. Predation by mammals, however, was much lower at PW (28%) than at WCP (41%). Nevertheless, the pink-lipped spider orchid was more successful at WCP with 18% of the tagged population producing seeds, than at PW, with less than 6%. It is important to note that we had not tagged buds that had been eaten; consequently, the estimates of 18% and 6% success are overestimates for the total population.

At this time, the identity of the pollinator is still not known and we can only speculate about its abundance and requirements. It is starting to become apparent that tall, straight flowers tend to be pollinated more often than others, and so rain and wind that affect the position of the stems may also affect pollination. In addition, such weather pushes certain flowers outside the protection offered by grass trees. Predation on the orchids is related to the degree of protection offered by other plants, mostly grass trees. Most of the predation seems to result from the grazing of kangaroos, with a minor impact by rabbits. Insect damage is very low.

Important conservation considerations for *Caladenia behrii* in South Australia include: the abundance of pastures around protected areas (maintenance of kangaroos), the maintenance of insect biodiversity, the availability of mycorrhiza, and the general health of the native ecosystem. The orchid may be considered an indicator of ecosystem health.

### Acknowledgements

We thank David Wilson for his precious assistance. The following grants have allowed us to realise the fieldwork: Australian Technology Network Small Research Grant 2001, University of South Australia Division of Information Technology, Engineering, and the Environment Small Grants 2000 and 2001.

## 2. Chemical communication in sexually deceptive orchids – implications for evolution and conservation.

Florian P. Schiestl, Jim Mant, and Rod Peakall\*

Division of Botany and Zoology, Australian National University, Canberra ACT 0200 Australia. Email: florian.schiestl@anu.edu.au

Sexually deceptive orchids do not provide any food reward for their pollinators. Instead, they imitate the shape and the sex pheromone of female insects. Only male insects, usually of a single species, pollinate the orchids as they attempt to copulate with the flower. Australia is well known as a centre for the evolution of this pollination syndrome with more than 100 species involved. While the importance of floral fragrances has long been recognised, until now there has been no investigation of this component of the pollination system. Our initial investigations have focussed on the genus *Chiloglottis* in which all species are pollinated by male Thynnine wasps. We extracted flowers and analysed the odour by gas chromatography with electroantennographic detection. Biologically active compounds (i.e. compounds eliciting receptor potentials in insect antennae) were identified by gas chromatography with mass spectrometry. We found one biologically active peak in *Chiloglottis trapeziformis* labella and sepals and the same active peak in females of the pollinator species *Neozeleboria cryptoides*. Two closely related, sympatric, *Chiloglottis* species, *C. trilabra* and *C. reflexa* emit one identical and one distinct biologically active peak each. The finding that just one or two compounds are involved in the attraction of pollinators, contrasts with the European sexually deceptive genus *Ophrys*, where an array of compounds are involved. If the relative simplicity of chemical communication in *Chiloglottis* is typical of Australian sexually deceptive orchids in general, further study of floral odours will provide a powerful tool for exploring outstanding systematic and evolutionary questions. It may also be possible to use synthetic chemicals to monitor pollinator populations of rare orchids, providing a novel new tool for conservation.

### 3. Specific pollinators and Australian sexually deceptive orchids: General principles and implications for conserving their biodiversity.

Colin C. Bower

Orchid Research, PO Box 300, Orange, NSW 2800, Australia. Email: colin.bower@agric.nsw.gov.au

The sexual deception pollination strategy is prominent among Australian terrestrial orchids, encompassing up to 300 species. Sexually deceptive orchids attract male insects by emitting odours (kairomones) mimicking the sex pheromones of their females. Pollination occurs when males attempt to mate with female-mimicking structures on the labellum. This paper reports the results of extensive field investigations of pollinator specificity in the sexually deceptive genus *Chiloglottis*, involving several hundred field experiments on 27 taxa and the capture of over 5000 attracted male thynnine wasps. The following findings and conclusions were made:

- The hypothesis that each sexually deceptive *Chiloglottis* species has a specific one to one relationship with a single pollinator species, was validated experimentally for the first time.
- However, most *Chiloglottis* species may also weakly attract other thynnine species as non-pollinating minor responders.
- Sympatric *Chiloglottis* species always have different pollinators, and hence different attractant kairomones, and are therefore reproductively isolated.
- However, while allopatric *Chiloglottis* species also always have different pollinators, the geographically separated orchids (and their pollinators) may share similar attractant odours.
- New cryptic species of *Chiloglottis* and thynnine wasps can be detected via specific pollinator relationships.
- Conversely, pollinator specificity can detect cases where two morphological species belong to the same biological species.

The ability to use specific pollinator relationships to detect cryptic *Chiloglottis* and thynnine wasp species is important for understanding biodiversity in these groups. That a relatively high number of cryptic *Chiloglottis* species was detected by studies of pollinator specificity demonstrates the potential importance of these approaches for identifying species diversity in other groups of sexually deceptive orchids. The highly specific pollinator relationships of sexually deceptive orchids have obvious important implications for orchid conservation. Recovery Plans for threatened species will need to include specific strategies to ensure the long term viability of pollinator populations.

*Terrestrial* → dipter  
*labellum* → hemiphetes  
*labellum* ~ female wasp  
 male attracted by pheromones  
 copulatory behavior  
 appear rapidly  
 males avoid flowers  
 specific relation to one of single pollinator species

Thynnine taxa  
 Principles  
 1. sympatric deception related  
 have + pollinators  
 2. share similar attractive odours  
 3. pollinator species specific  
 detection of new cryptic taxa  
 4. are distinct species related  
 with similar morphology

#### 4. Pollination of *Chiloglottis* by sexual deception of Thynnine wasps: ecosystem interactions and implications for conservation.

Jim Mant, Florian Schiestl, Peter Weston and Rod Peakall

Division of Botany and Zoology, Australian National University, Canberra 0200, ACT, Australia. Email: jim.mant@anu.edu.au

The pollination by sexual deception in orchids involves an intricate and highly specialised set of interactions. The majority of the more than 100 sexually deceptive Australian species are pollinated by Thynnine wasps, a diverse, mainly southern hemisphere group of Tiphids. Thynnines, in turn, are (most probably generalist) parasitoids of soil borne scarab beetle larvae. The Scarab hosts are themselves typically phytophagous on *Eucalyptus* spp. Additionally, male thynnines provide the wingless females essential food resources from honeydew (eg. scale insects) or floral nectar. The interdependence of these diverse interactions highlights the need for a broad ecological context to the conservation of orchid and pollinator species diversity. The extreme pollinator specialisation found among sexually deceptive orchids makes them particularly susceptible to disruption of pollinator and orchid habitat requirements. From an evolutionary perspective, sexually deceptive orchids at first appear to show considerable flexibility in their adaptation to a diverse range of pollinators. Some five hymenopteran families pollinate the Australian taxa. Similarly, in Europe the sexually deceptive *Ophrys* attracts insects from across three hymenopteran superfamilies. We investigate the extent of pollinator specialisation in a group of sexually deceptive Diurids using molecular sequence data on both orchid and wasp taxa. A pattern of constraint in the use of pollinators at a range of taxonomic levels is revealed and switching between unrelated pollinator taxa is rare. Specialisation at the specific level may translate to higher level specialisation to particular groups of pollinators. The orchids' chemical mimicry of female wasp pheromones ensures changes in specific pollinators are most likely to occur amongst related wasp species.

Q. Correlation among sexually deceptive orchids and their pollinators?

- Phylogenetic association not due to co-speciation
  - & related branch lengths
  - evidence of pollinator switching

## **Friday: Session D**

Conclusions and recommendations. Chair: **Stephen Hopper**

The key outcomes from each session will be compiled by session chairs and passed to the secretariat by the Friday lunch break.

If you are a session chair, please ensure that the key points (no more than five) are completed on time.

**Pectic zymogram analysis of orchid fungal endophytes.**

Nura Abdul Karim<sup>1</sup>, K. Sivasithamparam<sup>1</sup>, K.W. Dixon<sup>2</sup>

1. Faculty of Agriculture, The University of Western Australia, Crawley, Western Australia 6097. Email: nura@cyllene.uwa.edu.au

2. Science Directorate, Botanic Gardens & Park Authority, Kings Park, West Perth, Western Australia 6005.

An important step in studies of orchid mycorrhizal systems is the identification of the mycobionts. A diversity of endophytic fungi has been found associated with orchids. The endophytic fungi were mainly isolated from the roots of adult orchid plants. Generally, the largest group of fungi associating with orchids have been with members of the Subdivision Basidiomycotina (Basidiomycetes). The heterogenous assemblage of the form genus *Rhizoctonia* from this subdivision have been most commonly isolated and proven to be compatible with many orchid seed *in vitro*. Other common associates of orchid roots are fungal species of the Subdivision Ascomycotina (Ascomycetes) and the Fungi Imperfecti. The identification of the orchid endophytes is limited by the difficulty for inducing isolates to form teleomorphs (the perfect state) since the majority of the isolates are sterile in culture. Most orchid endophytic genera were established on morphological features, nuclear cytology and septal ultrastructure characteristics. Simple enzymatic tests such as pectic zymogram analysis, have been used in separating and clustering the endophytes of many pathogenic fungi into homogeneous groupings to assess the diversity of fungal strains.

Pectic zymogram (PZ) analysis is a technique involving electrophoresis of extractable pectic iso-enzymes produced by the fungi and is used as a taxonomic tool in distinguishing groups of fungi. In this study, over 100 isolated endophytic fungi of various orchid species of the Western Australian Kimberley region were rapidly grouped and specificity studies of endophytes were determined using the PZ technique. Previous studies of PZ on pathogenic fungi have shown that fungi sharing a zymogram banding pattern constituted a zymogram group and were found to share characteristics of morphology, number of nuclei per cell, teleomorph state, anastomosis grouping and pathogenicity. This study also agreed that zymogram patterns broadly reflected the differences/similarities in morphological and cultural characteristics of the isolated orchid endophytes. The PZ method allowed rapid characterisation and grouping of large numbers of isolates and allowed comparison of the diversity of isolates.



## Herbarium orchid database – Museu Nacional, Rio De Janeiro.

Ruy José Válka Alves\*, Regina Braga de Moura & Luiza Carla Trindade de Gusmão.  
Departamento de Botânica, Museu Nacional, Quinta da Boa Vista, Rio de Janeiro, Brasil. 20940-040. Email: ralves@acd.ufrj.br

The Herbário do Museu Nacional – (R) was established in 1831 being Brazil's first institutional and currently largest herbarium, with 500,000 specimens of vascular plants and 4,600 types. The Orchidaceae represent about 40% of all monocotyledons and many authors consider it the largest family of flowering plants. In Brazil, the Orchidaceae are represented by approximately 200 genera with some 2,500 described species. In this paper we present the results, benefits and difficulties of the first effective orchid database elaborated at the herbarium.

The database experiment was conducted on a single computer in order to determine the time and resources needed to informatise the entire herbarium. All data was obtained directly by examination of specimens, between January and June 1997. Microsoft Works 4.5 for Windows was used for creating the database, mainly because it was already available to the senior author who had previous experience with it. Commercial software is also easier and cheaper to translate by end-users world-wide, as opposed to programs tailored for specific purposes. A regular Windows 95-based, IBM-compatible Pentium 133 mainframe belonging to the author was used.

3,142 specimens of Orchidaceae were analysed, representing 1.6% of the entire herbarium. Of these, 2,201 (70.1%) were found to be in good, 643 (20%) in medium and 285 (9.1%) in bad condition. 13 specimens could not be studied at the time as they were on loan abroad. During database creation, 7 types were located in the general collection and added to the 52 types already separated in the type cabinet of the R - herbarium. Among the orchids, 2,638 specimens (84%) were collected in almost all states and territories of Brazil (fig. 1). These represent 1045 native species in 180 genera and 86 species in 21 exotic genera. The number of annual orchid collections from 1814 to 1997 included at the herbarium is plotted in fig. 2.

This result provided us with subsidies to elaborate a project for the entire herbarium and estimate the money involved. The hardware (computers, software, printer, a scanner and a digital camera, were provided by the Brazilian National Research Council "CNPq"). The herbarium database BRAHMS 5, developed at Oxford, and the HISPID standard for data entry shall be used in subsequent years.

### Acknowledgements

Research Supported in part by the Brazilian National Research Council (CNPq). Collaboration: Dr. Jirí Kolbek, Institute of Botany, Academy of Sciences of the Czech Republic. Special Acknowledgement: Ruy Antônio Reis Alves, for all the support including expenses with the trip to Perth.

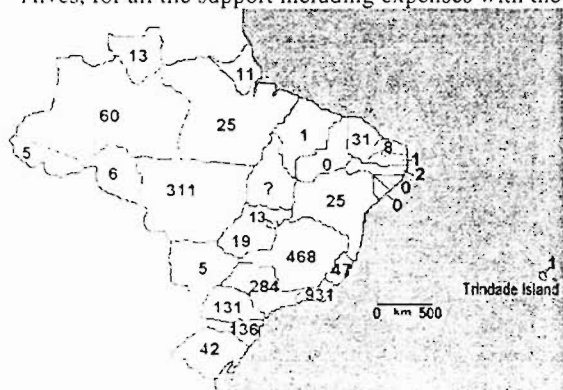


Figure 1. Number of orchid species per state.

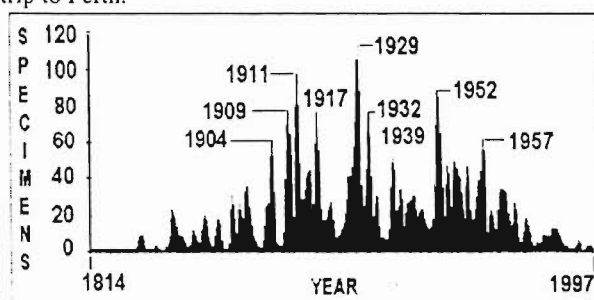


Figure 2. Number of collections per year.

# Orchids as agents of primary succession on rocks in Brazil.

Ruy José Válka Alves

Departamento de Botânica, Museu Nacional, Quinta da Boa Vista, Rio de Janeiro, Brasil, 20940-040. Email: ralves@acd.ufjf.br, ruyvalka@yahoo.com

The Campos Rupestres, which are an extraazonal, savannah outcrop vegetation with high plant diversity and rich in narrow-endemic species, occur as a mosaic in the Cerrado biome (savannahs on latossol) in Southeastern and Central Brazil. Several species of Orchidaceae, especially *Bifrenaria*, *Bulbophyllum*, *Encyclia*, *Pleurothallis johannensis* Barb. Rodr., *P. teres* Lindl. and *Sophranitis* (incl. *Laelia* sect. *Parviflorae*) have been observed as the first vascular plants to colonise quartzite outcrops, not necessarily preceded by lichens nor moss. The complex has an outstanding successional role throughout its relatively wide range (Alves & Kolbek, 2000), restricted to outcrops in the states of Bahia, Espírito Santo, and Minas Gerais.

Papers on tropical outcrop vegetation referred by Larson, Matthes & Kelly (1999) and Sarthou & Villier (1998) and the internet range from 1 to 5 per decade (1920-1980), leaping to 48 in the 1990s. Of 156 papers cited, only 19 are clearly from tropical, hot or desert biomes, of which the only 2 papers from South America do not deal with succession. The studied orchids, though, are preceded only by endolithic cyanobacteria such as *Stigonema*, defying the traditional models in which lichens, mosses and ferns precede, followed by vascular species. Established clusters of *Pleurothallis* have a radial growth, in which the "fronts" form a circle or semicircle on the rock surface. By trapping small rocks, leaves and twigs which would otherwise have rolled down the slope, these orchids build up fine soil, thus opening the way for other plants. Mosses benefit from the surplus water held by the new soil and an herb layer soon develops, followed by woody subshrubs. The initial orchid species now growing at the perimeter frequently succumb to this surplus humidity.

Rock dwelling plants are adapted to an almost nutrient-free substrate (quartzite) with extreme day-night temperature fluctuations (-5 to 70 °C) and availability of water is restricted to short periods of time (condensed morning dew evaporates quickly and rainwater runs off the bare rock surfaces). Cattle-raising adds nutrients to the poor litholic soils allowing invasive plant species to compete with and eliminate native s-strategists. As cattle-ranching is a deeply rooted tradition in Brazil, there is little perspective for a short term reversal of this destructive process.

## References

- Alves, R. J. V. & Kolbek, J. 2000. Primary succession on quartzite cliffs in Minas Gerais, Brazil.- *Biologia* 1(55):69-83, Bratislava.
- Larson, D. W., Matthes, U. & Kelly, P. E. 1999. Cliff Ecology, Pattern and Process in Cliff Ecosystems.- 340 pp., Cambridge University Press, Cambridge. ISBN 0521554896
- Sarthou, C. & Villier, J. F. 1998. Epilithic plant communities on inselbergs in French Guiana. *J. Veg. Sci.* 9(6):847-860.

## Acknowledgements

Research Supported in part by the Brazilian National Research Council (CNPq). Collaboration: Dr. Jirí Kolbek, Institute of Botany, Academy of Sciences of the Czech Republic. Special Acknowledgement: Ruy Antônio Reis Alves, for all the support including expenses with the trip to Perth.

## Identification of threatened *Caladenia* species (Orchidaceae) using traditional and molecular techniques to aid in their conservation.

Georgia Basist<sup>1\*</sup>, A.C. Lawrie<sup>1</sup>, R.E. Raleigh<sup>1,2</sup> and R.G. Cross<sup>2</sup>

1. Dept of Biotechnology & Environmental Biology, RMIT University, GPO Box 2476V, Melbourne, Victoria, 3001, AUSTRALIA. Email: s9709115@student.rmit.edu.au, aclawrie@rmit.edu.au, rrarei@rbgmelb.org.au

2. Plant Sciences & Biodiversity, Royal Botanic Gardens, Birdwood Avenue, South Yarra, Victoria 3141, AUSTRALIA. Email: rcross@rbgmelb.org.au

Both traditional and molecular techniques have been used to study a polymorphic population of terrestrial orchids at a field site in western Victoria. This population shows flower characters that combine features of *Caladenia fulva* G.W. Carr (Tawny Spider Orchid) and *C. reticulata* Fitzg. (Veined Spider Orchid), both of which are present on site, and it has been suggested that they may constitute a hybrid swarm (Backhouse and Jeanes, 1995). The population was classified into five groups on petal and labellum coloration (Table 1).

**Table 1.** Categorisation of the polymorphic types within a presumed spider orchid 'hybrid swarm'.

Category	Petal Colouration	Labellum Colouration
Type I	yellow or light yellow with crimson stripes	Crimson
Type II	yellow, light yellow or light green with crimson stripes or blotches	Crimson blotches or crimson with a central white stripe
Type III	yellow, light yellow, light green or dark yellow with slight crimson stripes or blotches	Yellow, dark yellow, light green or white
Type IV	light pink, yellow pink, rosy or peachy with crimson stripes	Crimson
Type V	yellow or light green with a thick crimson stripe	Crimson with or without a central white stripe

Fertility within and between groups was studied by hand-pollination and assay of seed viability by the fluorescein diacetate method. Differences were observed in capsule formation, seed set and seed viability within and between crosses. The seed viability results varied from 0-94%.

Molecular differences were also compared. DNA was extracted from tips of young leaves, using the QIAGEN DNeasy<sup>®</sup> Plant Mini Kit. Several techniques were employed to compare the extracted DNA. Products from Polymerase Chain Reaction (PCR) of the Internal Transcribed Spacer (ITS) region was obtained for Restriction Fragment Length Polymorphism (RFLP) analysis, where preliminary results show no differences in the ITS region across the five categories and *C. fulva*. Randomly Amplified Polymorphic DNA (RAPD) analysis and sequencing are being performed to confirm or reject these findings.

Furthermore, in conjunction with Dr Adrian Dyer (Monash University), UV imaging of flowers is being conducted to infer what the insect pollinator sees compared with the five phenotypic categories.

### Reference

Backhouse G, Jeanes J. 1995. The Orchids of Victoria. Melbourne University Press: Carlton, Vic.

## Pollination studies on *Bipinnula fimbriata* (Poep.) Johnst. in Central Chile.

M. A. Cisternas<sup>1</sup> & C. A. Lehnebach<sup>2\*</sup>

1. Escuela de Agronomía, Universidad Católica de Valparaíso, Casilla 4-d, Quillota, V Región, Chile. Email: m.cisternas@eudoramail.com

2. Ecology Group, Institute of Natural Resources, Massey University, Palmerston North, New Zealand. Email: C.lehnebach@massey.ac.nz

The endemic orchid *Bipinnula fimbriata* (Poep.) Johnst. is restricted to the coastline biotopes and low elevation areas of the Andes of Central Chile. Currently, due to dramatic alterations of its habitat by urbanisation and recreational projects, good-sized populations are rarely found. However, a large population has been recently located in the stabilised dunes of the V Region of Chile (33°S).

*B. fimbriata* is one of the first orchids to flower in Chile (late July to mid-October). Flowering individuals develop a single spike with up to 20 long-lived flowers (flower life-span:  $22.8 \pm 7.39$  days). It was observed that under natural conditions of pollination, fruit-set is rather low (30.5%), but considerably higher when flowers are hand self- and hand cross-pollinated (100%). Since direct self-pollination is also involved in the fruit-set (15.7% of bagged flowers formed capsules), this self-compatible orchid depends partially on the pollinators' service. The insects considered as probable pollinators are *Colletes musculus* and *Caenohalictus* sp. (Hymenoptera), both with a low visitation rate (0.001; and 0.0001 visits/spike/min., respectively).

## Recovery planning for three endangered orchid species in SE New South Wales - Crimson Spider orchid (*Caladenia concolor*), Tarengo Leek orchid (*Prasophyllum petilum*) and Rhyolite Midge orchid (*Genoplesium rhyoliticum*).

Stephen S. Clark

Senior Threatened Species Officer, Threatened Species Unit, Southern Directorate, NSW National Parks and Wildlife Service, PO Box 733 Queanbeyan, NSW 2620, AUSTRALIA. Email: stephen.clark@npws.nsw.gov.au

Recovery Teams have been established for each of these species by the NSW National Parks and Wildlife Service. The task of the teams is to draw together available information about the known populations, their ecology, habitat and existing threats. From this background, recovery actions which will ensure the continued survival of the species are developed and implemented. Representatives of various government authorities are represented on these teams as well as landowners and community groups.

Habitat description is an important part of all three of these Recovery Plans and can serve as an important guide to other areas where searches for new populations of the species should be carried out. Such information needs to be interpreted with caution, however, since, as is the case for the Crimson Spider Orchid, historical information points toward the species being abundant in a somewhat different habitat type than the one in which it now occurs. Survey based on habitat modelling is identified as an action in these plans. For Tarengo Leek Orchid, for example, BioClim modelling has been used to generate a map of potential habitat to guide field survey effort.

All of these species are characterised by small population size and hence, it is vital to know with some accuracy ongoing trends in numbers with time. This helps us to know if threats continue to operate and to assess the effectiveness of recovery actions being implemented. In the case of the Crimson Spider Orchid, in particular, attempts to augment population numbers are an important part of the Recovery Plan. These include hand cross-pollination, isolation and culturing of the fungal associate and seed germination trials.

It is often the case that information on which to base management decisions is lacking for endangered orchid species. While both fire and grazing have been a part of the environment of Tarengo Leek Orchid, it remains to be determined what the optimum regime would be for maintaining population numbers. The large population at Tarengo makes it possible to establish a series of management trials to obtain answers to such questions. The Rhyolite Midge Orchid is confined to rhyolite outcrops and all three known populations are on State Forest. While these sites are protected to some extent, there is still cause for concern regarding fire. Periodic fire has shaped the communities on these outcrops, however, it is not clear what fire intensities and frequencies are desirable for the maintenance of the Rhyolite Midge Orchid. Research to provide a basis for sound management decisions is a high priority.

It is important to consider the current tenure for all of the sites on which these three orchid species occur and whether it will facilitate or at least permit the implementation of important recovery actions. National Park status is generally highly regarded because conservation management of endangered species is accorded a high priority on these lands. Where populations occur on land managed by other government authorities or in private ownership, the *Threatened Species Conservation Act 1995* makes provision for Joint Management Agreements or Voluntary Conservation Agreements to be entered into by interested parties. The establishment of links with the local community is considered to be important for all of these species. This can take the form of both informing people of the value and importance of the species and involving interested and committed individuals in the implementation of recovery actions.

# Australasian Native Orchid Society Inc.: The role of the ANOS groups in New South Wales in orchid conservation.

Alan Dash, Ruth Rudkin, Peter Eygelshoven and Russell Wales.

The Australasian Native Orchid Society Inc., 23 Yeramba Crescent, Terrigal 2260, New South Wales, Australia.

## Poster 1. Field trips.

There are 11 ANOS Groups in New South Wales and range from the far north coast to south of Nowra. Most organise field trips, both on their own, or combined with other groups, as educational and social outings. They offer the members the opportunity to see the range of native orchids, and to understand their growing conditions. As the population of the State increased, more and more land was being developed for housing and it was realised that much of this land contained orchids. Sydney in particular is fortunate to have a great deal of bushland in many suburbs because of the rugged nature of the environment, so travelling is not often a problem. Examples of such Field Trips are to: Strickland State Forest (Central Coast), Mt. Wilson (Blue Mountains), Royal National Park (South of Sydney), Southern Highlands, the South Coast, and the Far North Coast. Groups record their findings and the habitats and check with Botanic Gardens when new or unidentifiable orchids are found. They often work in conjunction with botanists to study variation between species and genera. Some groups are now working with National Parks and Wildlife Service by recording the findings for the NPWS Atlas of NSW Wildlife.

## Poster 2. Preserving habitats.

The following activities are monitored and if necessary reported to the relevant authority: proposed land developments or inappropriate land use (public and private), clearing of native vegetation, inappropriate herbicide/insecticide use, unnecessary mowing or animal impact, inappropriate fire regimes, and any other threat to rare or endangered orchid populations. Other activities are to physically remove weeds and erect protective fencing if necessary.

Finally, Groups co-operate and engage in joint activities with other Community and Conservation Groups.

Examples of recent activities involving rare and endangered orchids or orchid habitats are:

*Diuris* sp. aff. *chrysantha* at Byron Bay in conjunction with local residents and others.

*Diuris arenaria* at Tomaree Peninsula, under threat from a proposed highway construction. Recovery is under way.

*Pterosylis* sp. aff. *plumosa* (Botany Bay) where ANOS is involved with the recovery team.

*Pterostylis gibbosa* in Illawarra Region; has been recovering under the stewardship of the Illawarra Group for more than a decade and is a model for all recovery plans in NSW.

*Prasophyllum affine* in Vincentia where it was under threat of proposed urban development.

*Calanthe triplicata*, although not an endangered species, was with many other orchids, endangered from a proposed use of the rain forest habitat as a refuse tip for Sydney. The proposal was defeated.

*Microtis angusii*, in Ingleside (Sydney region) and Sunny Corner. A recovery plan is in progress.

## Poster 3. Educating group members and the public about our native orchid heritage.

First and foremost is the ANOS Journal "The Orchadian" with its articles on botany, new species found, horticulture, Show results and so on. Secondly, there is the Website, [anos.org.au](http://anos.org.au). John Riley, of Macarthur Group has made available some of his botanical artwork which shows the structure of various orchid plants. Locally, the native orchid shows, usually 2 a year for each group, attract a lot of interest. Judging at the shows has lead to the breeding of more horticulturally desirable "species" and hybrids. This is further encouraged by the Australia wide competition of the Ira Butler Trophy Committee which was set up in memory of Ira Butler, one of the earliest hybridisers of our native orchids by which he hoped to stop the collection of species in the wild. Winners of shows or of awards from the Judging Panel are encouraged to submit photos of their plant for one or other of the trophies available.

## Conservation threats and the current status of South African terrestrial orchids with respect to *in vitro* germination.

Edwards, T. J.\* Piper, S. & Thompson, D.I

School of Botany & Zoology, University of Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa. Email edwards@nu.ac.za

The Orchidaceae of South Africa number 433 spp. with the vast majority belonging to the subfamily Orchidoideae which is represented by 2 tribes, the Orchideae and the Diseae. We deal with the South African component of the latter tribe. The Diseae is subdivided into 5 subtribes and comprises 250 southern African species in 11 genera (Linder & Kurzweil 1999). Many of these terrestrials display high levels of endemism and estimations of conservation status within the family have been compiled by (Hilton-Taylor *et al.* 1996). It is clear that the best form of conservation is *in situ* and by using GIS information on species distributions it is possible to earmark areas of high diversity for the tribe.

A disturbing factor relating to the conservation of the Diseae is our level of ignorance regarding the germination and cultivation requirements of species within the tribe. A false notion of germinability exists for the genera *Satyrium* and *Disa* (Wodrich 1997) where a few of the Cape species are tractable in cultivation. The vast majority have never been successfully cultivated or germinated *in vitro*. These factors do not bode well for conservation within the tribe. Our presentation overlays data (GIS) of land usage, orchid distribution and geographic species-richness to develop a better idea of species which are threatened with extinction. This forms the basis for future studies on population dynamics, management strategies and *in vitro* germination research.

We outline Diseae species that have never been successfully germinated. Clearly such species are extremely vulnerable to habitat destruction and the conservation options are limited. The *in vitro* germination and artificial cultivation of threatened species provides a management tool that may be used to augment natural plant recruitment. This is important in the light of habitat destruction. We consolidate the current knowledge of *in vitro* germination of Diseae in South Africa in relation to their vulnerability to urban sprawl, silviculture and agriculture.

### Acknowledgments

The National Research Foundation and Natal University Research Fund are acknowledged for financial assistance.

### References

- Hilton-Taylor, C. 1996. Red Data List of southern African plants. Strelitzia 4.
- Linder, H.P. & Kurzweil. 1999. Orchids of southern Africa. Rotterdam: A.A. Balkema
- Wodrich, K. 1997. Growing South African indigenous orchids. Rotterdam: A.A. Balkema.

## Endangered *Prasophyllum* R.Br. species in south-eastern Australia.

Karina J. FitzGerald\* and D.L. Jones

Technical Officer, Orchid Research Group, Centre for Plant Biodiversity Research, CSIRO, P.O. Box 1600, Canberra, ACT, 2601. Email: Karina.FitzGerald@pi.csiro.au

Since European settlement 99.5% of Australia's native grasslands have been destroyed by clearing for urbanisation or agriculture. This extensive change has had a dramatic impact on the plants which occur naturally in the temperate grasslands of south-eastern Australia resulting in the remaining populations of many species being left vulnerable or threatened by processes such as habitat fragmentation, weed invasion, feral animals, rubbish dumping and road works.

*Prasophyllum* R.Br. is a specialised genus of native orchids many species of which inhabit the grasslands and grassy woodlands of Australia. Much of this habitat has been cleared and many *Prasophyllum* species have been reduced to relict, isolated populations. The population size and specific threats to seven species of *Prasophyllum* from south-eastern Australia are discussed.



*Prasophyllum petilum* D.L.Jones et. R.J.Bates

Photo: J.Jeanes



## ***Thelymitra matthewsii* – A specialist orchid maintaining a precarious ‘toe-hold’ in New Zealand’s far north?**

Elizabeth A. Fraser

Ongarue-gakonui Road, R. D. 4 Tamarunui, New Zealand. Ph. [NZ] 07 894 5991

*Thelymitra matthewsii* is a naturally uncommon species in New Zealand, with a sparse distribution and potential to become threatened, and susceptible to extirpation within its range. First discovered by R.H. Matthews near Kaitaia c. 1910, and described by Cheeseman, *Thelymitra matthewsii* was not recorded again until 1987 when D. McCrae, in a survey of the orchids of Te Paki Farm Park observed a plant near Surville Cliffs. A plant recorded in 1995 recorded in 1995 confirmed its presence in this locality. Subsequent ephemeral populations are recorded by New Zealand Native Orchid Group members in localities within the Farm Park, 1996-2000. *Thelymitra matthewsii* also occurs in an isolated and sporadic distribution in Australia, and generally, is considered uncommon. This poster presents the known morphological and ecological aspects of *Thelymitra matthewsii* in New Zealand. Photographs illustrate habitat and plant morphology. Three-dimensional photography enhances flowers and reproductive systems. Hand drawn line drawings complete the pictorial record. It is hoped that exposure will draw comparable data, and furnish valuable contacts for the Australian taxa, which will facilitate a projected study of the species for a Masters thesis.

## Conservation through propagation.

Wesley E. Higgins

Marie Selby Botanical Gardens, 811 South Palm Avenue. SARASOTA, Florida, 34236, USA.

Email: whiggins@selby.org

Marie Selby Botanical Gardens has developed a program for orchid conservation through propagation. The emphasis is on native Florida orchids and World Conservation Union (IUCN) Red Book species. Native orchids are being hand-pollinated in the Fakahatchee Strand State Preserve, east of Naples. The 80,640-acre preserve in Collier County has a mixed subtropical-tropical flora unique within North America. It is known for 50 species of orchids as well as rare ferns and bromeliads growing in swamp lakes, marl prairies, hammocks, and cypress domes. With a state permit from the Florida Department of Environmental Protection, Selby Garden associates are collecting seed capsules and propagating them aseptically in flasks. From the 15 native species now growing in flasks, many plants will be donated to the Orchid Conservation Committee and other non-profit groups for re-establishment in approved wild areas. Dr. Dale Jenkins, Selby Research Associate and former Director of Ecological Programs at the Smithsonian Institute, heads the ex situ conservation of Red Book orchids. By comparing IUCN Red Book orchids extinct, endangered, or threatened in the wild with plant records of living orchids at Selby Gardens, he has identified 92 listed species: 1 species extinct in the wild, 22 endangered, 40 vulnerable, and 29 rare in the wild. After hand-pollination in the greenhouse, capsules are sent to a private lab for germination. These plants will be distributed to other botanic gardens and institutions for ex situ conservation.

## Orchid population dynamics under human influence.

Jana Jersakova & Pavel Kindlmann\*

Faculty of Biological Sciences, University of South Bohemia, Branisovska 31, 370 05 Ceske Budejovice, Czech Republic; Email: pavel@entu.cas.cz

Plants from the family Orchidaceae, which are jewels of any regional flora, are sensitive indicators reacting to external chemical and mechanical treatments. In addition, many species of terrestrial orchids have been adapted to secondary forest-free area, meadows and pastures created by humans. Therefore, appropriate vegetation management of the site is essential for the orchid populations to persist. Vegetation management, usually summer mowing, helps to maintain the relatively low-growing herbaceous vegetation that the orchids require. Thus, in order to prevent the existing orchid populations from extinction, conservation only by law but without appropriate management may have results strongly differing from the aims of nature conservation authorities. Sites, which are once abandoned, lose their orchid flora due to lack of traditional management. The sunset of old orchards, which were suitable for orchid populations, is an explicit example of this process and a sad evidence of neglectful protection of landscape character in the Czech Republic. Green-winged Orchid (*Orchis morio* L.) represents a formerly abundant vernal species in the Czech Republic, which has recently disappeared from many areas of the whole Europe due to loss of habitat through the agricultural improvement of old hay meadows. Considerable population decline is well documented by former and recent distribution maps of the species in the Netherlands, Switzerland, East Germany and eastern part of the Czech Republic. During the recent six years we monitored 44 sites in South Bohemia, where *Orchis morio* populations still survive and established field experiments, testing effects of different types of management on orchid population dynamics. Our main results are: i) Irregular mowing strongly weakens and destabilises *O. morio* populations. ii) Absence of mowing has a serious negative effect on the number of flowering plants and leads to changes in species composition in the vegetation. iii) Mowing twice a year improves performance of orchid populations, especially in fertile sites with high vegetation cover in autumn. iv) Removal of litter and surface disturbance is more suitable for thick sod places. v) *O. morio* reacts very quickly in a negative way to lack of mowing and very slowly in a positive way to management restoration.

## Cryopreservation of protocorm-like bodies of *Dendrobium* by vitrification technique.

Saleh B. Kadzimin\* and Philip Sipen

\*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Email: saleh@agri.upm.edu.my

A study was undertaken to elucidate the effects of various levels of treatments involved in the cryopreservation of protocorm-like bodies through vitrification technique in an attempt to develop a protocol for long-term preservation of the orchid species. The study examined viability and survival of proliferating protocorm-like bodies of a *Dendrobium* hybrid subjected to various levels of pre-culture, loading and dehydration treatments prior to freezing. Viability was defined as those protocorm-like bodies remaining green 7 days after return to culture. Survival was assessed after 60 days and was based on cultures remaining green and differentiating. At the onset of the study, protocorm-like bodies were established and multiplied through the standard culture for shoot tips in a modified Vacin and Went (1949) medium.

Pre-culturing protocorm-like bodies in medium supplemented with 0.06, 0.1, 0.3 and 0.5 M sucrose for 3 days yielded viability that ranged from 3.7 to 23.3% after freezing. No viability was obtained from the control (0 M) and 0.7 M treatments. There was no survival at all levels of sucrose tested. Pre-culturing in a standard medium of 0.3 M sucrose for 1 to 9 days prior to freezing improved the viability range from 11.1 to 26.3%, with the highest obtained after 5 days. No viability was recorded from the control. There was no survival after freezing.

Protocorm-like bodies, pre-cultured for 5 days in medium supplemented with 0.3 M sucrose, were cryoprotected for a duration of 15 minutes with 6 different loading solutions of various combinations of sucrose, glycerol and dimethyl sulfoxide. Survival after freezing ranged non-significantly from 3.7 to 11.1%, and none from the control (medium without sucrose) and 2.0 M glycerol with 0.4 M sucrose treatments. The highest survival was obtained using loading solution containing 0.5 M glycerol, 10% dimethyl sulfoxide and 0.3 M sucrose. Cryoprotecting for 5 to 30 minutes with this solution increased survival to a range of 11.1% to 30.3%, the highest was obtained at 20 minutes. The control (without cryoprotection) and 30 minutes cryoprotection treatments had no survival.

Following cryoprotection, protocorm-like bodies were dehydrated for 10 minutes using 5 types of vitrification solutions of various combinations of glycerol, ethylene glycol, dimethyl sulfoxide, sucrose and calcium chloride. Survival range of between 7.4 and 26.3% were recorded except the control (medium without sucrose) which had none. The best vitrification solution was one with 50% glycerol plus 50% sucrose. Dehydrating for 5 to 30 minutes with this combination gave viability and survival rates of 20.1 to 53.2% and 3.7 to 48.5% respectively with the highest at the 30-minute duration.

For long-term preservation of genetic resources of orchids, this study recommends preculturing protocorm-like bodies for 5 days in Vacin and Went (1949) medium supplemented with 0.3 M sucrose, followed by 20-minute cryoprotection in a loading solution containing 0.5 M glycerol, 10% dimethyl sulfoxide and 0.3 M sucrose. Subsequently, they should be dehydrated for 30 minutes in a 50% glycerol plus 50% sucrose vitrification solution. The high survival rate of 48.5% obtained in the present study suggests that cryopreservation by vitrification technique can be a promising tool for long-term conservation of orchid germplasm.

## *In vitro* symbiotic germination of *Bletia urbana*.

Susana Luna-Rosales<sup>1\*</sup>, P. Ortega-Larrocea<sup>2</sup>, V. Chávez<sup>3</sup>, A. Barba-Alvarez<sup>1</sup>

1. Unidad de Investigación en Biología Vegetal, Facultad de Estudios Superiores-Zaragoza, UNAM, 09230, AP 09-20, México D.F. Email: barbaral@servidor.unam.mx

2. Unidad de Ambientes Controlados, Facultad de Ciencias, UNAM, 04511, AP 70-359, México D.F.

3. Laboratorio de Cultivo de Tejidos Vegetales, Jardín Botánico IB-UNAM, Ciudad Universitaria, 04510, AP 70-614, México D.F. Email: victorm@mail.ibiologia.unam.mx

*Bletia urbana* Dressler is an endemic terrestrial orchid of Mexico and native south Mexico City and Oaxaca (Sosa, 1994). This orchid is categorised as vulnerable (IUCN, 1997). Terrestrial orchids are often highly adapted to specific environments and the disturbance or disappearance of their habitats threaten their survival. The germination of *B. urbana* seeds on nutrient media has been reported by Rubluo *et al.* in 1989. Attempts to propagate orchids from seed using symbiotic fungi have been successful for species in Australia, Europe and United States. The use of the symbiotic method is now preferred over asymbiotic techniques for the seed of terrestrial orchids. Relatively few species have been propagated in this manner and few studies have addressed the requirements for successful soil establishment of symbiotic seedlings from *in vitro* conditions (Zettler and McInnis, 1993). The objectives of this investigation were to compare *in vitro* symbiotic and asymbiotic germination methods of the same lot of seeds of *B. urbana* that were used to germinate and propagate with the asymbiotic method fourteen years later.

Seeds of *B. urbana* were collected in September 1984 and stored for 14 years at 4°C. Knudson C medium (KC) was used for asymbiotic germination, on basic Oat medium (Clements *et al.* 1986) with *Rhizoctonia* sp. (O+) isolated from roots (Mitchell, 1989) of *Bletia* sp. for symbiotic germination, with basic Oat medium (O) as the control. Approximately 100 seeds were sown in December 1998 onto the surface of the media in Petri dishes. 5 replicates of each medium were seeded and incubated at 25±2°C under 16 hr photoperiod. Observations were recorded weekly. After 30 days symbiotic seedlings were subcultured onto fresh O medium. Seedlings were ready to be transferred to soil two weeks later.

*In vitro* symbiotic seed germination of *Bletia urbana* was achieved with the same lot of seeds that were asymbiotically germinated in 1989. Seeds germinated (protocorm formation) on all media. After 42 days in culture 100% of vigorous seedlings with roots, bulbs, and leaves have been formed only in symbiotic culture. Seedlings were obtained 48 days earlier than seedlings generated asymbiotically fourteen years earlier. Symbiotic seedling survival under greenhouse conditions was 100 percent after one year.

### References

- Clements, M., P.J. Cribb and H. Muir, 1986. Preliminary report on the symbiotic germination of European terrestrial orchids. *Kew Bull.* 41: 437-445.
- Mitchell, R. 1989. Growing hardy orchids from seeds at Kew. *Plantsman* 2(3): 152- 169.
- Sosa, V., 1994. A revision of the *Bletia reflexa* complex (Orchidaceae). *Lindleyana* 9(1): 7-17
- Walter, K.S. and Gillett, H.J. [eds] (1998). *1997 IUCN Red List of Threatened Plants*. Compiled by the World Conservation Monitoring Centre. IUCN - The World Conservation Union, Gland, Switzerland and Cambridge, UK. lxiv + 862pp.
- Zettler, L.W. and T.M. McInnis, Jr., 1993. Symbiotic seed germination and development of *Spiranthes cernua* and *Goodyera pubescens* (Orchidaceae: Spiranthoideae). *Lindleyana* 8(3): 155-162.

### Acknowledgements

This study was supported in part by Programa de Apoyo a Proyectos de Investigación e Investigación Tecnológica (PAPIIT) IN201800.

## **Paternity analysis and gene flow in a lithophytic orchid using AFLP.**

Barbara Rodríguez Marcano<sup>1\*</sup>, Raymond L. Tremblay<sup>1,4</sup>, Elvia Meléndez-Ackerman<sup>2</sup>, Carla Cortés<sup>3</sup> and Owen Mcmillan<sup>3</sup>.

1. Department of Biology, 100 carr. 908, University of Puerto Rico, Humacao, Puerto Rico, 00791-4300.

2. Institute of Tropical Ecosystem Studies, University of Puerto Rico-Rio Piedras PO BOX 363682 San Juan PR 00936-3682.

3. Department of Biology, University of Puerto Rico, Rio Piedras Campus, PO BOX 23360. San Juan, Puerto Rico, 00931-3360.

4. Contact person. Email: raymondtr Tremblay@hotmail.com

Our ability to define what is a population in orchids has been (in most cases) limited to different subjective definitions of the spatial distribution of clusters of individuals. Our definition of a population will influence our ability to make prediction on the importance of natural selection and genetic drift as relevant evolutionary mechanisms in natural conditions. For processes such as natural selection and genetic drift to occur the amount of migrant per generation into a population or cluster of individuals must not exceed one. Gene flow rates larger than one individual per generation will otherwise homogenise genetic characteristics between clusters. Previous work on *Lepanthes rupestris* Stimson using allozymes as genetic markers has suggested that gene flow can be variable and is weakly related to distance in this lithophytic orchid. In this study we develop a more sensitive molecular technique using Amplified Fragment Length Polymorphism (AFLP) to measure the distance of pollen flow within and among populations of this species. This technique can be used to identify with reliable validity the likely father of seeds. We collected one leaf of each individual in five nearby populations within the Luquillo Experimental Forest in Puerto Rico and all mature fruits still attached to plants. Using fluorescent AFLP with an automated sequencer, we are characterising each individual with a unique set of genetic characters. Results on the success of this fingerprinting technique to exclude all but one possible father are discussed.

## **The orchid flora of Orissa: status and strategies for conservation.**

Sarat Chandra Misra

Orchid Study Centre, C – 89, HIG, Baramunda Housing Board Colony, Bhubaneswar 751 003, Orissa, India.

The state of Orissa, located in the east of peninsular India in the tropics, has a very rich and diverse vegetation due to a wide range of edaphic and climatic conditions. 128 species of orchid (61 terrestrial and 67 epiphytic) belonging to 43 genera and three sub-families namely, Spiranthoideae, Orchidoideae and Epidendroideae are reported to occur in the tropical moist deciduous and semi-evergreen montane forests of Orissa. This includes 21 taxa that are endemic in India; 5 of these are confined to the state of Orissa. Besides, there are two species reported as new plant records for the flora of India. The orchid flora of the state is interesting from a phytogeographical point of view as the state forms the meeting ground for the flora of the Himalayas and the peninsular India. More numbers of species are distributed in the north-eastern districts of Mayurbhanj, Keonjhar and Sundargarh followed by the south eastern districts of Koraput and Gajapati. The wild habitats of orchids are rapidly decreasing due to unregulated deforestation bringing many species under the category of rare, vulnerable or endangered. It is essential to identify orchid habitats rich in diversity and initiate prompt conservation measures by preserving the area as botanical reserves, protectorates or orchid sanctuaries. The Similipal (93 species, 2 endemics) and Rebana (2 endemics) are good examples for this. Removal of orchids from their wild habitats needs to be checked and orchid species occurring in forest areas where deforestation is inevitable could be collected and replanted in botanical reserves. The state forest personnel should be made aware of the importance and need for protection of orchids and appropriate legal measures be taken to prevent collection of orchids from the wild. Ex situ conservation should be encouraged by way of establishment of orchidaria in parks and tourist resorts.

## Ensuring survival of Kenyan orchids: the *ex situ* conservation interventions

George K. Mugambi

National Museums of Kenya, East African Herbarium, PO Box 45166, Nairobi, Kenya.

Email: gmugambi@avu.org

The current threats to the natural habitats in Kenya stress the need for active orchid conservation. The country's high population growth has resulted in over-exploitation of natural resources, which has led to complete destruction or alteration of certain ecosystems. This has made *ex situ* conservation an important undertaking particularly for the species found in high-risk areas. For almost a decade, the Plant Conservation Program of the National Museums of Kenya (NMK) has been carrying out *ex situ* conservation activities for orchids. The outcome has been an *ex situ* orchid conservation program that includes: live plant collections in greenhouses, conservation of orchid seeds, research into seed biology and physiology and the related programs for conservation education and public awareness.

The *ex situ* collection comprise over 300 seed accessions from various orchid species in Kenya and over 1000 live orchid specimens comprising over 120 species held in the Nairobi Botanic Garden greenhouses. To support this, an active orchid seed propagation program has been established, which has ensured that the viability of orchid seeds is tested before storage as well as producing material for display in the greenhouses and possible future reintroduction. Orchids being a flagship group for conservation can play a major role in promoting and popularising plant conservation. Orchid display areas accessible to the general public have therefore been developed in the Nairobi Botanic Garden for the purposes of conservation, education and awareness.

To ensure appropriate *ex situ* conservation, it is essential to understand the biology of the species targeted for conservation. For this reason NMK has initiated research in the field of seed biology (seed storage behaviour and seed physiology), seed propagation and establishment of seedlings. Research is also carried out on orchid mycorrhiza and the importance of mycorrhizal fungi in orchid seed germination, documenting the country's orchid flora and database on complete Kenyan herbarium specimens of orchids. Of particular interest are optimum conditions for seed storage and propagation, seed viability testing and the role of mycorrhizal fungi in germination and seedling establishment. Although some knowledge may exist in these fields, the current investigations are species specific because of the great variations observed in orchids.

*In vitro* orchid seed propagation is difficult and often requires the use of elaborate laboratory techniques to achieve success. Quite often germination will only be achieved by ensuring an appropriate mycorrhizal fungus is inoculated together with the seeds on the growth medium. This requirement for a mycorrhizal fungus is pronounced in certain terrestrial species. The appropriate fungus will differ from species to species and even within a particular species that isolated from a mature orchid individual may be different from that for germination. Knowledge of the biology of orchid mycorrhiza is scant with past studies restricted to relatively few orchid species. Even less study has been carried out for tropical African species and this research program will therefore play a major role in improving the knowledge available. Research into seed propagation therefore targets establishing the germination procedures for Kenya's threatened orchid species. This knowledge is particularly important for seed viability testing before storage.



# Conservation biology of an achlorophyllous orchid: A case study on the underground orchid (*Rhizanthella gardneri*).

Sofi Mursidawati<sup>1,2</sup>, K. Sivasithamparam<sup>2</sup>, K.W. Dixon<sup>1</sup>, M.C. Brundrett<sup>1,2</sup>

1. Faculty of Agriculture, The University of Western Australia, Crawley, Western Australia 6097.

Email: mursis01@agric.uwa.edu.au

2. Science Directorate, Botanic Gardens & Park Authority, Kings Park, West Perth, Western Australia 6005.

Fungal symbiosis is a critical component of orchid biology that determines their development and survival. Their tiny seed can not carry adequate food reserves for their establishment. The degree of their dependency to associated fungal symbiont varies with the species, however higher level of dependency shown by achlorophyllous orchids, since they lack capability for photosynthesis. Former studies concluded that *Rhizanthella gardneri* has an obligate requirement of fungal endophytes that also form ectomycorrhizas with the green plant *Melaleuca uncinata* to nourish them. Nevertheless, there are still many biological aspects regarding the identity of the fungal symbiont and nutritional association that remain unclear.

Further studies on the identity of the fungal symbiont are urgently required and the result of the study will contribute greatly to the conservation management of this rare orchid. Molecular and biochemical approaches may permit the investigation of fungal symbionts and specificity among numerous orchid rhizoctonias, especially those which are difficult to isolate and grow axenically. Bioassays and examination of field collected roots will be carried out at under glasshouse conditions to identify the nutritional and ecological significance of the symbiotic relationship between *Rhizanthella gardneri*, *Melaleuca uncinata* and the fungal endophytes.

## Is there a one-to-one specificity between *Diuris* species and mycorrhizal fungi?

L. Quay\*<sup>1</sup>, P.A. O'Brien<sup>1</sup>, J.A. McComb<sup>1</sup>, K. Sivasithamparam<sup>2</sup> & K.W. Dixon<sup>3</sup>

1. School of Biological Science & Biotechnology, Murdoch University, Murdoch, WA 6150, AUSTRALIA.

Email: lquay@central.murdoch.edu.au

2. Soil Science & Plant Nutrition, The University of Western Australia, Nedlands, WA 6009, AUSTRALIA.

3. Kings Park & Botanic Garden, West Perth WA 6005, AUSTRALIA.

Specificity of *Diuris magnifica* D. Jones and related species to their mycorrhizal fungi was examined. These terrestrial species, endemic to Western Australia, regenerate from non-infected tubers, and mycorrhizal fungi infect the roots and form hyphal coils (pelotons). Fungal endophytes were characterised by cultural appearance, seed germination ability and DNA analysis.

Flowering plants of *D. magnifica*, *D. corymbosa*, *D. magnifica* x *D. corymbosa* hybrid, *D. brumalis* and *D. laxiflora* were collected from July to October from locations within 50km of Perth Western Australia. Pelotons were isolated after cutting roots and tubers into 1cm sections and plating onto isolation medium (1). Endophytes were recovered by subculturing tips of fungal outgrowths onto PDA. Germination was assessed 8 weeks after inoculating seed with fungal isolates from their respective and other *Diuris* species (1,2). DNA was extracted from mycelium grown on V8 broth, and amplified by polymerase chain reaction (PCR) using ITS1 and ITS4 primers (3) which are complementary to the internal transcribed spacer (ITS) region of the rDNA. Amplified DNA products were subjected to restriction fragment length polymorphism (RFLP) analysis using the enzymes *Bam*HI, *Cfo*I, *Hae*III, *Msp*I and *Taq*I. Fragments were separated by agarose gel electrophoresis and visualised under UV light after staining with ethidium bromide.

Mycelial growth on PDA from most root isolates from all *Diuris* species was similar (creamy white, round colonies with aerial mycelia), while a few isolates from both roots and tubers had white, pink or brown aerial mycelia. Germination tests between seed and fungi of their respective species demonstrated that not all isolates were efficacious. Some isolates had the ability to germinate seeds of *Diuris* species other than the one from which they were isolated (Table 1). Differences in cultural appearance of the fungi correlated with seed germination ability, and differences in the size of the amplified ITS fragments and RFLPs. Our results suggest that there is not a one to one relationship between *Diuris* species and their mycorrhizal fungi, but a varying degree of specificity.

**Table 1.** Number of plates with germinated seed when inoculated with fungal isolates from *D. magnifica* (DM), *D. corymbosa* (DC), *D. magnifica* x *D. corymbosa* hybrid (DMC), *D. brumalis* (DB) & *D. laxiflora* (DL). Numbers in brackets represent total replicates; DM1, DM2 etc different fungal isolates.

Fungi	Orchid Species Seed				
	DM	DC	DMC	DB	DL
DM1	3(3)	-	0(2)	3(3)	3(3)
DM2	3(3)	-	0(2)	1(3)	3(3)
DC1	5(5)	4(4)	0(3)	5(5)	5(5)
DC2	5(5)	4(4)	0(3)	4(5)	5(5)
DMC1	0(5)	-	3(3)	5(5)	5(5)
DB1	5(5)	-	0(3)	2(2)	5(5)
DB2	0(5)	-	0(3)	2(2)	5(5)
DB3	0(5)	-	-	2(2)	5(5)
DL1	0(5)	-	-	5(5)	2(2)
DL2	5(5)	-	0(3)	2(5)	2(2)
DL3	5(5)	-	0(2)	2(5)	2(2)

### REFERENCES

1. Ramsay RR, Dixon KW & Sivasithamparam K. (1986). *Lindleyana* 1, 203-4.
2. Wilkinson KG, Dixon KW & Sivasithamparam K. (1989). *New Phytologist* 112, 429-35.
3. White TJ, Bruns T, Lee S & Taylor J. (1990). In: Innis MA, Gelfand DH, Sninski JJ & White TJ (eds) *PCR Protocols: a Guide to Methods and Applications*. New York: Academic Press, 315-22.

## The genus *Dactylorhiza* in the flora of Belarus.

LV Scmerenko & IV Shvets

Akademicheskaya Str., 27, V.F. Kuprevich Institute of Experimental Botany of National Academy of Sciences of Belarus, Minsk, Belarus 220733. Email: flora@biobel.bas-net.by

The genus *Dactylorhiza* Nevski is taxonomically complicated and to assist in identifying species it is highly desirable to apply caryological methods in addition to comparative morphological studies to resolve specific variation. For more than 10 years we researched this genus over a wide geographical range to determine the limits on species.

Root meristems of flowering plants served as suitable material for cytological analysis. The chromosome numbers were determined in a sample of 380 plants. This revealed two cytotypes in *D. fuchsii* (Druce) Soo (with  $2n = 40$  and  $80$ ). Tetraploid plants forming quite numerous populations were located in a spruce or spruce-broad-leaved forest ecosystem. Populations (or some individuals) of diploid plants occur throughout the republic area in diverse but predominantly forest ecotypes: from dry pine forests in uplands to black alder swamp forests. Tetraploids and diploids exhibit typical traits of *D. fuchsii*: broad-elliptic or obovate basal leaves and deep trisected flower lips.

Application of caryological methods also revealed of hybrid forms, determination of limits of intraspecific phenotypic variability and selection of major species diagnostic characters. *D. longifolia* (L. Neum.) Aver. that was considered earlier to be rather rare species in the republic is not rare at present in river valleys of Belarus. Our own collections and critical examination of herbarium ones have changed a notion about *D. majalis* (Reichenb.) P.F. Hunt et Summerhayes distribution in the republic.

At the present stage of cytotaxonomic investigations in the area of Belarus we have identified by phenotype and verified caryologically the following *Dactylorhiza* species: *D. incarnata* (L.) Soo –  $2n = 40$ , *D. fuchsii* (Druce) Soo –  $2n = 40, 80$ , *D. longifolia* (L. Neum.) Aver. –  $2n = 80$ , *D. maculata* (L.) Soo –  $2n = 80$ , *D. majalis* (Reichenb.) P.F. Hunt et Summerhayes –  $2n = 80$ , as well as numerous hybrid forms. *D. traunsteineri* (Saut.) Soo and *D. crenata* (O.F. Muell.) Soo were identified only by herbarium specimens.

## Unusually high genetic variability revealed through allozyme polymorphism of eastern (*Pterostylis gibbosa*) and Western Australian (*P. aff. picta*) endangered orchid species.

Ish K. Sharma<sup>1</sup>, D.L. Jones<sup>1</sup>, M.A. Clements<sup>1</sup>, C.J. French<sup>2</sup>

1. Centre For Plant Biodiversity Research, Division of Plant Industry, CSIRO, Canberra, ACT 2601.

Email: ish.sharma@pi.csiro.au

2. 2 Rowney Close, Bateman, W.A. 6150.

Starch gel electrophoresis was employed to survey the allozyme polymorphism among the known populations of the eastern Australian orchid species *Pterostylis gibbosa* and western Australian *P. aff. picta* both of which currently have endangered species status. The aim of our investigation was to assess the genetic diversity within and among populations of these orchids. Results showed a high level of genetic diversity both at population and species level. The percentage of polymorphic loci ( $P$ ), the number of alleles per locus ( $A$ ), observed and expected heterozygosity ( $H_o$ ,  $H_e$ ) were 69%, 2.21, 0.210, 0.261 in *P. gibbosa* and 69%, 2.04, 0.275, 0.284 in *P. aff. picta* respectively. The  $G_{st}$  value ranged from 5-15% suggesting that substantial variation resides within populations. The average gene flow ( $N_m$ ) value of  $>4$  in both species indicate that gene flow is sufficient to impede genetic structuring. High genetic variability and low population divergence are present despite a restricted distribution in both species and a recent rapid decline in the populations, possibly due to ecological factors. Higher genetic variability may also be due to extensive gene flow maintained by seed and pollen movement. Although endangered and restricted to only a few geographical sites, the populations of *P. gibbosa* and *P. aff. picta* are viable as a consequence of higher levels of genetic variation when compared with other orchids which have larger populations.

# Conquering coat-imposed seed dormancy in the South African *Disa* (Orchidaceae): old ideas and new techniques.

Dave I. Thompson, Trevor J. Edwards\* and Johannes van Staden

Research Centre for Plant Growth & Development, School of Botany & Zoology, University of Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa.

The *in vitro* seed culture of South Africa's terrestrial orchids has historically met with limited success (Arditti, 1982; LaCroix and LaCroix, 1997). Conventional seed culture techniques result in unpredictable germination that is achieved only rarely or in low percentages (Wodrich, 1997). *Disa*, southern Africa's largest orchid genus, was investigated in this context. Easily germinated winter rainfall species have led to invalid conclusions regarding seed culture across the genus (Collett, 1971; Vogelpoel, 1980; Arditti, 1982; Vogelpoel, 1987; LaCroix and LaCroix, 1997; Wodrich, 1997). Germination is unrecorded for over 90% of *Disa* species including all endemics from summer-rainfall areas. These seeds possess a comparatively thick, continuous and phenol-rich testa, subjecting the undifferentiated embryo (16- 32 cells) to a moisture-deprived dormancy. Coat-imposed seed dormancy is a strategy suited to extended winter drought. Modified techniques have resulted in first time germination reports for various South African species, viz. *D. cooperi*, *D. nervosa*, *D. pulchra* and *D. stachyoides*. Immature seed sowing, agitated liquid cultures and culture media supplemented with activated charcoal were instrumental in the germination of these species. Hypochlorite or peroxide scarification, stemming from prolonged surface decontamination, also decreases the time taken to and results in elevated levels of germination. Increased testa permeability, through scarification or leaching, also allowed accurate determination of seed viability through embryo staining. The above protocol modifications have led to the development of dual-phase seed culture media - a novel technique of this research. The time taken to and total percentage germination (signalled by protocorm emergence) was most favourable in dual-phase cultures (6 weeks & > 85% for *D. cooperi*; 8 weeks & > 60% for *D. nervosa* and *D. pulchra*). *D. sagittalis*, germinated from immature seed by Crous (pers. comm.) in 6 months, responded in 11 weeks under dual-phase conditions, albeit in low percentages (<30%). Germination synchrony was enhanced in all species, being spread over only several weeks.

## Acknowledgements

The National Research Fund (South Africa) and the Natal University Research Fund are acknowledged for financial Assistance.

## References

- Arditti, J. 1982. Orchid Seed Germination and Seedling Culture - a manual. In: *Orchid Biology - Reviews and Perspectives II.*, (ed.) Arditti, J. Cornell University press, New York.
- Collett, A. 1971. Notes on growing *Disa uniflora*. *Journal of the Royal Horticultural Society* 96: 358-361.
- LaCroix, I.F. and LaCroix, E. 1977. *African orchids in the wild and in cultivation*. Timber press, Portland.
- Vogelpoel, L. 1980. *Disa uniflora* - its propagation and culture. *American Orchid Society Bulletin* 49: 961-974.
- Vogelpoel, L. 1987. New Horizons in *Disa* breeding. The parent species and their culture I. *Orchid Review* 95: 176-181.
- Wodrich, K. 1997. *Growing South African indigenous orchids*. A.A. Balkema, Rotterdam.

## Micropropagation of jarrah forest plant species at Marrinup Nursery (ALCOA World Alumina-Australia).

David Willyams

Alcoa World Alumina Australia, Marrinup Nursery, PO Box 52, Dwellingup, WA 6213, Australia.

Email: david.willyams@alcoa.com.au

Alcoa World Alumina Australia operates two bauxite mines in the Northern Jarrah Forest. Bauxite is sent to three alumina refineries on the Swan Coastal Plain. Following mining the land is rehabilitated – the aim is to re-establish a self-sustaining jarrah forest ecosystem. Botanical diversity of the rehabilitated mines is a key measure of success. Alcoa's Marrinup Nursery plays a crucial role in many of the rehabilitation procedures developed to achieve high botanical diversity.

Many plant species re-establish on the rehabilitated mines from seed contained in topsoil. Best results are achieved when fresh topsoil is transferred directly from areas being prepared for mining to rehabilitation areas. For other species, locally collected seed is broadcast to supplement the soil seed store. Marrinup Nursery manages the collection, storage, treatment and mixing of this seed. For plants with low seed production or viability seed is grown at the nursery for hand-planting. These seeds may require pre-treatment to encourage germination (e.g. smoke, gibberellic acid). The jarrah forest understorey also includes several 'recalcitrant' species that produce very few viable seeds. In nature they usually reproduce and spread vegetatively. For these species, clonal propagation and planting is necessary.

Orchids naturally recolonise the mined areas as the rehabilitation ages. It appears that the obligate symbiotic fungi the orchids require, only re-establish once a litter layer develops.

Many of the 'recalcitrant' species have never been grown for the horticultural trade. Hence propagation methods have had to be developed specially for this project. The simplest, most reliable and economical vegetative propagation alternatives are used. Thirteen woody dicot species are propagated using cuttings. Several monocots however are best reproduced *in vitro*.

The micropropagation unit at Marrinup Nursery is producing over 100,000 plants annually for use in mine rehabilitation. There are 14 species in production, mostly members of the Cyperaceae, Restionaceae and Dasypogonaceae. These dryland rushes, sedges and strap-leaved monocots are a major component of the jarrah forest understorey, fulfilling the role of the grasses that are found in less ancient ecosystems.

Production protocols have been developed for a further seven species, with work progressing on another twenty. Species are only added to the mass production list when they can be reliably and economically propagated.

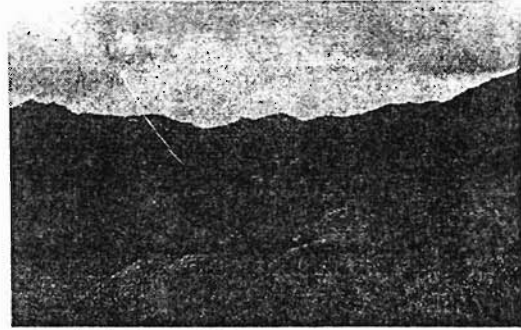
*In vitro* propagation methods and media have been developed at Marrinup Nursery in collaboration with the Micropropagation Unit at the Botanic Gardens & Parks Authority in Perth. Many of these methods are applicable to propagating other monocots.

# The orchids of the Northwest Yunnan, China

Jin Xiaohua and Qin Haining

Institute of Botany, Chinese Academy of Sciences, Beijing, China

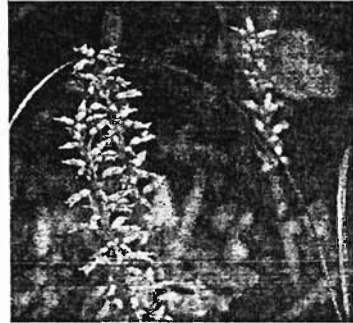
The northwest Yunnan represents a complex vegetation in form of forests, grasslands, marshes, deserts, and meadows. Associated with this complex vegetation is the diverse niche, which provides many habitats for orchids.



The Atlas and Environments of Northwest Yunnan.



*Epipactis* sp.



*Neottia* sp.



*Orchis* sp.



*Calanthe* sp.



*Holcoglossum* sp.



*Cypripedium* sp.

## Abstracts in Absentia.

Due to circumstances beyond their control, the following delegates were unable to attend. Their abstracts are included here to provide a more complete picture of global orchid conservation issues.

## Analysis of orchid endemism in eastern Indochina.

Leonid V. Averyanov

Komarov Botanical Institute of the Russian Academy of Sciences, Prof. Popov Str., 2, St.-Petersburg, 197376, Russia. Email: av@herb.bin.ras.spb.ru

Six floristic regions are outlined on the territory of eastern Indochina on the base of analysis of modern data on geology, geomorphology and climate of the region, as well as numerous original data on flora and vegetation. They are: Sikang-Yunnan, South Chinese, North Indochinese, Central Annamese, South Annamese and South Indochinese Provinces. Sikang-Yunnan Province embraces western Szechwan, Yunnan Plateau, northern mountains of Myanmar, Laos and Vietnam. This highland area represents a SSE extension of Himalayas with monsoon tropical climate, rainy warm summer and cold foggy winter. Main vegetation here is tropical montane forests with considerable influence of temperate Asian elements. 117 orchid species from 46 genera are reported from this area (1 genus and 11 species are local endemics). South Chinese Province includes tropical regions of S. Yunnan, S. Guangxi, the Luichow Peninsula, Hainan and northeastern portion of Vietnam with monsoon tropical climate, relatively cool dry winter and summer rains. Specific tropical dry forests on highly eroded limestone are typical for landscapes of this floristic province. 295 species from 88 genera are known in this province (among them 41 species are local endemics). Large parts of NW. Vietnam and N. Laos belong to the North Indochinese Province. Highland forests closely related with flora of S. Himalayas are often formed on silicate rocks and limestone. Climate in the area is very variable and belong to tropical types with summer or autumn rains. About 201 orchids from 76 genera are reported for the province (among them 11 species are local endemics). Central Annamese Province is outlined by montane areas of central Vietnam and eastern Laos. This area has monsoon tropical climate with warm winter and summer or autumn rains. Wet tropical mountain forest on silicate rocks are most widespread here. 335 species of orchids from 99 genera inhabit area of Central Annamese floristic province (among them 21 local endemics of the area). South Annamese Province is outlined by isolated highland mountain area situated in the centre of southern Vietnam. Mountains here composed mainly with granite and gneiss support rich and specific tropical montane forests. Monsoon tropical climate with warm winter and summer rains is typical for the area. About 502 orchids from 112 genera are known on this territory, 58 of which are local endemics. Alluvial plain and hilly lowlands of Cambodia, southern Laos and southern Vietnam belong to South Indochinese Province where dominate dry deciduous and semi-deciduous forests and woodlands. Monsoon hot sub-equatorial climate with summer and autumn rains is typical for largest part of this province. 273 species from 91 genera are reported from this area (23 orchid species here are local endemics). Preliminary floristic analysis reveals clear connections of Sikang-Yunnan and North Indochinese orchid flora with flora of S. Himalayas. South Annamese and especially South Indochinese Provinces has obvious floristic relations with W. Malesia. At the same time orchid floras of all defined floristic regions has very specific features. All they have outstanding level of local orchid endemism, which outlines these areas as very important centers of plant diversity. Obviously species composition of regional orchid floras may serve as appropriate and representative marker in biogeography generalisations in tropical areas of the southern Asia. As one of the most sensitive component of the flora these plants represent also effective marker for monitoring of natural conditions in different kinds of habitats all over the area. Deforestation and commercial collecting are the main factors of catastrophic orchid extinction and rapid decrease of the general biodiversity in the eastern Indochina. Most endangered orchids in eastern Indochina are species of *Aerides*, *Anoectochilus*, *Arachnis*, *Cymbidium*, *Dendrobium*, *Holcoglossum*, *Paphiopedilum*, *Papilionanthe*, *Phalaenopsis*, *Pleione*, *Renanthera*, *Thunia* and *Vanda*.



## Pollination ecology of four *Dracula* species growing sympatrically in northwestern Ecuador.

Lorena Endara A.

Herbarium QCA- Pontifical Catholic University of Ecuador. Email: lendara@puceuo.puce.edu.ec,  
clendara@hotmail.com

The reproductive biology was investigated in four orchid species in the genus *Dracula* growing sympatrically in a mid-elevation Andean forest in central Ecuador. The central goal of the research was to determine whether *D. lafleurii* and *D. pubescens*, which have small geographic ranges, differ in their floral biology from *D. felix* and *D. chiroptera*, which are distributed throughout the northwestern Andes. In particular, the hypotheses were tested that the rare species had more highly specific pollinators than the common species, and that the pollinators of the rare species were themselves restricted to small geographic areas.

The *Dracula* flowers observed in the field received a large array of visitors, mainly belonging to the mycophagous dipteran genus *Zygothrica*, currently the largest genus of the family Drosophilidae. Neither hypothesis was supported: *D. lafleurii* and *D. chiroptera* shared visitors as well as pollinators, and some pollinators of the rare species are widely distributed throughout South America.

In view of the complex relationships between the four species of *Dracula* and their visitors and pollinators, the poorly understood interactions between the species themselves, and the threat of habitat loss and commercial over-harvesting in Ecuador, I propose a revision of actual status of the genus in CITES.

## Conservation status of endemic Ecuadorian orchids.

Lorena Endara A\*, Renato Valencia R., Susana León-Yáñez  
Herbarium QCA - Pontifical Catholic University of Ecuador, P.O.Box 17-01-2184, Quito, Ecuador

If you walk through Ecuadorian forests you have the chance that one out of every five plants species you find is an orchid, with 40% chance that this orchid is locally endemic. Additionally, that endemic orchid you could have in your hands has an 83% of chance of becoming extinct in the next twenty years.

The megadiverse Ecuadorian orchid flora comprises 3,314 (Dodson & Luer, in press) species of which 1,318 are endemic. Endemic species tend to have restricted geographic distributions that might be indicators of species prone to extinction (Gaston 1994, Rabinowitz 1981). Thereupon a national effort to analyse Ecuadorian endemic species was overtaken and the final result was the "Red Book of the Endemic Plants of Ecuador" (Valencia *et al.* 2000), which presents the categories for threatened species using IUCN parameters, with a brief description of the known range of abundance of each species.

Two percent of the endemic orchid species are Critically Endangered (CR), 4% are Endangered (EN) and 79% are Vulnerable (Vu) (Endara, 2000). Ecuadorian orchids situation is perhaps more difficult than any other Ecuadorian plant group. The high number of orchid species, which occupy an extreme diversity of habitats and microclimates are threatened by two main reasons: a devastating high annual deforestation rate, 1.8%, currently the highest in South America (Wunder 1997), and an implacable over-harvesting. Additionally, 84% of the endemic orchids have not been registered in the Ecuadorian System of Protected Areas.

Our next step after knowing which species are most threatened, is building a National Strategy for the Conservation of Endangered Orchids in Ecuador, a pilot project for the Northern Andes Ecoregion.

## Colonisation and diversity of epiphytic orchids on trees in disturbed forests.

Mohammed Kamrul Huda<sup>1\*</sup>, Christopher Charles Wilcock<sup>2</sup> And Mohammad Atiqur Rahman<sup>1</sup>

1. Department of Botany, University of Chittagong, Chittagong 4331, Bangladesh.

Email: mkhuda@ctgu.edu and marahman@abnetbd.com

2. Department of Plant and Soil Science University of Aberdeen, Aberdeen AB24 3UU, UK.

Email: c.wilcock@abdn.ac.uk

Epiphytes predominate in tropical or subtropical regions. Orchids are the most diverse group of epiphytes with more than two thirds of all their species being epiphytic, yet they are little studied compared to their terrestrial counterparts. The sparse information which is currently available suggests that the presence of suitable host plants, host age and area available for colonisation may affect epiphyte abundance and community composition. Data from epiphytic orchids in Costa Rica has shown that populations are generally small and composed of scattered or clustered individuals. However little is known of the elevation of the epiphyte (which could affect pollination) or the size of the clump (which could indicate its age on the host).

The colonisation and diversity of epiphytic orchids on trees in disturbed forests of Bangladesh were investigated. Of the total orchid flora reported from Bangladesh some 65% (106 species) are reported to be epiphytic. Data from 41 of these species have been obtained. These species were found growing on a total of 57 different host tree species (phorophytes), indicating a wide range of potential hosts for the family.

Larger host trees accumulated more orchids on them than smaller ones. In old forests more than 60% of the largest trees may be colonised by orchids whereas the smaller trees in the same forest have less than 10%. There was no evidence of host specificity, but three native host trees (*Syzygium grande*, *Stereospermum chelonoides* and *Syzygium cumini*) had a higher than expected number of individual clumps per tree and a wider diversity of orchids on them. The data show that colonisation of an epiphytic orchid is a rare and random event, the presence of one orchid neither attracting nor repelling others on the same host. Most orchids were found on host trees only as single individuals/clumps (76% of all occurrences on host trees). Colonisation was not affected by leaf persistency or status of host (i.e. whether native or introduced) but was higher in areas of Bangladesh with greatest orchid richness.

The data suggest that the frequency of colonisation of epiphytic orchids is primarily a function of, 1) *age of the host*, allowing both more time and more surfaces to accumulate seeds on them, and 2) the existing *orchid richness* of an area, allowing for a higher colonisation rate from local seed input. Selective logging of the oldest trees in area would therefore cause a selective decline in epiphytic orchid abundance and further loss in orchid richness of the area. Data are presented which show that epiphytic orchids of Bangladesh have generally lower reproductive success in the field than terrestrial species and that smaller populations have lower fruit set than larger ones. The knock-on effect of loss of habitat and potential for natural recovery of epiphytic orchids in disturbed forests may be more serious than for terrestrial species.

## Achlorophyllous orchids of the Indian Region and their conservation needs.

Udai C. Pradhan

Chair, Indian Sub-continent Regional Orchid Specialist Group, SSC, IUCN. Email: himalaya@dte.vsnl.net.in

When we talk about conservation of orchids, more often than not, we are focussing our attention on to the aesthetically appealing members of the Orchidaceae which we can grow and enjoy in our homes and gardens. Just a mention that *Paphiopedilum druryi*, *Sepiopedilum fairrieianum*, *Renanthera imschootiana* or *Vanda coerulea* are endangered by habitat destruction or excessive commercial exploitation, could make us co-relate to these subjects and create a lot of fuss about their conservation needs and studies. But when we mention saprophytic orchids like *Epipogium sessanum*, *Galeola cathcartii*, *Gastrodia arunachalensis*, *Risleya atropurpurea*, *Yoania prainii*, *Didymoplexis pallens* or *Evrardianthe asraoa*, very few of us would know or care much about them – why? The main reason is that they are unknown in cultivation, occur sporadically in their ranges and often hide or disappear when their habitats undergo demographic changes perhaps never to reappear again! How fortunate that the former species are now grown widely are independent of mycotrophic dependence as an adult and are among the few plants favoured in CITES and Wildlife Protection Acts in India!

Habitat destruction looms as the single most important factor in the disappearance of this group of orchids as they are hardly known as traded plants. Natural calamities like earthquakes, excessive rainfall resulting in flash floods and heavy landslides are some of the other natural factors. Periodic forest “harvesting” and road construction, and clearance of virgin forests for making dams, expanding population and other mono-cultural activities add to the destruction.

The Indian Region is estimated to have 1200 species of orchids in 170 genera. This study enumerates the 40 saprophytic orchid species of the Indian Region, evaluates what is known of them and urges more intensive study of these unique plants before it is too late and their habitats gone. The primary aim is to direct attention on these botanical curiosities with a view to seeking out the possibilities of cultivating them in our greenhouses and gardens, so that they come to be appreciated more and scientific studies can be carried out to determine the nature of their function in the complex web of life. Understanding these entities intimately and knowing how to cultivate them can alone assure their long-term survival in natural habitats or protected areas.

### Bibliography

- Bhattacharya, U.C. & A.K. Goel (1982). *Aphyllorchis parviflora* King & Pantl. New distribution record of a rare orchid from Tehri Garhwal. J. Econ. Tax. Bot. 3(2): 588-590.
- Deori, N.C. (1979). *Pantlingia serrata* sp.nov. (Orchidaceae) – a second species of the genus from Meghalaya. Bull. Bot. Surv. India, 20(1-4): 175-176.
- Hegde, S.N. & A.N. Rao (1985). *Gastrodia arunachalensis* Hegde & Rao – A new orchid from Arunachal Pradesh, India. Orch. Rev. 93(1099): 171-172.
- Joseph, J. & N.R. Abbareddy (1985). *Evrardia asraoa* Joseph & Abbareddy – A new orchid species from Khasi Hills, Meghalaya, with incidental first record of the genus from India. Bull. Bot. Surv. India, 25(1-4): 232-234.
- King, G. & R. Pantling (1898). The orchids of the Sikkim-Himalaya. Ann. Roy. Bot. Gard. Calc. 8: 1-342. Bengal Secretariat Press, Calcutta, India.
- Malhotra, C.L. & B. Balodi (1985). A new species of *Corallorhiza* Gagnep. From Gori Valley. Bull. Bot. Surv. India, 26(1-2): 108-109.
- Pradhan, U.C. (1976,1979). Indian orchids: guide to identification and culture. Vols. 1 & 2. Kalimpong & Faridabad.
- Fao, A.N. (1988). A taxonomic review of the saprophytic orchids of Arunachal Pradesh, India. J. Orch. Soc. Ind. 2(1,2): 9-18.
- Shish Kumar, C. & K.S. Manilal (1994). A catalogue of Indian orchids. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.
- Smith, W.W. (1911). *Aphyllorchis pantlingii*. Rec. Bot. Surv. India 4:243.

## Population studies in the epiphytic orchid *Sarcochilus japonicus*.

Irina V. Tatarenko

Moscow Pedagogical State University, Karakozava St. 58, Mozhaisk, Moscow Province, 143200, RUSSIA. Email: irina@mozhaysk.net tel.++7-09638-24535

*Sarcochilus japonicus* (Reichenb. f.) Miq. is an epiphyte growing in a temperate climatic zone. It was studied in Hiroshima Prefecture, Japan in 2000. The population of *S. japonicus* was located on the 400-years old tree of *Pinus thunbergii* and two younger ones. Population structure was analysed according to the age class groups (Rabotnov, 1950): l – seeds; p – protocorm; p1 – protocorm with first green leaf; j – juvenile, im – immature; v – virginal; g1 – young generative (sexual mature), g2 – adult generative; g3 – old generative; s – senile.

Seeds of *S. japonicus* can germinate at once after dispersion from capsule or stay latent for one year inside the ripe non-opened capsule, as just on the surface of orchid leaves or *Pinus* bark. Successful germination of seeds is clearly indicated by numerous protocorms existed in population all around a year. At the first stage of protocorm development (p) the primary shoot axis elongates along the surface of substrate and forms leaf-like flat structure on the dorsal side of protocorm body. The first root emergents at the same time with first green leaf. The primary protocorm dies soon after roots reach the length 0,5-1,5 cm and could keep the plant on the tree. Number and size of leaves, length of monopodial shoot, number and length of roots consequently increase from juvenile to generative age stages. Lifespan of adult shoot is 8-10 years or more. Total ontogenesis takes near 15 years.

*S. japonicus* demonstrates sexual reproduction dominated in reproductive strategy of species. Majority of adult plants in population comes to flowering simultaneously, once in 3-4 years. Every shoot produces at once 2-4 flower stalks with 2-3 flowers after plant has been keeping the buds undeveloped during 2-3 years. Vegetative germination in *S. japonicus* exists owing to proliferation of lateral buds. However, less than 3% of the old generative and senile plants come to sprouting of lateral vegetative shoots instead of inflorescences.

Absolute number of population studied was near 1000 plants. Age spectrum of population reflects the relative ratio of age groups (%): 12: 22: 7: 3: 9: 5: 32: 6: 4. At the end of summer population age spectrum has two clear maxima: on the protocorms and adult generative plants. However, the protocorm number varies seasonally much more than another age classes. Age structure of investigated population of *S. japonicus* is rather similar to some terrestrial orchids, which are characterised by predomination of generative reproduction (Tatarenko, 1996).

Spatial structure of epiphytic orchid populations strongly depends on the host tree architecture. Dimensions of *S. japonicus* were concentrated on the nine lower branches of 1-3 levels on the *Pinus* tree: more than 60% – on the first level; near 20% – on the second, 15% on the third. Few separated plants and small groups were situated on the branches of 4-th level. Consequent decrease of orchid number from the bottom to the top of tree was related on more dry and hot conditions in the upper part of *Pinus*. Orchid location was obviously depended on the direction of branch growth. The largest dimensions of *S. japonicus* grew on the North and South-West oriented branches. Southern branches have only a few orchids because of too dry conditions.

Area of orchid roots spreading upon the surface of branches is many times more than the area occupied by hanging shoots. "Root substrate" was very favourable for germination of orchid seeds because of active mycorrhizal fungi come from the roots and promote the seeds development. The most of protocorms were settled upon and between the orchids roots. In terrestrial orchid populations seeds also preferably germinated close to the roots of mother plant (Nikitina, Denisova, 1980; Tatarenko, 1991; Batalov, 1998), where mycorrhizal fungi seems to be physiologically more compatible to interact with protocorms.

Stable population life of *S. japonicus* in habitat studied was guaranteed by direct interactions of orchid with mycorrhizal fungi, unicellular green algae, 3 species of epiphytic lichens and 3 species of green mosses. Analysis of functional relations between all these organisms would be useful in many ways.

## General Outcomes

- integrated conservation. celebration of the concept of,
- orchid as flagship species. the "funny waddy" factor
- allateral conservation
- Re-invigoration of our combined passion and enthusiasm - the ET factor (we are not alone!)
- An orchid Renaissance - the large number of young delegates. succession planning

## Specific Outcomes

### Measure diversity

- Importance of good taxonomy, appropriate sampling
- Vouchering
- In some cases genetic markers fail to discriminate accepted taxa (pollinators)

## Threatening and Ecological Processes

- Threatening processes - fire
- Trade: > 97% live plants are artificially propagated yet 0.5 million wild pl traded annually.
- Mycorrhizal associations and orchids
- Pollinator specificity
- Population dynamics and long term studies

## Ex situ Conservation

- Genetically representative sample
- Seed science - dormancy, viability, quality
- Mycorrhizal specificity - epiphytes?
- Germoplasm conservation → UK, Kew

## Recovery Operations

OK  
Cypripedium calceolus to Caladenia hastata



The First International  
**Orchid Conservation Congress**

**Perth, Western Australia, 24-28 September 2001**  
C/- Kings Park & Botanic Garden, West Perth 6005, Western Australia  
<http://www.bgpa.wa.gov.au/OrchidCongress/OrchidCongress.html>

This is to certify that

***Miss Ximena Alvarez***

Biotechnologia Vegetal S.A.  
CHILE

Attended the

**1<sup>st</sup> International Orchid Conservation Congress**

Held from the 24<sup>th</sup> – 28<sup>th</sup> September 2001

At the Hyatt Regency Perth, Western Australia

Date: *28<sup>th</sup> September*

Signed: *Ximena Alvarez*



The First International  
**Orchid Conservation Congress**

**Perth, Western Australia, 24-28 September 2001**  
C/- Kings Park & Botanic Garden, West Perth 6005, Western Australia  
<http://www.bgpa.wa.gov.au/OrchidCongress/OrchidCongress.html>

This is to certify that

***Mr Mauricio Cisternas Baez***

Universidad Catolica De Valparaiso  
CHILE

Attended the

**1<sup>st</sup> International Orchid Conservation Congress**

Held from the 24<sup>th</sup> – 28<sup>th</sup> September 2001

At the Hyatt Regency Perth, Western Australia

Date: *28<sup>th</sup> September 01*

Signed: *Elva Navarro*





The First International  
**Orchid Conservation Congress**

**Perth, Western Australia, 24-28 September 2001**  
C/- Kings Park & Botanic Garden, West Perth 6005, Western Australia  
<http://www.bgpa.wa.gov.au/OrchidCongress/OrchidCongress.html>

This is to certify that

***Dr Ximena Calderon Baltierra***

Unidad De Talca  
CHILE

Attended the

**1<sup>st</sup> International Orchid Conservation Congress**

Held from the 24<sup>th</sup> – 28<sup>th</sup> September 2001

At the Hyatt Regency Perth, Western Australia

Date: 28<sup>th</sup> September 01

Signed: *[Signature]*

## General outcomes

- integrated conservation. Celebration of the concept of
- orchid as flagship species. The "funny waddy" factor
- lateral conservation
- Re-invigoration of our combined passion and enthusiasm - the ET factor (we are not alone!)
- An orchid renaissance - the large number of young delegates. Succession planning

## Specific outcomes

### Measure diversity

- Importance of good taxonomy, appropriate sampling
- Vouchering
- In some cases genetic markers fail to discriminate accepted taxa (pollinators)

## Threatening and Ecological Processes

- Threatening processes - fire
- Trade: > 97% live plants are artificially propagated yet 0.5 million wild pl traded annually.
- Mycorrhizal associations and invertebrates
- Pollinator specificity
- Population dynamics and long term studies

## Ex situ Conservation

- Genetically representative sample
- Good science - dormancy, viability, quality
- Mycorrhizal specificity - epiphytes?
- Cryoplasma conservation → UK, Kew

## Recovery operations

UK  
Cypripedium calceolus to Caladenia hastata