More than symbioses: orchid ecology, with examples from the Sydney Region

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Abstract: The Orchidaceae are one of the largest and most diverse families of flowering plants. Orchids grow as terrestrial, lithophytic, epiphytic or climbing herbs but most orchids native to the Sydney Region can be placed in one of two categories. The first consists of terrestrial, deciduous plants that live in fire-prone environments, die back seasonally to dormant underground root tubers, possess exclusively subterranean roots, which die off as the plants become dormant, and belong to the subfamily Orchidoideae. The second consists of epiphytic or lithophytic, evergreen plants that live in fire-free environments, either lack specialised storage structures or possess succulent stems or leaves that are unprotected from fire, possess aerial roots that grow over the surface of, or free of, the substrate, and which do not die off seasonally, and belong to the subfamily Epidendroideae.

Orchid seeds are numerous and tiny, lacking cotyledons and endosperm and containing minimal nutrient reserves. Although the seeds of some species can commence germination on their own, all rely on infection by mycorrhizal fungi, which may be species-specific, to grow beyond the earliest stages of development. Many epidendroid orchids are viable from an early stage without their mycorrhizal fungi but most orchidoid orchids rely, at least to some extent, on their mycorrhizal fungi throughout their lives. Some are completely parasitic on their fungi and have lost the ability to photosynthesize. Some orchids parasitize highly pathogenic mycorrhizal fungi and are thus indirectly parasitic on other plants.

Most orchids have specialised relationships with pollinating animals, with many species each pollinated by only one species of insect. Deceptive pollination systems, in which the plants provide no tangible reward to their pollinators, are common in the Orchidaceae. The most common form of deceit is food mimicry, while at least a few taxa mimic insect brood sites. At least six lineages of Australian orchids have independently evolved sexual deception. In this syndrome, a flower mimics the female of the pollinating insect species. Male insects are attracted to the flower and attempt to mate with it, and pollinate it in the process.

Little is known of most aspects of the population ecology of orchids native to the Sydney Region, especially their responses to fire. Such knowledge would be very useful in informing decisions in wildlife management.

Introduction

An important element of ecology is the biology of symbioses. All plants rely upon other organisms for their role in nutrient cycling and other processes that maintain environmental stability. Most plants also depend on animals for pollination and/or dispersal, and have less visible relationships with soil micro-organisms for nutrient uptake. If one wanted to furnish concrete, fascinating examples of the importance of such interactions, the Orchidaceae would provide a richer source of illustrations than any other family: all orchids are dependent upon symbioses with specific organisms for their survival and reproduction. This essay explores our knowledge of orchid life cycles, to complement the accompanying treatment of Orchidaceae in the *Ecology of Sydney plant species* (Benson & McDougall 2005). Consequently, examples are given, where available, from orchids of the Sydney Region (based on the Central Coast plus Central Tablelands botanical subdivisions), emphasising how partnerships with other organisms are crucial in the continuation of those life cycles.

This essay also highlights the substantial gaps in our knowledge of orchid ecology. While we have relatively detailed knowledge of some aspects of this subject, notably the pollination biology and mycorrhizal associates of terrestrial orchids, little scientific research has been published on other aspects of orchid ecology such as demography and its interaction with disturbance regimes such as fire. The imperative to fill these gaps is rendered starkly apparent if one reads any of the numerous recovery plans that have been prepared for threatened species of Australian orchids. They read as a litany of our ignorance of aspects of ecology that bear importantly on the management of orchid habitats. We have listed what we regard as crucial gaps in ecological knowledge in text boxes throughout the essay.

Firstly, we outline some ecological generalisations about the orchids as a whole and about particular subgroups.

Diversity and distribution

Orchids are one of the largest, most diverse, and most cosmopolitan families of flowering plants, including somewhere between 20 000 and 35 000 species, which together occupy almost every kind of habitat on land. Although all are herbaceous plants, some grow to the size of a small truck, while others are moss-like plants that would happily grow in a thimble. The flowers of some are huge and flamboyant while others are no larger than a pinhead. Many orchids are 'terrestrials', living in soil like 'normal' plants but most species are epiphytes — plants that perch on, or hang suspended from, the trunks, branches and twigs of trees, mostly in tropical rainforests (see Dressler 1981, 1993). Many epiphytes also grow on rocks and some ('lithophytes' or 'epiliths') grow exclusively on them. More than 800 orchid species are native to Australia, where they grow in lowland rainforests to alpine meadows, from coastal sand dunes to the semi-arid regions of the continent (see e.g. Jones 1988). The great majority of Australian species are confined to non-arid regions of the continent. In contrast to the world-wide dominance of epiphytes, the orchid flora of the Sydney Region (and of Australia more generally) is numerically dominated by terrestrial species. Of the 38 genera and 220 species of Orchidaceae reported from the Sydney region, nine genera and 28 species are epiphytic, lithophytic or climbers, but the great majority — 76% of genera and 87% of species — are 'terrestrial'.

The categorisation of orchids as either terrestrial or epiphytic/lithophytic plants is an over-simplification but it does have substantial predictive power, both functionally and taxonomically. The great majority of terrestrial orchids native to the Sydney Region:

- are deciduous plants,
- live in fire-prone environments,
- die back seasonally to dormant underground root tubers (usually during the summer),
- undergo most vegetative growth during the autumn or winter,
- possess exclusively subterranean roots, which die off as the plants become dormant,
- belong to the subfamily Orchidoideae.

Most of the epiphytes and lithophytes native to the Sydney Region:

- are evergreen plants,
- live in relatively fire-free environments,
- either lack specialised storage structures or possess succulent stems or leaves that are unprotected from fire,
- undergo most vegetative growth during the summer,
- possess aerial roots that grow over the surface of, or free of, the substrate, and which do not die off seasonally,
- belong to the subfamily Epidendroideae.

The few exceptions to these categories include:

- terrestrial, leafless, mycoheterotrophic Epidendroideae of fire-prone environments, the only above-ground parts of which are inflorescences, which re-sprout seasonally from fleshy underground root systems or tubers (*Dipodium*, *Gastrodia*);
- terrestrial, leafless, mycoheterotrophic Orchidoideae of fire-prone environments, the only above-ground parts of which are inflorescences which resprout seasonally from fleshy underground root systems or tubers (*Prasophyllum flavum*, *Genoplesium baueri*, *Rhizanthella slateri*, *Arthrochilus huntianus*, *Cryptostylis hunteriana*);
- terrestrial, leafless, mycoheterotrophic, apparently monocarpic Orchidoideae of fire-prone environments, the only above-ground parts of which are inflorescences (*Burnettia cuneata*: D.L. Jones pers. comm.)
- leafless, mycoheterotrophic lianas, of fire-prone environments, which die back to fleshy underground root systems, and which belong to the subfamily Vanilloideae (*Erythrorchis cassythoides*);
- terrestrial, evergreen Epidendroideae of fire-free environments (*Calanthe triplicata*);
- epiphytic Epidendroideae of fire-prone environments that resprout from stems protected inside the trunk of their host tree (*Cymbidium suave*);
- lithophytic Epidendroideae that are capable of surviving fires of low intensity by resprouting from stems protected either by plant bulk or in rock crevices (*Liparis reflexa*, *Thelychiton speciosus* (*Dendrobium speciosum*));
- terrestrial, evergreen Orchidoideae which live in fire-prone environments and which resprout after fire from fleshy underground root systems (most species of *Cryptostylis*);
- terrestrial, evergreen Orchidoideae which live in fire-free environments and which lack specialised storage organs (*Adenochilus nortonii*);
- lithophytic, evergreen Orchidoideae that live in fire-prone environments, and which resprout after fire from fleshy root systems protected in rock crevices (*Rimacola elliptica*).

Environmental characteristics that distinguish the Sydney Region from surrounding orchid habitats

Sydney is located in a transitional climatic zone, between dominant winter rainfall in the south and summer rainfall to the north. This position, and Sydney's moderately high relative humidity with low seasonal variation, provide habitats that are conducive to the growth of both orchidoid (summer dormant, terrestrial) and epidendroid (summer growing, mostly epiphytic) species. Diversity of orchidoid species in the Sydney Region is higher than in summer rainfall areas to the north, while diversity of epidendroid species is higher than in areas to the south. Of 263 orchidoid

Table 1. Taxa occurring in Sydney Region grouped by broad habitat category (PlantNET 2003).

Habit Grouping	Subfamily	Tribe	Subtribe	Genus	No. of species
Climber	Vanilloideae	Vanilleae		*Erythrorchis*	1
Epiphytic or lithophytic	Epidendroideae	Dendrobieae		*Dockrillia*	6
				Thelychiton	3
				Tropilis	1
				Tetrabaculum	2
				Bulbophyllum	4
		Cymbidieae		*Cymbidium*	1
		Malaxideae		*Liparis*	2
		Vandeae	Aeridinae	*Papillilabium*	1
				Plectorrhiza	1
				Sarcochilus	4
Terrestrial	Orchidoideae	Diurideae	Diuridinae	*Diuris*	17
				Orthoceras	1
			Cryptostylidinae	*Cryptostylis*	4
			Drakaeinae	*Arthrochilus*	2
				Caleana	2
				Chiloglottis	10
				Lyperanthus	1
				Pyrorchis	1
				Rimacola	1
				Burnettia	1
			Thelymitrinae	*Thelymitra*	15
				Calochilus	4
			Acianthinae	*Acianthus*	7
				Corybas	8
				Cyrtostylis	1
			Prasophyllinae	*Genoplesium*	18
				Microtis	4
				Prasophyllum	13
			Rhizanthellinae	*Rhizanthella*	1
			Caladeniinae	*Adenochilus*	1
				Caladenia	26 (2 extinct)
				Cyanicula	1
				Eriochilus	2
				Glossodia	2
		Cranichideae	Pterostylidinae	*Pterostylis*	41
			Spiranthinae	*Spiranthes*	1
	Epidendroideae	Arethuseae *p.p.*		*Calanthe*	1
		Cymbidieae		*Dipodium*	4
		Gastrodieae		*Gastrodia*	2
		Epidendreae	Laeliinae	**Epidendrum*	1 (naturalised)
Totals				**38**	**220**

species listed for New South Wales in Harden (1993), 173 occur in the Sydney Region, while only 161 are recorded for the North Coast plus Northern Tablelands botanical subdivisions and 170 are recorded for the South Coast plus Southern Tablelands. Conversely, of 80 epidendroid species listed for New South Wales in Harden (1993), 79 are recorded for the North Coast plus Northern Tablelands botanical subdivisions while only 32 are recorded from the Sydney Region, and 22 from the South Coast plus Southern Tablelands (Table 1).

The Sydney Region is also located just south of the Hunter River Valley, a significant barrier to range expansion for epiphytic and lithophytic species. Eight epiphytic species have their southern limits of distribution in the coastal ranges or tablelands just north of the Hunter and one has its northern limit just south. Much of the Hunter Valley is a wide alluvial plain, dominated by eucalypt forests and woodlands, and devoid of rainforests and rocky outcrops. It thus provides no suitable habitats for most epiphytic and lithophytic species. Interestingly, of 25 epiphytic or lithophytic species native to the Sydney region, only one is not recorded further north. This suggests that the Hunter Valley has primarily been a barrier to southward, not northward expansion of epiphytes and lithophytes.

Geologically, the Sydney Region is dominated by the Sydney Basin, a sedimentary basin that includes overlying strata of sandstones and shales, dissected by erosion to produce a mosaic of contrasting soils (Benson & Howell 1990). As seems to have been the case for other plant taxa, this topographic and edaphic complexity has probably facilitated speciation in terrestrial orchid taxa within the region, particularly in those species adapted to growing on soils derived from sandstone. Of 13 species endemic to the Sydney region, 11 are restricted to sandstone (which occupies about half the total area).

The orchid life cycle: demography and natural history

Seeds

Orchid seeds are very small and dust-like — usually ranging from 0.05–6.0 mm long and 0.01–0.9 mm wide and weighing from 0.31–24 µg (Arditti & Ghani 1999). They are, however, produced in large quantities — usually in the thousands, but as few as 50 per fruit in the large-seeded Western Australian species *Rhizanthella gardneri* (Dixon et al. 1990) and as many as 4 000 000 seeds per capsule in *Cycnoches chlorochilon*, a neotropical epiphyte (Arditti 1992). Endosperm is occasionally scanty but is usually lacking altogether and the seed has no cotyledon. However, cells of the tiny embryos are packed with lipid and protein bodies (Rasmussen 1995), so they have some nutrient reserves.

Orchid seeds are highly buoyant, with the embryo occupying only a minor proportion of the volume inside the testa of most species that have been examined, the rest of the seed being filled with air. The seeds of most orchids can thus be modelled as miniature balloons and, indeed the seeds of some species resemble tiny balloons in form (Arditti & Ghani 1999). Not surprisingly, orchid seeds are generally thought to be wind-dispersed and there seems little doubt that this is the predominant mechanism of dispersal in most species. Very few species have been subject to empirical investigation of the distribution of dispersal distances under realistic conditions but the studies that have been conducted (Carey 1998, Murren & Ellison 1998) have tested the adequacy of general mathematical models in simulating observed patterns. Given the general similarity in gross physical characteristics of seeds of different orchids species, corroborated general models can be extrapolated to estimate the likely distributions of dispersal distances in species for which the physical attributes of seeds are known. In reviewing these studies and the much more extensive array of published data on orchid seed morphology, Arditti and Ghani (1999: 406) concluded that 'Given the many seeds produced by orchids, it is reasonable to assume that even if a small proportion will constitute the large variance and long tail [of the distribution of dispersal distances], a sufficient number will travel far enough to colonize new areas…'

The testas of orchid seeds that have been examined in detail are covered in a water-repellent lipoid layer that maintains the buoyancy of the largely air-filled seed in water. Moreover, the testa is also often sculptured, causing it to trap air bubbles. These characteristics enable orchid seeds to float on the surface of water for long periods of time (references cited in Arditti & Ghani 1999). Kurzweil (1994) observed that the seeds of several species of *Disa* that inhabit moist stream banks are dispersed by water. Rasmussen (1995) speculates that other species of bog orchids might also be primarily dispersed by water. Whether rain drops or intermittently running water are significant factors in seed dispersal in most orchid species is unknown. The effect of seawater on the viability of orchid seeds seems not to have been tested. In any case, seawater seems an unlikely dispersal vector for orchid seeds because no species grow in the intertidal zone and the probability of beached seeds dispersing further to suitable habitats would seem to be low.

Arditti and Ghani (1999) speculate that orchid seeds might also be transported on the feet and plumage of birds. They point out that this mode of dispersal could potentially involve the simultaneous transport of both orchid seeds and the spores of appropriate mycorrhizal fungi. While this is plausible, no empirical studies have tested whether it actually happens, or its relative importance. Dispersal in the guts of birds and mammals seems likely in the few species (including *Rhizanthella slateri*, a Sydney native) in which the fruit is succulent and indehiscent (a berry) and the seeds have a crustose, presumably resilient testa.

Dormancy, imbition

Growth in plant seeds begins with the process of germination. In orchids, germination of seeds in the field is poorly under-stood because of the microscopic size of the seed. Orchid seeds are therefore difficult to locate and observe *in situ* and most of our knowledge of their biology is attributable to *in vitro* laboratory experiments and *in situ* seed sowing experiments (Masuhara & Katsuya 1994, Rasmussen 1995, Brundrett et al. 2003).

The first step in the process of gemination is imbition. This involves the uptake of water by the seeds from the surrounding environment. Imbition in most seed plants allows enzymatic activity to begin breaking down storage compounds in the endosperm, thus initiating embryo growth. In the majority of orchids, imbition may be initially delayed by a combination of exogenous and endogenous seed dormancy mechanisms. At present, there is limited scientific knowledge of the factors that may contribute to breaking dormancy in seeds of Australian orchid species.

One of the most important exogenous seed dormancy barriers is the hydrophobic nature of the seed coat that surrounds the embryo. In the laboratory this can be overcome by soaking the seed in water for extended periods of time, but in the field seed must land in a suitably moist location. In addition to their hydrophobic nature, the seed

coats of some orchids contain inhibitory compounds, such as abscisic acid (Kinderen 1987). These compounds must be leached out of the seed coats with water before the seeds can germinate and it has been speculated that this may be a major factor in long dormancy periods experienced by many orchid species *in situ* (Rasmussen 1995). Under *in vitro* conditions these inhibitory seed coat compounds are easily leached out through the process of surface sterilisation (Rasmussen 1995).

Endogenous seed dormancy mechanisms are also important in delaying germination in orchid seeds. Many terrestrial orchids that experience cold winter climates require cold stratification of the seed to break dormancy (Rasmussen 1995). Most of this research has been conducted on orchid species native to the northern hemisphere. For example, a number of American and European species of *Cypripedium* require cold stratification temperatures of 4–5°C for 2–5 months for most of the seeds to germinate *in vitro* (Rasmussen 1995). We can speculate that Australian orchids growing under similar climatic conditions may also require cold stratification to break dormancy.

The first morphological change that an orchid seed undergoes during germination is swelling to form a protocorm, a rootless, shootless 'blob'. Germination in orchids is more complicated than in many plant groups, due to dependence on mycorrhizal fungi for early protocorm (and seedling) development. Endogenous dormancy mechanisms may also be broken down in many orchids through chemical 'signalling' between a specific orchid mycorrhizal fungus and its host orchid (Rasmussen 1995). It has been speculated that symbiotic association or infection is a precursor to germination in some species but in other orchids, a functional mycorrhiza may form after the seed has imbibed and produced epidermal hairs at the basal end of the protocorm (Rasmussen 1995, Warcup 1990). It is known that chemical 'signalling' is vital for the appropriate association to develop *in situ,* but the chemistry and interactive genetic regulation between the associates is poorly understood.

> **Research questions:**
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> 1. What environmental factors break the dormancy of Australian orchid seeds?
> 2. What controls and influences the initial interaction between orchid seed and fungus?

Mycotrophy

Orchids in the wild are known to form associations with specific fungi, called orchid mycorrhizas. The orchid fungi form an intracellular relationship with their hosts, usually in the roots and/or tubers and are thus classed as endomycorrhizas. Orchid mycorrhizal fungi form hyphal coils, called pelotons, in the cells of the orchid and are beneficial to the orchid in that they provide the host plant with nutrients such as soluble sugars (Rasmussen 1995, Warcup 1990). The duration of the association varies according to the life history of the particular orchid species.

For example, achlorophyllous orchids (e.g. *Rhizanthella slateri*) rely totally on specific mycorrhizal fungi because of their inability to photosynthesise (Taylor & Bruns 1997, Warcup 1990). Many other orchids are capable of photosynthesing but remain partially mycotrophic throughout their lives (e.g. *Dipodium punctatum*) (Warcup 1990). In true autotrophic orchids, the mycorrhizal fungi are vital in allowing orchid seeds to develop into photosynthetic seedlings, as the embryos (within orchid seeds) are physiologically unable to develop into seedlings on their own. Orchids ancestrally gained aerial mobility through extreme reduction in the size of their seeds through the loss of some essential food reserves, particularly carbohydrates (Rasmussen 1995, Warcup 1990).

When orchid seeds are dispersed into the air, their mycorrhizal fungi are not transported with them, so the seed must land in close proximity to suitable fungi. Usually, suitable sites would be some kind of moist substrate, such as soil or moist bark. In contrast to the orchids, the mycorrhizal fungi survive successfully on their own in the soil as saprophytes or as pathogens on other plants (Rasmussen 1995, Sneh et al. 1991, Warcup 1990). However, when the orchids and mycorrhizal fungi come into contact there is cell-to-cell recognition and the orchid host allows the fungi to infect the orchid cells in a controlled fashion. Prior to fungal infection, orchid seeds will often germinate in the presence of water and develop into an unspecialised developmental stage called a protocorm. The suspensor cells at the base of the protocorm have been observed as the site through which the orchid fungi initiate mycorrhizal infection (Clements 1988, Rasmussen 1995, Warcup 1990). Pelotons (hyphal coils) form within the basal half of the protocorm and essential nutrients are transferred from the fungus to its host. Growth within the protocorm commences, in which the basal portion forms a root and the apical portion produces the first juvenile leaf. A combination of photosynthesis and uptake of nutrients from the fungus allows the young plantlet to develop into a seedling and later to a mature plant (Rasmussen 1995, Warcup 1990). The genetic and chemical interactions underlying this symbiotic process are still poorly understood.

In many epiphytic and lithophytic orchids and evergreen terrestrial orchids, mature photosynthetic plants can survive successfully without the aid of the mycorrhizal fungus and the presence of pelotons in the roots become less frequent or absent. In contrast, autotrophic terrestrial orchids growing in nutrient-poor soil substrates and dry conditions, often possess small leaves, underground tubers and poorly developed roots systems. These orchids commonly have a summer or dry season dormancy and they continually rely on a close association with their fungal symbionts when they are in active vegetative or reproductive growth. In Australia, the majority of native orchids are terrestrial species that exhibit all of these life history strategies. They also show strong dependence on their fungal associates throughout their life cycles (Masuhara & Katsuya 1992, Perkins & McGee 1995).

Research questions:

3. What are the critical processes underlying the symbiotic relationship between orchid and fungus?

4. What is the frequency and importance of fungal symbiotic relationships in mature orchid plants?

Taxonomy and fungal specificity

Research into mycorrhizal fungi in orchids has revealed that a relatively small number of fungal genera form associations with orchids. Identification of these fungi has mostly been achieved through the *in vitro* isolation of the fungi from parts of mature orchid plants (roots, tubers and protocorms) and subsequent morphological and genetic comparisons with known teleomorphic test cultures (Kristiansen et al. 2001, Lilja et al. 1996, Roberts 1999, Sneh et al. 1991). However, 'seed baiting' techniques (Rasmussen & Whigham 1993, Brundrett et al. 2003) have recently been shown to be effective in both isolating mycorrhizal fungi and in allowing experimentation on germinating orchids *in situ*.

The great majority of true orchid mycorrhizal fungi belong to the fungal Division Basidiomycota (mushrooms). Fungal endophytes are either binucleate or multinucleate and are members of the form genus *Rhizoctonia*, and species of the teleomorphic genera *Ceratobasidium, Tulasnella, Sebacina, Thanatephorus, Oleveonia* and *Serendipita* (Roberts, 1999, Sneh et al. 1991, Warcup 1971, 1973, 1981, 1990, Warcup & Talbot 1966, 1967, 1971, 1980). Rare endophytes include species of *Laccaria* (Kristiansen et al. 2001), *Russula* (Taylor & Bruns 1999) and *Armillaria* (Campbell 1962) (Basidiomycetes), as well as endophytes belonging to the Division Ascomycota (Currah et al. 1997, Rasmussen 1995).

Isolations made from Australian orchids sampled from the wild have revealed a high degree of specificity between the species of orchid and the associated species of mycorrhiza. Extensive research across a broad sample of orchid species has also revealed that there are patterns of specificity shared across different species with the same genus and across different genera. For example, within the Australian terrestrial orchids, species of *Pterostylis, Prasophyllum* and *Genoplesium* associate with *Ceratobasidium*; *Thelymitra, Calochilus, Diuris, Orthoceras,* and *Acianthus* associate with *Tulasnella*; and *Caladenia, Eriochilus,* and *Glossodia* associate with *Sebacina* (Warcup 1971, 1973, 1981, 1990, Warcup & Talbot 1966, 1967, 1971, 1980). Species of *Microtis, Spiranthes* and *Lyperanthus*, are less specific and can associate with *Ceratobasidium, Tulasnella* or *Sebacina* (Masuhara & Katsuya 1994, Perkins et al. 1995, Warcup 1971, 1973, 1981, 1990, Warcup & Talbot 1966, 1967, 1971, 1980).

This high degree of specificity in the field is not necessarily maintained when the orchids are grown *ex situ* or raised *in vitro* (Masuhara & Katsuya 1994, Perkins & McGee 1995, Perkins et al. 1995). Laboratory and glasshouse grown plants have been demonstrated to form functional mycorrhizae with a range of different *Rhizoctonia* strains, including some known to be virulent pathogens of agricultural crop plants (Sneh et al. 1991, Perkins & McGee 1995, Pope & Carter 2001). It appears that a number of as yet unidentified ecological factors may facilitate the high degree of specificity between the symbionts existing *in situ*. What causes this degree of specificity to break down *in vitro* and *ex situ* is poorly understood. Examination of ecological specificity between orchids and their mycorrhizas (Masuhara & Katsuya 1994, Perkins & McGee 1995, Perkins et al. 1995) has recently been identified as a vital component of managing rare and endangered orchid populations in Australia and abroad.

The biology of their mycorrhizal associates may strongly influence the distributions and habitats in which orchid species grow. For example, the mycorrhizal fungus of the Western Australian underground orchid *Rhizanthella gardneri, Thanatephorus gardneri,* is ectomycorrhizal on the roots of myrtaceous shrubs in the *Melaleuca uncinata* species complex (Warcup 1991, Craven et al. 2004). *Rhizanthella gardneri* only grows in association with *Melaleuca scalena*, *M. uncinata* and *M. hamata* and it seems likely that the orchid's distribution is constrained by that of its mycorrhizal fungus. However, *Melaleuca uncinata* also occurs in south eastern Australia, where this underground orchid is absent, so factors other than fungal distribution may need to be invoked to provide a more complete explanation of the orchid's distribution. Similar, 'host plant' associations are known for other terrestrial, lithophytic and epiphytic species of orchids (Table 2). It is tempting to speculate that these associations are mediated by species of fungi that are symbiotic with both orchids and 'host plants' but the mycorrhizal associate(s) of none of these orchids is known.

Saprophytes and epiparasites

Some taxa of terrestrial orchids have evolved to be so dependent on their mycorrhizal associates that they no longer photosynthesize at all. A native of the Sydney region, the eastern Australian underground orchid, *Rhizanthella slateri*, exemplifies this extreme form of parasitism, developing no chlorophyllous tissues and emerging slightly above ground level only to flower and fruit. Such plants have been incorrectly termed 'saprophytes' (organisms that live on dead or decaying organic matter) but this term is probably correctly applied to the fungi on which the orchids depend. *Rhizanthella* is more correctly termed 'mycoheterotrophic' (see e.g. Molvray et al. 2000). Whether this term is also applicable to other Sydney orchids is less clear. Although no orchid is completely autotrophic, a range of states of autotrophy/heterotrophy is found, ranging from those orchids that are only parasitic as seedlings to those that rely completely on their mycorrhizal associates for all supplies of organic nutrients. Other leafless orchids such as *Gastrodia sesamoides*, *G. procera*, *Prasophyllum flavum* and *Arthrochilus huntianus* seem to produce no green tissues and may well be holoparasites too. Leafless species of *Dipodium*, however, usually produce chlorophyllous tissues in their

Table 2. Associations of orchid species in the Sydney region with other species of plants

Orchid	Associated plant	Kind of association
Adenochilus nortonii	*Sphagnum* sp.	Co-occurring terrestrial plants
Burnettia cuneata[2]	*Melaleuca squarrosa*	Co-occurring terrestrial plants
Corybas fordhamii[1]	*Melaleuca squarrosa*	Co-occurring terrestrial plants
Dockrillia cucumerina[1,2]	*Casuarina cunninghamiana*	Epiphyte/host
Dockrillia teretifolia[1,2]	*Casuarina glauca*	Epiphyte/host
Pterostylis sp. aff. *parviflora*[1]	*Melaleuca squarrosa*	Co-occurring terrestrial plants
Rimacola elliptica[1]	*Gleichenia* sp.	Co-occurring lithophytic plants
Tropilis aemula ('ironbark form')[1,2]	*Eucalyptus paniculata* and relatives	Epiphyte/host

References:
1. Nicholls (1969)
2. Bishop (2000)

stems. Although these species decline and die if isolated from their mycorrhizal fungi (Jones 1988), they are apparently capable of photosynthesising to a limited extent.

The fact that *Rhizoctonia* taxa are commonly parasitic on autotrophic plants, and orchids are commonly parasitic on *Rhizoctonia*, including some strains known to be crop pathogens, suggests that many orchids may be epiparasites — organisms that are indirectly parasitic on autotrophic plants. The close association of the Western Australian underground orchid, *Rhizanthella gardneri* with species in the *Melaleuca uncinata* complex, mediated by their shared mycorrhizal associate *Thanatephorus gardneri*, illustrates this concept well. In this case, the orchid is parasitic on a fungus involved in a mutualistic association with an autotrophic plant. Carbon fixed by the autotroph and sequestered by the fungus is, in turn, sequestered by the mycoheterotrophic orchid, which is thus indirectly parasitic or 'epiparasitic' on the autotroph. Warcup (1991) was able to demonstrate that this transfer of resources happens by providing a radioactively labelled source of carbon to cultivated plants of *Melaleuca uncinata sensu lato* on which the association of *Thanatephorus gardneri* and *Rhizanthella gardneri* had already been established. Traces of labelled carbon were then detected in tissues of *Rhizanthella gardneri*. Although epiparasitism has rarely been directly demonstrated, it seems likely to be a common kind of symbiotic relationship involving orchids.

Research questions:

5. What are the mycorrhizal associates of Sydney orchids?

6. Do these mycorrhizal associates have specialised symbiotic relationships with other, associated, species of plants?

7. Are there shared phylogenetic patterns between symbiotic fungi and orchid species that would help interpret the systematic and ecological relationships between these two groups of organisms?

8. How do mycorrhizal associates limit the distributions and habitats of orchid species?

Demography and phenology of growth, dormancy, flowering and fruiting

Our knowledge of orchid phenology is based almost exclusively on data gleaned from herbarium collections (see e.g. Harden (ed.) 1993, Bishop 2000) and anecdotal reports by orchid enthusiasts (see e.g. Harrison 1982, 1983a, 1983b, 1984, 1985). Few scientific studies have been published that document any aspect of orchid demography based on observational data for significant events in the life cycles of wild populations. Some of the best available information in Australia comes from amateur enthusiasts with a strong interest in their local bushland reserve or property.

A Victorian example:

An example of a short-term but intensive study, is a series of observations over nine years to 1997 (and continuing) on a small patch of the naturally occurring hybrid *Chiloglottis × pescottiana*, and a transplanted patch of *Chiloglottis valida*, at Anglesea in western coastal Victoria (N. Tucker pers. comm.). These two species occur in the Sydney region. The ten individuals of *C. valida* planted in 1988 had spread, presumably by vegetative proliferation, to over 400 plants by 1996, but the percentage flowering had dropped from 70% to 25%. A presumed 'natural' population of *C. × pescottiana* (which includes *C. valida* as one of its parents…) was first detected in 1995, consisting of 32 individuals of which 40% were in flower. The following year there were 37 individuals, 62% in flower, then in 1997, 93 individuals of which 63% were in flower. So a similar increase in population size, but the reverse correlation with flowering success.

Do these two taxa have differing ecological strategies, or is there a difference between the responses in planted and 'natural' populations? Clearly greater detail, including meteorological and other environmental correlations, and studies of replicate populations, are needed before we can hypothesise about population dynamics. But one can envisage scientific studies of hybridity and pollinator specificity using a system such

as this. Tucker also studied the spatial extent and spread of the colonies by mapping each individual until the population became too large.

Anecdotal reports suggest that most species of subfamily Orchidoideae native to the Sydney region are deciduous, tuberous, terrestrial herbs that commence active growth in mid to late summer, the new shoots emerging from the soil surface in late summer to early autumn. Green leaves develop quickly in most species and survive until late spring or early summer. Then the whole shoot system withers, as does the tuber from which it grew, and the plant 'dies back' to one or more newly developed dormant tubers. During the period of active growth, one or more new, 'replacement' tubers develop, usually with one developing beside the old tuber and sometimes with one or more additional new tubers (or 'droppers') developing at the tips of lateral roots (see Pridgeon & Chase 1995). Whether one or more replacement tubers develop is biologically (and horticulturally) important because species in which more than one new tuber develops are capable of vegetative (asexual) multiplication whereas those that produce only one new tuber each year multiply entirely through the (presumably sexual) production and growth of seeds.

Flowering in most deciduous, tuberous, terrestrial orchid species native to the Sydney region occurs between autumn and later spring. Almost nothing in the way of scientific research has been published on the fruiting phenology of orchid species native to the Sydney region. Anecdotal accounts suggest that fruit maturation usually occurs within 2 months of fertilisation in most species of subfamily Orchidoideae but this process usually takes longer (up to 32 months in some species) in most species of subfamily Epidendroideae (Arditti 1992).

Fire

The 'typical' Orchidoid annual growth cycle, involving annual tuber replacement, followed by a period of dormancy in which all organs other than the subterranean tuber senesce, probably evolved many millions of years before fire became a regular component of the Australian environment. Subterranean dormancy during the hot, relatively dry summer of the Sydney region now allows most native orchidoid species to avoid the potentially lethal effects of fire but seasonal dormancy is a pre-adaptation to other kinds of seasonal stress in other environments. Consequently, seasonal dormancy in orchidoid orchids should not be described as an adaptation to fire-prone environments but as a pre-adaptation or 'exaptation' to fire. In alpine Australia as well as much of the temperate holarctic region, seasonal dormancy allows orchidoid orchids to avoid freezing winters. In monsoonal tropical Australia, it allows them to avoid fire and drought during the dry, fire-prone winter period.

Fire stimulates flowering of many terrestrial orchid species (Jones 1988), and in some, such as *Pyrorchis nigricans* and *Burnettia cuneata*, flowering rarely occurs except in the growing season immediately following a fire. Fire is thought to cause a hormonal response in the plant, probably related to ethylene levels. Fire can also kill plants, particularly when they happen in autumn, winter or spring, when the plants are in active growth. So what is the answer to the question 'are fires good for terrestrial orchids?' It depends what we mean by 'good'. If we mean that fire stimulates a pleasant floral display for us to enjoy, then for many species, at least in the short-term (e.g. for one season), fire is 'good'. From a biological point of view, stimulation of flowering also provides the opportunity for increased sexual reproduction, and thus the potential for population increase and genetic recombination. However, we know little about the long-term consequences of frequent fires on populations of terrestrial orchids. One plausible consequence of increasing the resources that a plant puts into its floral display and seed development in response to fire could be the diversion of resources away from the development of replacement tubers. Frequent fires could then result in the decline of individual plants or the suppression of vegetative multiplication. Depending on the rate of recruitment of seedlings, frequent fires could cause population decline in species that superficially appear to be 'stimulated' by fire.

Fire is more obviously destructive of epiphytic orchids, most of which are not adapted to avoiding fires at all, and which are not usually found in fire-prone environments. A few species do grow in fire-prone environments and some of these fortuitously avoid destruction by growing in fire-free microhabitats — germinating on surfaces of rocks or trees that are sufficiently distant from flames. For example, plants of the 'ironbark form' of *Tropilis aemula* (*Dendrobium aemulum*) often grow high on the trunks and branches of their hosts, thus avoiding all but crown fires. Similarly, plants of *Thelychiton speciosus* often grow on large rock outcrops. The ability of epiphytic species to 'find' fire-free microhabitats will depend in the first instance on the aerodynamic properties of their seeds and the preferred microhabitats of their mycorrhizal partners. The identity of the mycorrhizal fungi of Australian epiphytic orchids is poorly known and even less is known of their ecology.

Another epiphytic species, *Cymbidium suave*, is partially protected from fire by its fire-resistant hosts. This species diverts a substantial proportion of its growth into roots and stems that grow in the decaying heartwood inside the stems of its host, usually a eucalypt or *Allocasuarina* tree. Although the leafy shoots that grow externally are destroyed when burnt, the plants can resprout vigorously from buds protected inside the hosts, supplied by nutrients stored in the extensive system of thick, fleshy roots. The time required for a plant of *Cymbidium suave* to replace its leafy shoot system is unknown.

Adams and Lawson (1993) were unable to find any reports of the effect of fire on pollinator activity or pollination rate, repeating only the common observation that there is a large seed set in many terrestrial species following fire.

Research questions:

9. What is the relationship between the demography of terrestrial orchid species and the frequency, intensity and seasonality of fires?

10. What is the relationship between the demography of epiphytic orchids that grow in fire prone environments and the frequency, intensity and seasonality of fires?

11. To what extent does fire limit the distribution and abundance of epiphytic and epilithic orchid species?

Drought

Anecdotal reports suggest that flowering and fruit set are reduced, the active growing season is shortened or cancelled and mortality is increased in most, if not all Sydney orchid species during droughts. However, this effect has not been quantified for any Sydney orchid species.

Research questions:

12. Using long-term monitoring, and where possible, experimentation, what are the demographic characteristics of orchid species in the Sydney region?

13. How do environmental factors such as fire and drought interact to determine actual and observed population structure?

14. How do pollination strategies effect response of particular species to fire and drought?

Pollination

More is known about the pollination of orchids than about any other aspect of orchid ecology, thanks largely to the observations of orchid enthusiasts, attracted by the fascinatingly specialised relationships between orchid species and their pollinators. Many, perhaps most, orchid species are each pollinated by just one (or a few closely related) species of animal, usually an insect. This high degree of specialisation can be explained as an evolutionary consequence of the way that all orchids, except the phylogenetically basal subfamily Apostasioideae, package their pollen grains. These are aggregated in coherent masses called pollinia, which may, in turn, be aggregated into pollinaria. The pollen grains are held together by pollenkitt or elastoviscin threads or callose, or by common cell walls and are dispersed as one or a few pollen dispersal units (see Pacini & Hesse 2002). Such coherent packages of pollen need to be deposited precisely on a receptive stigma to result in effective pollination and thus fertilisation. There has therefore been strong selective pressure throughout most of the evolutionary history of the orchids favouring specialised pollination systems, involving reliable, agile animal vectors and against pollination by generalist floral foragers and abiotic vectors. In the majority of orchid species for which

pollen vectors are known, they are species of Hymenoptera but species of Diptera, Lepidoptera, Coleoptera and nectar-feeding birds are pollinators of a significant minority of orchid species (Cingel 1995, 2001).

The extensive literature on pollination systems in Australian orchids has received two recent comprehensive reviews (Adams & Lawson 1993, Cingel 2001). Moreover, pollination biology in the tribe Diurideae has also been recently reviewed in great detail elsewhere (Bower 2001). Consequently, we see no need to review this subject in detail again here (but see table 3). An argument worth pursuing here, however, concerns the methodological criteria proposed by Adams and Lawson (1993). They argue that to 'confirm' an animal species as a pollinator, it is necessary to directly observe a pollinating animal remove pollen from the anther of one flower and then deposit it on the stigma of another. Pollinators that have only been observed removing pollinia but not depositing them should be classed only as 'probable' pollinators according to Adams and Lawson. Experimental inference is not discussed by them as providing useful evidence at all. This attitude seems to us to over-rate the value of direct observation and under-rate the role of inference in scientific method. Many insect visitors of orchid flowers are simply too fast, their floral visits too rare and their flights between flowers too long to allow reliable direct observation of the whole process of pollination. Like most other subjects of modern scientific research, identification of the plant species (if any) that these insects pollinate must rely on inferential methods, which may be just as reliable as direct observation if applied intelligently.

A notable feature of orchid pollination systems in general, and those of the Australian flora in particular, is the high proportion of 'deceptive' pollination systems relative to 'rewarding' systems.

Rewarding systems

'Rewarding' systems attract animal pollen vectors with tangible rewards such as food in the form of nectar, pollen, oils or specialised nutrient-containing structures such as pseudo-pollen (Faegri & Pijl 1979). Other forms of tangible reward are offered by some orchid taxa, the best studied of which are the fragrances produced by hundreds of species in several subtribes of the neotropical tribe Maxillarieae, which are gathered and stored by euglossine bees (Dressler 1982). Adams (1991) suggested that the species of *Trigona* that pollinate taxa in the *Thelychiton speciosus* (*Dendrobium speciosum*) complex analogously gather fragrances from these flowers.

Aggregation of pollen grains in pollinia apparently precludes the functioning of orchid pollen as a food reward because it would facilitate the consumption of the entire pollen production of a flower, leaving no pollen for pollination and fertilisation. However, protein- or starch-bearing particles derived from fragmentation of labella trichomes and which apparently function as pseudo-pollen have been investigated in detail in some non-Australian orchid taxa (see Davies &

Table 3. Putative pollinators for orchid taxa in the Sydney region

Orchid	Insect pollinators	Postulated attraction mechanism
Acianthus caudatus[1,2,3]	Fly (*Mycoma* sp.)	Nectar, musty perfume
Arthrochilus spp.[2]	Male thynnine wasps (*Arthrothynnus* spp.)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Caladenia subgenus *Caladenia p.p.* (*C. carnea*)[1,2,3]	Bees (*Trigona* sp.)	Food mimicry (labellum calli functioning as pseudo-anthers)
Caladenia subgenus *Caladenia p.p.* (*C. catenata*)[1,2,3]	Hover flies (*Syrphus* sp.)	Food mimicry (labellum calli functioning as pseudo-anthers)
Caladenia subgenus *Stegostyla* (*C. congesta*)[1,2,3]	Bees (*Hylaeus* sp.)	Food mimicry (labellum calli functioning as pseudo-anthers)
Caladenia subgenus *Calonema p.p.* (e.g. *C. tentaculata*)[1,2,3]	Male thynnine wasps (*Thynnoides* spp., *Lophocheilus* spp.)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Calanthe triplicata[3]	Lepidoptera?	Nectar in labellum spur?
Caleana major[1,2,3]	Sawfly (*Pterygophorus*)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Caleana minor[2] (*Thynnoturneria armiger*)	Male thynnine wasps	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Calochilus spp.[1,2,3]	Male scoliid bees (*Campsomeris* spp.)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Chiloglottis spp.[1,2,3]	Male thynnine wasps (*Neozeleboria* spp., *Eirone* sp.)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Corybas spp.[1,2,3]	Mycetophilid flies?	Brood-site mimicry (fungus-like odour and floral appearance)?
Cryptostylis spp.[1,2,3]	Ichneumonid wasp (*Lissopimpla excelsa*)	Sexual mimicry (Pseudo-pheromones and insectiform labellum)
Cymbidium suave[1,3] Jones, pers. comm..	Apid bees (*Trigona* spp.)	Fluid in easily punctured labellum callus, sweet perfume (D.L.
Dipodium punctatum[1,3]	Megachilid bees (*Chalicodoma derelicta*)	Food mimicry?
Diuris maculata[1,2,3]	Colletid bees (*Trichocolletes* spp., *Leioproctus* sp.)	Food mimicry (pea-like flower)
Dockrillia linguiformis (as *Dendrobium linguiforme*)[1]	Colletid bees (*Euryglossina* spp.)	Scent (a chemical reward system?)
Gastrodia sesamoides[1]	Apid bees (*Exoneura* sp.)	Edible pseudopollen, scent
Genoplesium spp.[2]	Chloropid and Milichiid flies	Nectar, scent, dark colours and/or hairy labellum that vibrates in breeze
Liparis reflexa[1]	Mycetophilid and sarcophagid flies	Scent (food mimicry)
Microtis spp.[1,2]	Ants, ichneumonid and braconid wasps	Nectar, scent
Prasophyllum[1,2]	Colletid and halictid bees, ichneumonid, tiphiid, scoliid and sphecid wasps, syrphid flies, beetles	Nectar, scent
Pterostylis spp.[1,4]	Male mycetophilid and culicid flies	Sexual mimicry (Pseudo-pheromones and insectiform labellum)?
Rhizanthella[1,2]	Phorid flies (*Megaselia* sp.)	Scent (food mimicry)
Thelychiton tarberi (as *Dendrobium speciosum* var. *hillii*)[1,3]	Apid bees (*Trigona* sp.)	Scent (a chemical reward system?)
Thelymitra spp.[1,2,3,]	Halictid bees (*Lasioglossum* spp.), syrphid flies	Pseudopollen, scent (food mimicry)

References:

1. Adams & Lawson (1993)
2. Bower (2001)
3. Cingel (2001)
4. Jones & Clements (2002)

Turner 2004 and references therein). Such detailed studies have not been conducted on any Australian species but Jones (1985) observed that labella calli in *Gastrodia sesamoides* produce fine, starchy grains that function as pseudo-pollen in rewarding its bee pollinator, a species of *Exoneura*.

Numerous orchid species produce nectar, usually from secretory tissues on the labellum or in elongated labella spurs. Nectariferous orchids of the Sydney Region are pollinated by species of bees (e.g. *Spiranthes sinensis*), Tiphiid wasps (e.g. *Prasophyllum elatum*), ants (*Microtis parviflora*) and flies (e.g. *Acianthus caudatus*).

Deceptive systems

Deceptive orchid pollination systems involve either Batesian mimicry by the orchid flower of a specific 'model' that is frequently visited by the pollinating animal, or more generalised, 'non-model' or 'guild' mimicry of a class of resources. The model may be a food source such as a kind of flower that offers a reward of edible pollen and/or nectar (food mimicry). It may be the kind of site preferred by a female pollinating animal as a suitable substrate on which to lay eggs (brood site mimicry). Or it may be a female of the same species as the male pollinating insect, an animal with which the pollinator seeks to mate (sexual mimicry). The essential feature of deceptive systems is that no tangible reward is provided by the plant, only the impression of a reward. This impression may involve visual, olfactory and tactile cues. All of these deceptive systems function by inducing the pollinating animal to perform the kind of stereotypical behaviour elicited by the model that is compatible with the deposition and removal of pollinia from the orchid flower.

The selective pressures responsible for the evolution of deceptive pollination systems have been the subject of much discussion (see e.g. Dafni 1984, Johnson & Nilsson 1999). Various fitness 'costs' such as energy expended in providing tangible rewards, the resources invested in 'advertising display', and inbreeding depression or pollen discounting caused by self-pollination have been adduced to explain the multiple origins of different kinds of deceptive pollination systems. Whether any of these provide a general explanation or whether all have shaped the evolution of at least some lineages are questions that have rarely been tested (e.g. Johnson & Nilsson 1999) but would reward further investigation, especially in genera such as *Diuris* that include both rewarding and non-rewarding species (James Indsto, pers. comm.).

Research question:

15. What are the models mimicked by deceptive orchid species, and how do they effect the distribution and population dynamics of individual species?

16. What is the character phylogeny of pollination systems in the Orchidaceae?

17. What selective pressures are responsible for the evolution of deceptive pollination systems?

Food mimicry

Many terrestrial and epiphytic orchids in the Sydney Region are thought to mimic food sources including pollen-bearing and nectariferous flowers and rotting carcasses. However, this kind of mimicry may be difficult to test for several theoretical and practical reasons. How does one measure similarity between a putative mimic and its putative model? Given an acceptable measure, what degree of similarity constitutes functional mimicry? Pollinators tend to be very rare visitors to mimics, presumably because they are capable of learning how to distinguish between rewarding models and non-rewarding mimics, so gaining a reasonable sample of observations may be an extremely laborious task. The genera *Diuris*, *Thelymitra*, *Eriochilus*, *Adenochilus*, *Glossodia*, *Cyanicula*, *Caladenia*, *Dipodium*, *Dendrobium sensu lato* (i.e. *Dockrillia*, *Thelychiton*, *Tropilis*, and *Tetrabaculum*) are thought by some authors to include food mimics native to the Sydney region.

Bernhardt and Burns-Balogh (1986), for instance, postulated that *Thelymitra nuda*, a nectarless orchid, mimics a guild of petaloid monocots in the family Anthericaceae. The orchid resembles the model species in having blue to mauve tepals but differs from them in having an ornate mitra concealing the gynostemium in the centre of the flower, where the model flowers have stamens. Hairy lobes of the mitra superficially resemble pollen-bearing anthers. The orchid is pollinated by the same pollen-collecting bees that pollinate the model species. Batesian mimicry is a plausible explanation for the orchid pollinating behaviour of the bees but the degree of resemblance between the orchid and its models was not quantified in this study.

The flowers of *Diuris maculata*, another nectarless orchid, are thought to mimic the similarly coloured flowers of 'egg and bacon' peas in various genera of Mirbelieae and Bossiaeeae. Beardsell et al. (1986) tested this by examining whether a Victorian population *Diuris maculata* was pollinated by the same pollinators as the co-occurring egg and bacon peas *Daviesia virgata*, *D. mimosoides* and *Pultenaea scabra*. They found that two unidentified species of *Trichocolletes* and one unidentified *Leioproctus* (Colletidae) did, in fact, pollinate both the orchid and its putative models. They concluded that the orchid is a Batesian mimic of the peas. Subsequently, James Indsto (pers. comm.) has tested the hypothesis of mimicry further by repeating the test at a location in the Sydney Region at which *Diuris maculata* occurs, but where a different species of egg and bacon pea, *Daviesia ulicifolia*, grows. Interestingly, Indsto found *Diuris maculata* and *Daviesia ulicifolia* to be pollinated by the same species of bee, *Trichocolletes venustus*. Moreover, this belongs to the same genus as two of the pollinators found by Beardsell *et al.* Further, he also showed that the similarity in appearance of the orchid to the putative model is striking in near-ultraviolet light, a range of wave-lengths that are visible to bees but not to humans. However, he also found *T. venustus* to be pollinating *Hardenbergia violacea*, a co-occurring purple-flowered pea. This example

appears to be intermediate between Batesian and non-model mimicry in that the deceptive orchid appears to be mimicking a subset of the species on which the pollinator feeds.

Brood site mimicry

There is strong evidence that some orchids are brood site mimics. For example, flowers of a Bornean species of slipper orchid, *Paphiopedilum rothschildianum*, are pollinated by females of the syrphid fly *Dideopsis aegrota*, as they escape from the orchid's bucket-shaped labellum (Atwood 1985). The flies are apparently attracted to the flower by its scent and hairy staminode, which superficially resembles a colony of aphids, on which females of this fly usually lay their eggs. There is no hard evidence for brood site mimicry in any orchids native to the Sydney region. However, it has been speculated that the mushroom-like flowers of *Corybas* species mimic fungi that are the brood sites of their putative pollinators: fungus gnats of the family Mycetophilidae (Bower 2001). As would be expected for deceptively pollinated species, the flowers are nectarless, pollinators of *Corybas* are rarely seen and the hooded flowers of most species preclude observation of the mechanics of pollination.

Sexual mimicry

The Australian orchid flora is the world's richest for sexual mimics. Several hundred Australian orchid species are known, or predicted to be pollinated by male insects that try to mate with their flowers (Cingel 2001). Moreover, sexual mimicry has evolved independently at least six times in different lineages (Kores et al. 2001). Within at least one sexually deceptive lineage, the subtribe Drakaeinae, pollinator switches seem to have occurred repeatedly (Mant et al. 2002). In several species of *Chiloglottis* the insects are known to be attracted by pseudopheromones released by the flowers (Mant et al. 2002, Schiestl et al. 2003), a form of attraction that is thought to be characteristic of all cases of sexual mimicry. Most of the insects involved are hymenopterans, of which the great majority are tiphiid wasps in the subfamily Thynninae (Bower 2001). However, several species of *Pterostylis* are known to be pollinated by male flies in the families Culicidae and Mycetophilidae (Adams & Lawson 1993, Bernhardt 1995), which are thought to be attracted to the nectarless flowers by pseudopheromones.

Self pollination

Although some orchid species are known to be self-incompatible, many are self-compatible and show varying levels of self pollination or autogamy. Obligate autogamy is found in a minority of species (e.g. *Orthoceras strictum*), although it has evolved independently in numerous orchid lineages, presumably as an adaptational response to scarcity or absence of pollinators. Completely autogamous species tend to produce smaller, less showy flowers than closely related outcrossers, a predictable evolutionary response to the loss of selective pressure for floral 'advertising'. Some autogamous species (e.g. *Caladenia cleistantha*) have dispensed with floral display altogether by becoming cleistogamous – producing self-pollinating flowers that never open.

A comparative study of *Thelymitra ixioides* and *T. circumsepta*, two morphologically very similar, non-rewarding species that both occur in the Sydney Region, showed that the former had, on average, five flowers per raceme with over half of them open on any given day, while the latter had about seven flowers per raceme but with only 15% open (Sydes & Calder 1993). In *Thelymitra ixioides* the flowers stayed open for up to a week, compared to the single-day blooms of *T. circumsepta*. Detailed studies of pollination and fertilisation (Sydes & Calder 1993) showed that, as expected, *Thelymitra ixioides* was cross-pollinating and *Thelymitra circumsepta* was self-pollinating. Sydes and Calder (1993) suggest that self-pollination may have been selected for in *T. circumsepta* due to its rarity (the chances of cross-fertilisation are low) and the lack of co-flowering species in its habitat (reducing the attractiveness to pollinators generally).

Apomixis

Asexual production of seeds has been demonstrated in few orchids (Davis 1966, Asker & Jerling 1992) and no embryological descriptions have been published of apomixis in orchids from the Sydney Region. However, detailed, unpublished embryological studies by M.A. Clements (pers. comm.) have demonstrated apomixis in *Genoplesium apostasioides* and *Caleana minor*. It had already been postulated (Jones & Clements 1989) that the former species was apomictic because its flowers usually have a sterile stamen and no functional stigma and yet develop almost invariably into fruits.

Research questions:

18. What animals, if any, pollinate the species of *Adenochilus*, *Bulbophyllum*, *Burnettia*, *Calanthe*, *Cyanicula*, *Cyrtostylis*, *Glossodia*, *Lyperanthus*, *Papillilabium*, *Plectorrhiza*, *Pyrorchis*, *Rhizanthella* and *Rimacola*?

19. What kind of experiment could decisively test for food mimicry?

20. What chemicals function as pseudo-pheromones in sexually deceptive orchids?

21. Is co-speciation or pollinator switching the predominant mode of evolutionary diversification of the relationship between sexually deceptive orchids and their pollinators?

Conservation

Species decline due to habitat destruction

Today many species of orchid have fragmented populations, mostly due to the destruction or alteration of habitat by

humans, both in Australia and elsewhere. Land clearing and altered land use seem to have been the main causes of local extinction. Herbarium and anecdotal evidence suggest that many Sydney species were once far more widespread. For example, 'Port Jackson', 'Sydney' and 'Parramatta' were cited by Robert Brown as the collection localities for many of the orchid species that he described early in the nineteenth century. While some of these species can still be found in fragments of urban bushland close to the city centre, a few (e.g. *Thelymitra media*) are now known only from the fringes of the Region or beyond. The Sydney Region is less affected by such threatening processes than many cities of comparable size because a relatively large proportion of its area (and particularly, a large proportion of its biologically diverse sandstone soils) is protected in national parks and nature reserves. Nevertheless, seven species of Orchidaceae native to the Sydney Region are listed as vulnerable, 13 as endangered and one as presumed extinct. Pleasingly, the last species, *Diuris bracteata*, has recently been rediscovered at several localities north and south of the Hawkesbury River (Eygelshoven 2000, D.L. Jones pers. comm.) and should be transferred to the endangered list in the near future. The number of threatened Sydney orchid species is likely to increase in the near future with the naming of newly resolved rare species in genera such as *Thelymitra* that are presently being taxonomically revised (J. Jeanes pers. comm.). Moreover, in habitats subject to further deterioration it is likely there will be further extinctions of local populations and species.

Orchids are not unusual in including many species that are geographically restricted or occurring in small populations that are reproductively isolated from one another, and the processes that threaten other such species are likely to threaten restricted orchids too. However, many orchids are unusual in relying on at least two highly specialised symbiotic relationships: one with a species of mycorrhizal fungus and another with a species of pollinating insect. One would expect this extreme specialisation to render such orchids more vulnerable to environmental fluctuations than species with more generalised habitat requirements. Perhaps the well documented loss in the Central Coast region of sexually deceptive species of *Caladenia* subgenus *Calonema* might be due to the decline there of one or other of the symbiotic partners on which these species depend? Herbarium records indicate the presence of *Caladenia tentaculata* at Gladesville in 1885, at Canterbury in 1891 and near Cooks River in 1903 but this species has not been collected in the Central Coast since then and is not common east of the Great Dividing Range. Similarly, *Caladenia fitzgeraldii* was collected at Gladesville in 1903 but has not been collected in the Central Coast since then.

Other threatening processes

The natural and anthropogenic processes that are considered to threaten rare orchid species are included in recovery plans for vulnerable and endangered species. Although such plans have been published for only two orchid species that are native to the Sydney region, many more recovery plans have been produced for other Australian orchids and at least some of the threatening processes that are discussed in those are probably relevant to Sydney species. After land clearing, the threatening processes that are most commonly cited relate to habitat degradation resulting from habitat fragmentation and other anthropogenic disturbance. These include weed invasion, inappropriate fire regimes, erosion, soil compaction, trampling by humans and other animals, grazing by livestock and feral herbivores, and pollution from adjacent agricultural, industrial or urban environments. While these are all plausible threats, little scientific research has been conducted to test the role of these processes in the decline of orchid species. Unauthorised collection by horticulturalists is considered a significant threat to many species, but these concerns have not been documented with much empirical evidence. Nevertheless, the prices that nurseries charge for the more spectacular epiphytic or lithophytic orchids such as *Thelychiton speciosus*, provide poachers with a strong incentive to collect these plants illegally.

Research questions:

22. Have species become locally extinct or rare due to local extinctions of pollinators or mycorrhizal species?

23. Is habitat disturbance and/or land clearing the major cause of local or species extinctions directly, or through its impact on insect pollinators or fungal symbionts?

24. What role do other postulated threatening processes have in the decline of orchid species?

Conclusions

Our knowledge of the ecology of orchids in general, and of those native to the Sydney Region in particular, is quite uneven. We have a much better understanding of the symbiotic relationships between orchids and their mycorrhizal associates and between orchids and their pollinators than we do of other aspects of orchid ecology such as demography, phenology, responses to disturbance and seed dispersal. To conserve orchids in their natural habitat, we must learn more about these relatively neglected areas of orchid biology.

Acknowledgements

References

Adams, P.B. (1991) Variation, multiple pollinators and breeding system in *Dendrobium speciosum* Smith: a biological review. *The Orchadian* 10: 124–140.

Adams, P.B. & Lawson, S.D. (1993) Pollination in Australian orchids: a critical assessment of the literature 1882–1992. *Australian Journal of Botany* 41: 553–575.

Arditti, J. (1992) *Fundamentals of orchid biology* (John Wiley & Sons: New York, U.S.A.).

Arditti, J. & Ghani, A.K.A. (1999) Tansley Review No. 110: Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist* 145: 367–421.

Asker, S.E. & Jerling, L. (1992) *Apomixis in plants* (CRC Press: Boca Raton, U.S.A.).

Atwood, J.T. (1985) Pollination of *Paphiopedilum rothschildianum*: brood-site deception. *National Geographic Research* 1: 247–254.

Beardsell, D.V., Clements, M.A., Hutchinson, J.F. & Williams, E.G. (1986) Pollination of *Diuris maculata* R.Br. (Orchidaceae) by floral mimicry of the native legumes *Daviesia* spp. and *Pultenaea scabra* R.Br. *Australia Journal of Botany* 34: 165–173.

Benson, D. & Howell, J. (1990) *Taken for granted: the bushland of Sydney and its suburbs* (Kangaroo Press: Kenthurst, Australia).

Benson, D. & McDougall, L. (2005) Ecology of Sydney plant species: part 10 Monocotyledon families Lemnaceae to Zosteraceae. *Cunninghamia* 9(1): 16–212.

Bernhardt, P. (1995) Notes on the anthecology of *Pterostylis curta* (Orchidaceae). *Cunninghamia* 4: 1–8.

Bernhardt, P. & Burns-Balogh, P. (1986) Floral mimesis in *Thelymitra nuda* (Orchidaceae). *Plant Systematics and Evolution* 151: 187–202.

Bishop, T. (2000) *Field guide to the orchids of New South Wales and Victoria*, second edition (UNSW Press: Kensington, Australia).

Bower, C.C. (2001) Pollination [of genera of Diurideae]. pp. 59–213. In: A.M. Pridgeon, P.J. Cribb, M.W. Chase & F.N. Rasmussen (eds) *Genera Orchidacearum* Volume 2: Orchidoideae (Part 1). (Oxford University Press: Oxford, U.K.).

Brundrett, M.C., Scade, A., Batty, A.L., Dixon, K.W. & Sivasithamparam, K. (2003) Development of *in situ* and *ex situ* seed baiting techniques to detect mycorrhizal fungi from terrestrial orchid habitats. *Mycological Research* 107: 1210–1220.

Campbell, E.O. (1962) The Mycorrhiza of *Gastrodia cunninghamii*. *Transactions of the Royal Society of New Zealand. Botany*. 1: 289–296.

Carey, P.D. (1998) Modelling the spread of *Himantoglossum hircinum* (L.) Spreng. at a site in the north of England. *Botanical Journal of the Linnean Society* 126: 159–171.

Cingel, N.A. van der (1995) *An atlas of orchid pollination — European Orchids.* (A.A. Balkema: Rotterdam, The Netherlands).

Cingel, N. A. van der. (2001) *An Atlas of Orchid Pollination — America, Africa, Asia and Australia* (A.A. Balkema: Rotterdam, The Netherlands).

Clements M.A. (1988) Orchid mycorrhizal associations. *Lindleyana* 3: 73–86.

Craven, L.A., Lepschi, B.J., Broadhurst, L. & Byrne, M. (2004) Taxonomic revision of the broombush complex in Western Australia (Myrtaceae, *Melaleuca uncinata s.l.*). *Australian Systematic Botany* 17: 255–271.

Currah R.S., Zelmer C.D., Hambleton S. & Richardson K.A. (1997) Fungi from orchid mycorrhizas. pp. 117–170. In: J. Ardittti & A.M. Pridgeon (eds) *Orchid biology: reviews and perspectives, VII* (Kluwer Academic Publications: Dordrecht, The Netherlands).

Dafni, A. (1984) Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* 15: 259–78.

Davies, K.L. & Turner, M.P. (2004) Pseudopollen in *Eria* Lindl. section *Mycaranthes* Rchb.f. (Orchidaceae) *Annals of Botany* 94: 707–715.

Davis, G.L. (1966) *Systematic embryology of the angiosperms* (John Wiley: New York).

Dixon, K.W., Pate, J.S. & Kuo, J. (1990) The Western Australian fully underground orchid *Rhizanthella gardneri*. pp. 37–62. In: J. Arditti (ed) *Orchid Biology Reviews and Perspectives,* V. (Timber Press: Portland, U.S.A.).

Dressler, R.L. (1981) *The Orchids: Natural History and Classification* (Harvard University Press: Cambridge, U.S.A.)

Dressler, R.L. (1982) Biology of the orchid bees (Euglossini). *Annual Review of Ecology and Systematics* 13: 373–394.

Dressler, R.L. (1993) *Phylogeny and Classification of the Orchid Family* (Dioscorides Press: Portland, U.S.A.)

Eygelshoven, P. (2000) In search of *Diuris bracteata* Fitzg. *The Orchadian* 13: 225–226.

Faegri, K. & Pijl, L. van der (1979) *The Principles of Pollination Ecology*, third revised edition (Pergamon Press: Oxford, U.K.)

Harden, G.J. (ed.) (1993) *Flora of New South Wales*. Vol. 4. (UNSW Press: Sydney, Australia)

Harrison, M. (1982) Cultivation based on habitat – IV *Dendrobium aemulum*. *The Orchadian* 7: 107–109.

Harrison, M. (1983a) Cultivation based on habitat observation – VI *Sarcochilus australis*. *The Orchadian* 7: 180–182.

Harrison, M. (1983b) Cultivation based on habitat – VII *Dendrobium tetragonum*. *The Orchadian* 7: 216–218.

Harrison, M. (1984) Cultivation based on habitat — IX *Cymbidium suave*. *The Orchadian* 7: 255–257.

Harrison, M. (1985) Habitats and cultivation XI *Bulbophyllum minutissimum*. *The Orchadian* 8: 51–55.

Johnson, S.D. & Nilsson, L.A. (1999) Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* 80: 2607–2619.

Jones, D.L. (1985) The pollination of *Gastrodia sesamoides* R.Br. in southern Victoria. *Victorian Naturalist* 102: 52–54.

Jones, D.L. (1988) *Native Orchids of Australia* (Reed Books: Frenchs Forest, Australia).

Jones, D.L. & Clements, M.A. (1989) Reinterpretation of the genus *Genoplesium* R. Br. (Orchidaceae: Prasophyllinae). *Lindleyana* 4: 139–145.

Jones, D.L. & Clements, M.A. (2002) A reassessment *Pterostylis* R.Br. (Orchidaceae). *Australian Orchid Research* 4: 3–63.

Kinderen, G. van der (1987) Abscisic acid in terrestrial orchid seeds: a possible impact on their germination. *Lindleyana* 2: 84–87.

Kores, P.J., Molvray, M., Weston, P.H., Hopper, S.D., Brown, A., Cameron, K.M. & Chase, M.W (2001) A phylogenetic analysis of Diurideae (Orchidaceae) based on plastid DNA sequence data. *Amer. J. Bot.* 88: 1903–1914.

Kristiansen, K.A., Taylor, D.L., Kjoller, R., Rasmussen, H.N. & Rosendahl, S. (2001) Identification of mycorrhizal fungi from single pelotons of *Dactylorhiza majalis* (Orchidaceae) using single-strand conformation polymorphism and mitochondrial ribosomal large subunit DNA sequences. *Molecular Ecology* 10: 2089–2093.

Kurzweil, H. (1994) The unusual seed structure of *Disa uniflora*, with notes on the dispersal of the species. pp. 394–396. In: A Pridgeon (ed) *Proceedings of the 14th World Orchid Conference, Glasgow*. (Her Majesty's Stationery Office: London, U.K.).

Lilja, A., Hietala, A.M. & Karjalainen, R. (1996) Identification of a uninucleate *Rhizoctonia* sp. by pathogenicity, hyphal anastomosis and RAPD analysis. *Plant Pathology* 45: 997–1006

Mant, J.G., Schiestl, F.P., Peakall, R. & Weston, P.H. (2002) A phylogenetic study of pollinator conservatism among sexually deceptive orchids. *Evolution* 56: 888–898.

Masuhara, G. & Katsuya, K. (1992) Mycorrhizal differences between genuine roots and tuberous roots of adult plants of *Spiranthes sinensis* var. *amoena* (Orchidaceae). *Botanical Magazine — Tokyo*. 105: 453–460.

Masuhara, G. & Katsuya, K. (1994) *In situ* and *in vitro* specificity between *Rhizoctonia* spp. and *Spiranthes sinensis*. *New Phytologist* 127: 711–718.

Molvray, M., Kores, P.J. & Chase, M.W. (2000) Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. Pp. 441–448. In: K.L. Wilson & D.A. Morrison (eds) *Monocots: Systematics and Evolution*. (CSIRO Publishing: Melbourne, Australia).

Murren, J.C. & Ellison, A.M. (1998) Seed dispersal characteristics of *Brassovola nodosa* (Orchidaceae). *American Journal of Botany* 85: 675–680.

Nicholls, W.H. (1969) *Orchids of Australia* (Thomas Nelson: Melbourne, Australia).

Pacini, E. & Hesse, M. (2002) Types of pollen dispersal units in orchids, and their consequences for germination and fertilization. *Annals of Botany* 89: 653–664.

Perkins, A.J., Masuhara, G. & McGee, P.A. (1995) Specificity of the associations between *Microtis parviflora* and its mycorrhizal fungi. *Australian Journal of Botany* 43: 85–91.

Perkins, A.J. & McGee, P.A. (1995) Distribution of the orchid mycorhiza1 fungus, *Rhizoctonia solani*, in relation to its host *Pterostyis acuminata*, in the field. *Australian Journal of Botany* 3: 565–575.

Pope, E. J. & Carter, D. A. (2001) Phylogenetic placement and host specificity of mycorrhizal isolates AG-6 and AG-12 in the *Rhizoctonia solani* species complex. *Mycologia* 93: 712–719.

Pridgeon, A.M. & Chase, M.W. (1995) Subterranean axes in tribe Diurideae (Orchidaceae): morphology, anatomy, and systematic significance. *American Journal of Botany* 82: 1473–1495.

Rasmussen, H.N. (1995) *Terrestrial Orchids: From Seed to Mycotrophic Plant* (Cambridge University Press: Cambridge, U.K.).

Rasmussen, H.N. & Whigham, D. (1993) Seed ecology of dust seeds *in situ*: a new study techniques and its application in terrestrial orchids. *American Journal of Botany* 80: 1374–1378.

Roberts, P. (1999) Rhizoctonia-*forming fungi. A Taxonomic Guide* (Royal Botanic Gardens, Kew, U.K.).

Schiestl, F.P., Peakall, R., Mant, J.G., Schulz, C., Ibarra, F. & Franke, F. (2003) The chemistry of sexual deception in an orchid-wasp pollination system. *Science* 302: 437–438.

Sneh, B., Burpee, L. & Ogoshi, A. (1991) *Identification of* Rhizoctonia *species* (American Phytopathological Society: St. Paul, U.S.A.).

Sydes, M.A. & Calder, D.M. (1993) Comparative reproductive biology of two sun-orchids; the vulnerable *Thelymitra circumsepta* and the widespread *T. ixioides* (Orchidaceae). *Australian Journal of Botany* 41: 577–589.

Taylor, L.D. & Bruns, T.D. (1997) Independent, specialized invasion of ectomycorrhizal mutualism by two nonphotosynthetic orchids. *Proceedings of the National Academy of Sciences, USA* 94: 4510–4515.

Taylor, L.D. & Bruns, T.D. (1999) Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids *Corallorhiza maculata* and *C. mertensiana*. *Molecular Ecology* 8: 1719–1732.

Warcup, J.H. (1971) Specificity of mycorrhiza association in some Australian orchids. *New Phytologist*. 70: 41–46.

Warcup, J.H. (1973) Symbiotic germination of some Australian orchids. *New Phytologist* 72: 387–392.

Warcup, J.H. (1981) The mycorrhizal relationship of Australian orchids. *New Phytologist* 87: 371–387.

Warcup, J.H. (1990) Mycorrhiza. pp. 21–26. In: R.J. Bates & J.Z. Weber (eds) *Orchids of South Australia* (Flora and Fauna of South Australia Handbook Committee: Adelaide, Australia).

Warcup J.H. (1991) The *Rhizoctonia* endophytes of *Rhizanthella* (Orchidaceae). *Mycological Research* 95: 656–659.

Warcup, J.H. & Talbot, P.H.B. (1966) Perfect states of some *Rhizoctonia*. *Transactions of the British Mycological Society* 49: 427–435.

Warcup, J.H. & Talbot P.H.B. (1967) Perfect states of *Rhizoctonias* associated with orchids I. *New Phytologist* 66: 631–641

Warcup, J.H. & Talbot, P.H.B. (1971) Perfect states of *Rhizoctonia* associated with orchids II. *New Phytologist* 70: 35–40.

Warcup, J.H. & Talbot, P.H.B. (1980) Perfect states of Rhizoctonias associated with orchids III. *New Phytologist*, 86: 267–272.