

1. Yumiko Bonnardeaux, Mark Brundrett, Andrew Batty, Kingsley Dixon, John Koch, K. Sivasithamparam,
Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions,
Mycological Research, Volume 111, Issue 1, January 2007, Pages 51-61, ISSN 0953-7562, 10.1016/j.mycres.2006.11.006.
(<http://www.sciencedirect.com/science/article/pii/S0953756206003170>)

Abstract: Summary

The diversity of mycorrhizal fungi associated with an introduced weed-like South African orchid (*Disa bracteata*) and a disturbance-intolerant, widespread, native West Australian orchid (*Pyrorchis nigricans*) were compared by molecular identification of the fungi isolated from single pelotons. Molecular identification revealed both orchids were associated with fungi from diverse groups in the *Rhizoctonia* complex with worldwide distribution. Symbiotic germination assays confirmed the majority of fungi isolated from pelotons were mycorrhizal and a factorial experiment uncovered complex webs of compatibility between six terrestrial orchids and 12 fungi from Australia and South Africa. Two weed-like (disturbance-tolerant rapidly spreading) orchids – *D. bracteata* and the indigenous Australian *Microtis media*, had the broadest webs of mycorrhizal fungi. In contrast, other native orchids had relatively small webs of fungi (*Diuris magnifica* and *Thelymitra crinita*), or germinated exclusively with their own fungus (*Caladenia falcata* and *Pterostylis sanguinea*). Orchids, such as *D. bracteata* and *M. media*, which form relationships with diverse webs of fungi, had apparent specificity that decreased with time, as some fungi had brief encounters with orchids that supported protocorm formation but not subsequent seedling growth. The interactions between orchid mycorrhizal fungi and their hosts are discussed.

Keywords: *Disa bracteata*; Fungal specificity; Mycorrhizas; Orchids; *Pyrorchis nigricans*; *Rhizoctonia*; Seed germination; Western Australia

2. Hans Jacquemyn, Rein Brys, Martin Hermy, Jo H. Willems,
Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands,
Biological Conservation, Volume 121, Issue 2, January 2005, Pages 257-263,
ISSN 0006-3207, 10.1016/j.biocon.2004.05.002.
(<http://www.sciencedirect.com/science/article/pii/S0006320704001995>)

Abstract: Summary

The long-term persistence of plant populations may partly depend on pollination processes and seed production. The exact role of pollination in determining plant population viability still remains largely unclear. Orchid species have often been shown to be pollinator limited. Especially, non-rewarding species are characterized by infrequent pollinator visits and low seed set. Therefore, it can be hypothesized that, if overall population fitness of orchid species is influenced by seed production, non-rewarding species should be more prone to local extinction than rewarding species. To study the importance of nectar reward on orchid persistence, we collected historical records about the distribution of 32 orchid species in Flanders (Belgium) and 37 orchid species in the Netherlands. For both regions, present distribution patterns were compared with historical data. Between 1930 (Flanders) or 1950 (the Netherlands) and 2000, 26 (81%) species showed

decreases in distribution range in Flanders, eight of which had gone extinct, whereas 29 (78%) species showed declines in distribution area in the Netherlands, five of which went extinct. Contrary to previously reported results, orchid distribution patterns were not related to nectar reward. There was also no significant relationship between nectar reward and extinction for both regions. Orchids typically occurring in wet grasslands and heathlands suffered greater losses than orchids typically confined to forest habitats or calcareous grasslands. We conclude that the production of nectar does not represent a safeguard for local extinction of orchid species. Habitat loss and other deterministic threats associated with habitat fragmentation and deterioration are more important determinants of orchid persistence.

Keywords: *Extinction risk; Nectar reward; Orchid species; Population viability; Seed production*

3. Wen-Chieh Tsai, Yu-Yun Hsiao, Zhao-Jun Pan, Chang-Sheng Kuoh, Wen-Huei Chen, Hong-Hwa Chen,
The role of ethylene in orchid ovule development,
Plant Science, Volume 175, Issues 1-2, July-August 2008, Pages 98-105,
ISSN 0168-9452, 10.1016/j.plantsci.2008.02.011.

(<http://www.sciencedirect.com/science/article/pii/S0168945208000484>)

Abstract: Summary

Orchids are known for their unique reproductive biology and ecological strategies. One of the remarkable reproductive characters of most orchid species is that ovule development is precisely initiated following pollination. Thus, orchids are attractive systems for investigating both the initiation and subsequent development of the ovule. Ethylene plays a critical role in ovary maturation and ovule differentiation in orchids. Genes corresponding to ethylene biosynthesis in orchid reproductive organs have been identified and characterized. The phosphorylation states of proteins involved in ethylene biosynthesis or ethylene signaling may be correlated with ovule development. Recently, an orchid GLOBOSA-like MADS-box gene was isolated from *Phalaenopsis* and shown to have a role in ovule development via auxin/ethylene signaling. Adopting functional genomics strategies will help us to get insights into the regulation network between ethylene signaling pathway and ovule developmental program in orchids.

Keywords: *Ethylene; Orchid; Ovule development; Phosphorylation; Pollination*

4. Samira Chugh, Satyakam Guha, I. Usha Rao,
Micropropagation of orchids: A review on the potential of different explants,
Scientia Horticulturae, Volume 122, Issue 4, 3 November 2009, Pages 507-520,
ISSN 0304-4238, 10.1016/j.scienta.2009.07.016.

(<http://www.sciencedirect.com/science/article/pii/S0304423809003641>)

Abstract: Summary

Orchids are among the most diverse of the flowering plant families, with over 800 described genera and 25,000 species. Orchids are prized for their beautiful long lasting flowers exhibiting an incredible range of diversity in size, shape and colour. Today growing orchids is more than just a hobby, it is an international business covering around 8% of the world floriculture trade and has the potential to alter the economic landscape of a country. Large-scale multiplication of exquisite and rare hybrids using tissue culture techniques has helped orchids occupy a position as one of the top ten cut flowers. As orchids are outbreeders, their propagation using seeds leads to the production of heterozygous plants. Hence, protocols providing regeneration from various vegetative parts of the plants are

needed. Though orchid micropropagation has shown spectacular development in the recent years, the wide spread use of micropropagation is believed to be still limited due to problems like exudation of phenolics from explants, transplantation to field, somaclonal variation etc. We endeavour to include the major investigations on explant-based orchid tissue culture starting from the pioneering works of Rotor [Rotor, G., 1949. A method of vegetative propagation of Phalaenopsis species and hybrids. Am. Orchid Soc. Bull. 18, 738-739] followed by Morel [Morel, G., 1960. Producing virus-free cymbidiums. Am. Orchid Soc. Bull. 29, 495-497] and Wimber [Wimber, D.E., 1963. Clonal multiplication of cymbidiums through tissue culture of the shoot meristem. Am. Orchid Soc. Bull. 32, 105-107] to date.

Keywords: *Explants; Mass propagation; Orchids; Protocorm-like bodies*

5. Dennis F. Whigham, John P. O'Neill, Hanne N. Rasmussen, Bruce A. Caldwell, Melissa K. McCormick,
Seed longevity in terrestrial orchids - Potential for persistent in situ seed banks,
Biological Conservation, Volume 129, Issue 1, April 2006, Pages 24-30,
ISSN 0006-3207, 10.1016/j.biocon.2005.10.029.

(<http://www.sciencedirect.com/science/article/pii/S0006320705004477>)

Abstract: Summary

Terrestrial orchids typically produce numerous small seeds that contain very small nutrient reserves. The seeds are structurally adapted for wind dispersal but little is known about their fate after dispersal. Some studies of seed viability in situ indicate survival for up to two years in temperate orchid species. Seeds stored in the laboratory may last much longer. We investigated seed viability of seven North American orchid species with seed packets buried in a range of soil and wood substrates within their natural habitats. In *Goodyera pubescens* most seeds germinated within one year. Four other species continued to germinate sparsely during the observation period, but after almost seven years many seeds were still viable. In one species, *Liparis liliifolia*, seeds that had been in situ for four years had germination rates as high as 68% when sown in vitro with a compatible fungus. The remaining two species did not germinate during the observation period but the seeds were judged to be intact and tested positively for viability after four years in the ground. These observations are interpreted as different species-specific strategies for in situ germination and their seed bank potential is discussed.

Keywords: *Soil seed bank; Terrestrial orchids; Maryland; Seed longevity; Seed viability*

6. J.F.X. Challe, P.C. Struik,
The impact on orchid species abundance of gathering their edible tubers by HIV/AIDS orphans: a case of three villages in the Southern Highlands of Tanzania,
NJAS - Wageningen Journal of Life Sciences, Volume 56, Issue 3,
December 2008, Pages 261-279,
ISSN 1573-5214, 10.1016/S1573-5214(08)80011-5.

(<http://www.sciencedirect.com/science/article/pii/S1573521408800115>)

Abstract: Summary

This paper examines the gathering of wild orchids and its effect on orchid species diversity and abundance in rural communities with high prevalence of HIV/AIDS and high numbers of orphans. The study was conducted in three villages in the Makete District of Tanzania. The study used a triangulation of ethno-botanical, anthropological and sociological methodologies. On the three gathering sites, we found a total of 12 different orchid species (7 edible and 5 non-edible ones) confirmed by an expert

botanist, although local gatherers identified many more species. The Shannon-Wiener diversity index significantly differed among the three gathering sites. Analyses of focus group discussions and household surveys revealed that HIV/AIDS orphans and non-orphans children were the main gatherers of wild orchids. HIV/AIDS orphans ($n = 55$) gathered significantly more frequently (4.1 ± 1.8 times per week) than non-orphan children ($n = 49$; 1.9 ± 1.3 times per week) ($P < 0.01$). There was a statistically significant interaction between village and type of gatherer ($P < 0.05$). Scattered observations of changes in orchid species abundance over time were done using gatherers' indigenous knowledge and opinions. Orchid abundance peaked during May 2006. The study showed increasing abundance of non-edible orchid species and decreasing abundance of edible ones. There were highly significant, linear negative relationships between gathering pressure on the one hand and total number of orchid plants, total number of orchid species, number of edible orchid plants and number of edible orchid species on the other. These relationships were not statistically significant for non-edible orchids. We surmise that gathering edible orchid tubers is likely to become unsustainable, because once its tuber is harvested the plant does not recover or survive.

Keywords: chikanda; edible orchids; gathering pressure; Orchidaceae; species diversity; survival strategies

7. Hans Jacquemyn, Katrien Vandepitte, Rein Brys, Olivier Honnay, Isabel Roldán-Ruiz,
Fitness variation and genetic diversity in small, remnant populations of the food deceptive orchid *Orchis purpurea*,
Biological Conservation, Volume 139,
Issues 1-2, September 2007, Pages 203-210, ISSN 0006-3207,
10.1016/j.biocon.2007.06.015.

(<http://www.sciencedirect.com/science/article/pii/S0006320707002571>)

Abstract:

In much of Western Europe, orchid species have suffered dramatic declines in abundance, whereas the remaining populations often tend to be small and isolated, occur in ecologically marginal habitats and may show decreased reproductive output due to altered pollinator interactions or the absence of specialized pollinators. Furthermore, small and isolated populations are expected to suffer from genetic erosion and increasing genetic divergence among populations, through the effects of random genetic drift, increased levels of inbreeding and reduced gene flow, potentially leading to reduced possibilities of population recovery in the future. In this study, genetic diversity and fitness variation were studied in nine fragmented populations of the food deceptive orchid *Orchis purpurea*. Within-population genetic diversity and among-population differentiation were investigated using dominant AFLP markers. All studied populations were relatively small (range 12-302; mean: 90 individuals/population). Despite their small size, genetic diversity within populations was rather high (mean gene diversity H_j : 0.21, range: 0.15-0.27) and genetic differentiation among populations was not higher than that typically observed in deceptive orchids ($F_{ST} = 0.09$). Nevertheless, Mantel tests showed a positive correlation between geographical and genetic distances indicating limited gene flow among populations. Fruit set, on the other hand, was very low (average fruit set: 5.5%), suggesting strong pollinator limitation, which, in turn, resulted in very low recruitment rates (average seedling recruitment per flowering individual: 0.12). Both measures significantly increased with increasing population size, indicating that a certain threshold value (>50 flowering individuals) has to be reached to produce a sizeable number of fruits and seedlings. These results suggest that although deceptive

pollination clearly results in a fitness cost, it may serve as an effective means to maintain high gene diversity within and to counter high genetic differentiation among small orchid populations. From a genetic point of view, our results further suggest that small populations of orchid species should not be neglected as they may harbour as much genetic diversity as large populations.

Keywords: *Deceptive pollination; Fragmentation; Gene flow; Orchid conservation; Orchidaceae*

8. Yan Huang, Fang Li, Kunsong Chen,
Analysis of diversity and relationships among Chinese orchid cultivars using EST-SSR markers, *Biochemical Systematics and Ecology*, Volume 38, Issue 1, February 2010, Pages 93-102, ISSN 0305-1978, 10.1016/j.bse.2009.12.018.

(<http://www.sciencedirect.com/science/article/pii/S0305197809001884>)

Abstract: Summary

Chinese orchid (*Cymbidium* spp.) is an important potted flower with extremely high ornamental value in China. It is important to identify the genetic diversity of its germ plasma resources for development and evaluation of its cultivars. A set of 13 expressed sequence tag (EST)-derived simple sequence repeat (SSR) was used to analyze 103 cultivars of six species of Chinese orchid, namely *Cymbidium goeringii*, *C. faberi*, *Cymbidium ensifolium*, *C. kanran*, *C. sinense* and *C. goeringii* var. *longibracteatum*. The 13 SSR primer pairs generated a total of 168 polymorphic bands, with an average of 12.92 bands per primer and a range of 6-24 bands which clearly revealed the difference between cultivars inter- or intra-species of Chinese orchid. Cluster analysis based on UPGMA, NJ and PCoA method showed a dendrogram with three basic clusters and splitting feature of *C. ensifolium* and *C. goeringii* which partially congruent with the current taxonomic classification.

Keywords: *Chinese orchid; EST-SSR; Genetic diversity; Genetic relationship*

9. Ursula Steinfort, Gabriela Verdugo, Ximena Besoain, Mauricio A. Cisternas,
Mycorrhizal association and symbiotic germination of the terrestrial orchid *Bipinnula fimbriata* (Poepp.) Johnst (Orchidaceae), *Flora - Morphology, Distribution, Functional Ecology of Plants*, Volume 205, Issue 12, December 2010, Pages 811-817, ISSN 0367-2530, 10.1016/j.flora.2010.01.005.

(<http://www.sciencedirect.com/science/article/pii/S036725301000054X>)

Abstract: Summary

In this study mycorrhizal fungi were isolated from the roots of the endemic terrestrial orchid *Bipinnula fimbriata*. Seven isolates were previously identified as the form-genus *Rhizoctonia*, a polyphyletic group known to form mycorrhizal associations with Orchidaceae. Two other isolates were included in the study: #793 isolated from *Chloraea crispa*, and #1325 *Rhizoctonia solani*, isolated from potato. After morphological and molecular characterization of the nine isolates, they were divided into three groups, *Ceratobasidium* sp., *Tulasnella calospora* and *Thanatephorus cucumeris*, to determine the diversity between isolates. Consensus ITS sequences were used for a blast search on the GenBank database, which confirmed the results of the morphological observations. Once the isolates were identified, an *in vitro* germination test was done with four plates of oatmeal agar inoculated with each fungus, plus an asymbiotic control. The germination stages of the seeds were recorded 30 days after sowing. All isolates obtained from *B. fimbriata*, and the isolate #793 from *Chloraea crispa*, promoted seed germination. However, the isolate #1325 *Rhizoctonia solani*, which is known as

both a pathogen and an orchid symbiont, did not promote germination. This shows that *B. fimbriata* is associated with more than one mycorrhizal fungus in its habitat and has a broader potential specificity in vitro. The results support the hypothesis that at least one fungal isolate promotes the germination of *B. fimbriata*, permitting the conservation of this species in ex situ conditions.

Keywords: *Endemic orchid; ITS1; ITS4; Rhizoctonia; CeratobasidiumTulasnella calospora*

10. Christiane Stark, Wiesław Babik, Walter Durka,
Fungi from the roots of the common terrestrial orchid *Gymnadenia conopsea*,
Mycological Research, Volume 113, Issue 9, September 2009, Pages 952-959,
ISSN 0953-7562, 10.1016/j.mycres.2009.05.002.

(<http://www.sciencedirect.com/science/article/pii/S0953756209000951>)

Abstract: Summary

The fungal community associated with the terrestrial photosynthetic orchid *Gymnadenia conopsea* was characterized through PCR-amplification directly from root extracted DNA and cloning of the PCR products. Six populations in two geographically distinct regions in Germany were investigated. New ITS-primers amplifying a wide taxonomic range including Basidiomycetes and Ascomycetes revealed a high taxonomic and ecological diversity of fungal associates, including typical orchid mycorrhizas of the Tulasnellaceae and Ceratobasidiaceae as well as several ectomycorrhizal taxa of the Pezizales. The wide spectrum of potential mycorrhizal partners may contribute to this orchid's ability to colonize different habitat types with their characteristic microbial communities. The fungal community of *G. conopsea* showed a clear spatial structure. With 43 % shared taxa the species composition of the two regions showed only little overlap. Regardless of regions, populations were highly variable concerning taxon richness, varying between 5 and 14 taxa per population. The spatial structure and the continuous presence of mycorrhizal taxa on the one hand and the low specificity towards certain fungal taxa on the other hand suggest that the fungal community associated with *G. conopsea* is determined by multiple factors. In this context, germination as well as pronounced morphological and genetic differentiation within *G. conopsea* deserve attention as potential factors affecting the composition of the fungal community.

Keywords: *FungiGymnadenia conopsea; ITS primers; Mycorrhiza; Orchids*

11. Nungruthai Kananont, Rath Pichyangkura, Sermsiri Chanprame, Supachitra Chadchawan, Patchra Limpanavech,
Chitosan specificity for the in vitro seed germination of two *Dendrobium* orchids (Asparagales: Orchidaceae),
Scientia Horticulturae, Volume 124, Issue 2, 15 March 2010, Pages 239-247,
ISSN 0304-4238, 10.1016/j.scienta.2009.11.019.

(<http://www.sciencedirect.com/science/article/pii/S0304423809005032>)

Abstract: Summary

The effects of different types of chitosan on seed germination and protocorm development were determined for two orchid species, *Dendrobium bigibbum* var. *compactum* and *Dendrobium formosum*. Six chitosan types derived from polymer or oligomer chitosan each with 70, 80 or 90% levels of deacetylation (P70, P80, P90, O70, O80 and O90, respectively), were evaluated as direct medium supplements at 0, 10, 20, 40 or 80 mg/L in modified VW medium by following seed germination and protocorm growth for 12 weeks. Chitosan of all six tested types and four concentrations were found to

significantly enhance the proportion of *D. formosum* seeds that germinated, when compared to these germinated without chitosan. In contrast, chitosan caused no enhanced germination rate was noted for *D. bigibbum* var. *compactum* with all tested chitosans and doses tested. However, almost all types of chitosan at 10 mg/L, except O90, were able to significantly improve the growth of *D. bigibbum* var. *compactum* protocorms, whilst 10 or 20 mg/L of P70 chitosan was the best formula to enhance the growth of *D. formosum* protocorms. It is concluded that chitosan responses in seed germination and protocorm development were somewhat species and developmental stage dependent. Therefore, the appropriate chitosan application for each plant species should be evaluated first before use.

Keywords: *Dendrobium*; Orchid; *In vitro* seed germination; Chitosan

12. Tiiu Kull, Michael J. Hutchings,

A comparative analysis of decline in the distribution ranges of orchid species in Estonia and the United Kingdom,

Biological Conservation, Volume 129, Issue 1, April 2006, Pages 31-39, ISSN 0006-3207, 10.1016/j.biocon.2005.09.046.

(<http://www.sciencedirect.com/science/article/pii/S0006320705004155>)

Abstract: Summary

National databases were interrogated to analyse and compare proportional alterations in the distribution ranges of orchid species between two surveys in the UK (surveys completed in 1969 and 1999) and in Estonia (surveys completed in 1970 and 2004). Nearly every species declined between the surveys in both countries, and two species may have become extinct in the UK. Mean decline in distribution range for 49 species in the UK was 50% (range 14-100%), and 23 species declined by over 50%. The mean decline for 33 orchid species in Estonia was 25% (range 0-62%), and three species declined by over 50%. These results corroborate serious range declines recently reported for orchids in other regions of Europe (the Netherlands and Flanders, Belgium). In contrast with these other regions, we found that species associated with calcareous grassland and woodland habitats had suffered greater mean contractions in range than species of wet grassland habitats. Greater decline was recorded for species found on drier soils, and for species characteristic of open habitats. In addition, greater decline was found in species with short inflorescences, and in species that were short-lived, and clonal. Our results suggest that levels of decline shown both by groups of species associated with specific habitat types, and by particular species of orchid, depend strongly on local policies and specific conservation action, and indicate the habitat types on which conservation efforts may need to be concentrated in the future. The results suggest that grazing and mowing of competing vegetation, and avoidance of substrate disturbance, will produce the greatest rewards for the most vulnerable species.

Keywords: Distribution ranges; Extinction risk; Orchid decline; Vulnerable species; Dynamic chorology

13. Fiona Coates, Ian D. Lunt, Raymond L. Tremblay,

Effects of disturbance on population dynamics of the threatened orchid *Prasophyllum correctum* D.L. Jones and implications for grassland management in south-eastern Australia,

Biological Conservation, Volume 129, Issue 1, April 2006, Pages 59-69, ISSN 0006-3207, 10.1016/j.biocon.2005.06.037.

(<http://www.sciencedirect.com/science/article/pii/S0006320705004179>)

Abstract: Summary

Natural and anthropogenic disturbances can have dramatic consequences for population growth, particularly for small populations of threatened plants. We analysed census data for the largest population (124 individuals) of the critically endangered orchid *Prasophyllum correctum* between 1992 and 2003, to identify environmental factors associated with annual changes in emergence and flowering, and to develop management prescriptions for its conservation. Fire frequency effects were analysed by comparing life stage transition matrices between plants subject to <3 year and >3 year fire intervals; climate effects were investigated using cross-correlation plots to relate total emergence, and numbers of sterile and flowering plants to rainfall, and grazing impacts were investigated by experimentally protecting plants in 1996-1998. Plants rarely emerged for more than two consecutive years or flowered for more than a single year. The total number of plants that emerged was significantly negatively correlated with autumn/winter rainfall in the previous year, perhaps due to on-going competitive effects of increased grass growth under wetter conditions. The proportion of reproductive adults was greater when fire intervals were <3 years, and a greater proportion of the population remained dormant and non-reproductive when fire intervals exceeded 3 years. Grazing had a significant negative effect on the orchid population in the first 2 years after fire. A management regime that includes frequent burning is likely to benefit conservation of the population by reducing competition from grasses, shortening dormancy periods, reducing mortality, enhancing flowering and, by implication, possibly increasing recruitment.

Keywords: *Endangered orchid; Fire; Dormancy; Grassland; Disturbance*

14. Nina Sletvold, Dag-Inge Øien, Asbjørn Moen,
Long-term influence of mowing on population dynamics in the rare orchid *Dactylorhiza lapponica*: The importance of recruitment and seed production,
Biological Conservation, Volume 143, Issue 3, March 2010, Pages 747-755,
ISSN 0006-3207, 10.1016/j.biocon.2009.12.017.

(<http://www.sciencedirect.com/science/article/pii/S0006320709005254>)

Abstract: Summary

Many orchids are currently red-listed due to changes in land use, and their future persistence will depend on management. Traditional land use like mowing is believed to favour orchids through increased survival and reproduction of established individuals, but the lack of data connecting their complex life cycle presently limits our ability to evaluate effects of management. Here we used data from 16 years of demographic monitoring to study how mowing affects population dynamics in two populations of the rare orchid *Dactylorhiza lapponica*. Both populations were characterized by long-lived individuals, low adult mortality and high seedling mortality. The traditional regime of mowing every second year strongly increased recruitment and reduced seed production in both populations, but had moderate effect on adult survival, growth rate and flowering probability. Population growth rate was positive for all population × treatment combinations. Traditional mowing significantly increased growth rate in both populations, and LTRE-analyses revealed that this primarily was a result of increased recruitment. The results indicate that demographic rates commonly associated with orchid persistence may be insensitive to traditional management, and underscores the importance of seed production and recruitment to maintain population growth in this long-lived species. The combination of low establishment success and no seed bank makes *D. lapponica* dependent on high seed input. Our results suggest that *D. lapponica* would benefit most from

traditional mowing performed after seed dispersal in the study areas, but also suggest a high probability of future survival in the absence of mowing.

Keywords: *Demography; LTRE-analyses; Management; Matrix models; Orchid populations; Recruitment*

15. Panisa Pornpienpakdee, Ronnawich Singhasurasak, Pongsathorn Chaiyasap, Rath Pichyangkura, Ruengwit Bunjongrat, Supachitra Chadchawan, Patchra Limpanavech,
Improving the micropropagation efficiency of hybrid *Dendrobium* orchids with chitosan,
Scientia Horticulturae, Volume 124, Issue 4, 1 May 2010, Pages 490-499, ISSN 0304-4238, 10.1016/j.scienta.2010.02.008.

(<http://www.sciencedirect.com/science/article/pii/S0304423810000658>)

Abstract: Summary

The appropriate chitosan types and concentrations for enhancing the in vitro micropropagation of *Dendrobium* 'Eiskul' were studied using 70, 80 and 90% N-deacetylated polymeric (P-70, P-80 and P-90) and oligomeric (O-70, O-80 and O-90) forms of crab (*Portunus pelagicus*) chitosan. For the initial protocorm-like body (PLB) multiplication, the application of 10 mg/L of P-70 or 20 mg/L of P-90 was optimal, although 10 mg/L of P-80 and O-70 were also effective, and attained maximal PLB replication rates without increasing the detectable levels of somaclonal variation. However, during PLB-shoot induction, 10 or 20 mg/L of O-80 was the most appropriate chitosan and also induced further PLB formation. For plantlet regeneration, the addition of 10 mg/L of O-80 or P-80 gave the best quantity and quality, respectively, of plantlets. Finally, 20 mg/L of P-70 chitosan as a supplement during exflasking enhanced both the survival rate and the growth of the plantlets at one month after exflasking. Together, these data reveal a potentially beneficial and applicable protocol for commercial orchid micropropagation.

Keywords: *Orchid; Dendrobium; PLB; Protocorm-like body; Chitosan; Genetic variation*
In vitro propagation

16. Craig F. Barrett, John V. Freudenstein,
Molecular evolution of *rbcL* in the mycoheterotrophic coralroot orchids (*Corallorhiza* Gagnebin, Orchidaceae),
Molecular Phylogenetics and Evolution, Volume 47, Issue 2, May 2008, Pages 665-679,
ISSN 1055-7903, 10.1016/j.ympev.2008.02.014.

(<http://www.sciencedirect.com/science/article/pii/S1055790308000869>)

Abstract: Summary

The RuBisCO large subunit gene (*rbcL*) has been the focus of numerous plant phylogenetic studies and studies on molecular evolution in parasitic plants. However, there has been a lack of investigation of photosynthesis gene molecular evolution in fully mycoheterotrophic plants. These plants invade pre-existing mutualistic associations between ectomycorrhizal trees and fungi, from which they obtain fixed carbon and nutrients. The mycoheterotrophic orchid *Corallorhiza* contains both green (photosynthetic) and non-green (putatively nonphotosynthetic) species. We sequenced *rbcL* from 31 accessions of eight species of *Corallorhiza* and hypothesized that some lineages would have pseudogenes resulting from relaxation of purifying selection on RuBisCO's carboxylase function. Phylogenetic analysis of *rbcL* and ITS gave high jackknife support for relationships among species. We found evidence of pseudogene formation in all lineages of the *Corallorhiza striata* complex and in some lineages of the *C. maculata* complex. Evidence includes: stop codons, frameshifts, decreased dS/dN ratios, replacements not observed in photosynthetic species, rate heterogeneity, and

high likelihood of neutral evolution. The evolution of *rbcL* in Corallorhiza may serve as an exemplary system in which to study the effects of relaxed evolutionary constraints on photosynthesis genes for >400 documented fully mycoheterotrophic plant species.

Keywords: *Pseudogene; Neutral evolution; Purifying selection; Corallorhizinae; Orchid*

17. Claire Micheneau, Barbara S. Carlswald, Michael F. Fay, Benny Bytebier, Thierry Pailler, Mark W. Chase, Phylogenetics and biogeography of Mascarene angraecoid orchids (Vandaeae, Orchidaceae), Molecular Phylogenetics and Evolution, Volume 46, Issue 3, March 2008, Pages 908-922, ISSN 1055-7903, 10.1016/j.ympev.2007.12.001.

(<http://www.sciencedirect.com/science/article/pii/S1055790307004228>)

Abstract: Summary

The large angraecoid orchid clade (subtribe Angraecinae sensu lato) has undergone extensive radiation in the western Indian Ocean, which includes Africa, Madagascar, and a number of Indian Ocean islands, such as the Mascarene Archipelago. To investigate systematics and biogeography of these Mascarene orchids, phylogenetic relationships were inferred from four plastid DNA regions, *trnL* intron, *trnL-F* intergenic spacer, *matK* gene, and *rps16* intron. Parsimony and Bayesian analyses provided identical sets of relationships within the subtribe; the large genus *Angraecum* as currently circumscribed does not form an exclusive clade. *Bonniera*, an endemic genus to Reunion, is shown to be embedded in part of *Angraecum*. Evidence from our research supports the main origin of Mascarene Angraecinae from Madagascar, and although there were many independent colonizations, only a few of the lineages radiated within the Mascarene Archipelago.

Keywords: *Angraecoid orchids; Biogeography; Mascarene Archipelago; Molecular phylogeny; Oceanic islands; Orchidaceae; Vandaeae*

18. Khin Lay Nge, Nitar Nwe, Suwalee Chandkrachang, Willem F. Stevens, Chitosan as a growth stimulator in orchid tissue culture, *Plant Science*, Volume 170, Issue 6, June 2006, Pages 1185-1190, ISSN 0168-9452, 10.1016/j.plantsci.2006.02.006.

(<http://www.sciencedirect.com/science/article/pii/S0168945206000537>)

Abstract: Summary

The effect of shrimp and fungal chitosan on the growth and development of orchid plant meristematic tissue in culture was investigated in liquid and on solid medium. The growth of meristem explants into protocorm-like bodies in liquid medium was accelerated up to 15 times in the presence of chitosan oligomer, the optimal concentration being 15 µg/ml. The 1 kDa shrimp oligomer was slightly more effective compared to 10 kDa shrimp chitosan and four times more active compared to high molecular weight 100 kDa shrimp chitosan. The 10 kDa fungal chitosan was more effective compared with 1 kDa oligomer. The development of orchid protocorm into differentiated orchid tissue with primary shoots and roots was studied on solid agar medium. The optimal effect, the generation of 5-7 plantlets in 12 weeks was observed in the presence of 20 µg/ml using either 10 kDa fungal or 1 kDa oligomer shrimp chitosan. The data are consistent with preliminary results from field experiments and confirm unequivocally that a minor amount of chitosan has a profound effect on the growth and development of orchid plant tissue.

Keywords: *Fungal chitosan; Meristematic bud; Orchids; Protocorm; Shrimp chitosan*

19. Hans Jacquemyn, Olivier Honnay, Thierry Paillet,
Range size variation, nestedness and species turnover of orchid species
along an altitudinal gradient on Réunion Island: Implications for
conservation,
Biological Conservation, Volume 136, Issue 3, May 2007, Pages 388-397,
ISSN 0006-3207, 10.1016/j.biocon.2006.12.008.
(<http://www.sciencedirect.com/science/article/pii/S0006320706005714>)

Abstract: Summary

Tropical oceanic islands contribute disproportionately for their area to global biodiversity and this is especially true for islands with strong altitudinal gradients. On these islands, species richness and composition usually change with altitude, but the way in which they do may vary from one system to the next. Better insights in how species richness and composition are related may have far-reaching conservation implications. At the one extreme, species composition of a species-poor site may be completely different from that of the most species-rich site. In this case, conservation of the species-poor site should be encouraged as it contains species that are not to be found elsewhere. At the other extreme, species composition of the species-poor sites may be a subset of the species-rich sites (i.e. nestedness or nested subsets). Focus on the most species-rich site may be sufficient to effectively conserve a maximum of species. In this study, we determined changes in species richness and composition of orchid species along an altitudinal gradient spanning more than 2500 m on Réunion Island (Mascarenes). A formal nestedness analysis was performed to investigate whether species-poor sites were a subset of species-rich sites and whether this was related to altitude. Differences in species composition along the altitudinal gradient were determined using two different measures of community similarity. The species-by-sites matrix was significantly nested when sorted by species richness, but not when sorted by altitude. Mean similarity indices were low at both low and high altitude sites, indicating significant turnover of orchid species. Our results suggest that reserve selection should be based on two basic principles: (1) maximize the number of vegetation zones in which reserves are to be placed and (2) within each zone, select the sites with the highest species richness. Given that at present only two nature reserves are present on the island, our results further suggest that they are not sufficient to effectively conserve the extremely high orchid diversity on Réunion Island.

Keywords: Altitude; Nestedness; Orchid conservation; Orchidaceae; Stevens' rule

20. Ingrid Kottke, Ingeborg Haug, Sabrina Setaro, Juan Pablo Suárez, Michael Weiß, Markus Preußing, Martin Nebel, Franz Oberwinkler, Guilds of mycorrhizal fungi and their relation to trees, ericads, orchids and liverworts in a neotropical mountain rain forest,
Basic and Applied Ecology, Volume 9, Issue 1, 2 January 2008, Pages 13-23,
ISSN 1439-1791, 10.1016/j.baae.2007.03.007.
(<http://www.sciencedirect.com/science/article/pii/S1439179107000291>)

Abstract: Summary

Mycorrhizas of vascular plants and mycorrhiza-like associations of liverworts and hornworts are integral parts of terrestrial ecosystems, but have rarely been studied in tropical mountain rain forests. The tropical mountain rain forest area of the Reserva Biológica San Francisco in South Ecuador situated on the eastern slope of the Cordillera El Consuelo is exceptionally rich in tree species, ericads and orchids, but also in liverworts. Previous light and electron microscopical studies revealed that tree roots are well colonized by structurally diverse Glomeromycota, and that

epiphytic, pleurothallid orchids form mycorrhizas with members of the Tulasnellales and the Sebaciniales (Basidiomycota). Sebaciniales also occurred in mycorrhizas of hemiepiphytic ericads and Tulasnellales were found in liverworts belonging to the Aneuraceae. On the basis of these findings, we hypothesized that symbiotic fungi with a broad host range created shared guilds or even fungal networks between different plant species and plant families. To test this hypothesis, molecular phylogenetic studies of the fungi associated with roots and thalli were carried out using sequences of the nuclear rDNA coding for the small subunit rRNA (nucSSU) of Glomeromycota and the large subunit rRNA (nucLSU) of Basidiomycota. Sequence analyses showed that Sebaciniales and Tulasnellales were only shared within but not between ericads and orchids or between liverworts and orchids, respectively. Regarding arbuscular-mycorrhiza-forming trees, however, 18 out of 33 *Glomus* sequence types were shared by two to four tree species belonging to distinct families. Nearly all investigated trees shared one sequence type with another tree individual. Host range and potential shared guilds appeared to be restricted to the plant family level for Basidiomycota, but were covering diverse plant families in case of Glomeromycota. Given that the sequence types as defined here correspond to fungal species, our findings indicate potential fungal networks between trees.

Keywords: *Sebaciniales; Tulasnellales; Andean clade of Ericaceae; Aneuraceae; pleurothallid orchids; nucSSU; nucLSU; fungal networks; Reserva Biológica San Francisco*