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Deception above, deception below: linking pollination and mycorrhizal biology of orchidsRichard J. Waterman^{1,2,*} and Martin I. Bidartondo^{1,2}¹ Imperial College London² Jodrell Laboratory, Royal Botanic Gardens, Kew TW9 3DS, UK

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Abstract

Several key characteristics of the species-rich orchid family are due to its symbiotic relationships with pollinators and mycorrhizal fungi. The majority of species are insect pollinated and show strong adaptations for outcrossing, such as pollination by food- and sexual-deception, and all orchids are reliant on mycorrhizal fungi for successful seedling establishment. Recent studies of orchid pollination biology have shed light on the barriers to reproductive isolation important to diversification in different groups of deceptive orchids. Molecular identification of orchid mycorrhizal fungi has revealed high fungal specificity in orchids that obtain organic nutrients from fungi as adults. Both pollinator and fungal specificity have been proposed as drivers of orchid diversification. Recent findings in orchid pollination and mycorrhizal biology are reviewed and it is shown that both associations are likely to affect orchid distribution and population structure. Integrating studies of these symbioses will shed light on the unparalleled diversification of the orchid family.

Key words: Mutualism, myco-heterotrophy, pollinator limitation, speciation.

Introduction

The biology of orchids was summarized by Benzing (1981) with the following five facts: (i) the Orchidaceae is the most diverse family of angiosperms with over 24 000 species (Dressler, 2005); (ii) orchid seeds are small, some of the smallest of any taxon (Arditti and Ghani, 2000); (iii) orchid seedlings are entirely dependent on

fungi for their energetic and nutritional needs (Smith and Read, 1997); (iv) many orchid species are rare or widely dispersed; (v) many orchids rely on specialized pollination systems for sexual reproduction. Two of these five facts relate directly to orchid symbiotic associations: symbiosis with pollinators for reproduction; and symbiosis with mycorrhizal fungi for successful seedling development. Another of these facts, the minuscule seeds, is also linked to their fungal associations. Orchids can afford to invest exceptionally few resources in huge numbers of individual seeds as the energy required for seedling establishment is provided by fungi. As will be explained, some aspects of orchid reproductive biology and mycorrhizal biology may combine to create the dispersed populations seen in many species. Whether these unique symbiotic relationships have also helped drive the unparalleled diversification of Orchidaceae has been the subject of extensive speculation and some research.

It has long been believed that pollination by animals has played an instrumental role in the diversification of the angiosperms (Darwin, 1876; Grant, 1949; Stebbins, 1970; Kiestler *et al.*, 1984; Coyne and Orr, 2004). However, direct evidence for pollinator preferences driving plant speciation is hard to come across. A recent study based on the well-studied *Mimulus* (Lamiales) system showed that pollinator preferences can drive ecological speciation in flowering plants under the right conditions (Gegeer and Burns, 2007). The diversity of plant–pollinator interactions in orchids is unrivalled by any other group of plants, and as such the family offers numerous possibilities to test these predictions and for comparative research into the effects of pollinators on diversification.

Knowledge of the orchid mycorrhizal symbiosis has accelerated greatly in the last decade, and some claim that this association may also drive diversification in the group

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(Otero and Flanagan, 2006). In this review, recent work in the still separate fields of pollination and mycorrhizas is summarized and it is suggested that orchids offer a unique system with which to improve and integrate our knowledge of symbiosis and test its effects upon diversification.

Insects and orchids

Despite the great diversity of Orchidaceae and the wealth of plant–animal interactions in the family, orchids are underrepresented in studies of speciation and reproductive isolation (Peakall, 2007). The spectacular floral diversity of orchids, and the fact that many are pollinated by a single species (Paulus and Gack, 1990), has led to an historic belief that pollinator isolation is the predominant reproductive barrier in the family (van der Pijl and Dodson, 1966; Gill, 1989). However, it has often proved difficult to provide direct evidence for this view, in part because other isolating barriers such as habitat preferences, differing phenologies, and post-zygotic barriers are also often in place (Dressler, 1981; Coyne and Orr, 2004). Identifying the first isolating barrier to evolve is a difficult but essential task for studying the primary factors involved in speciation (Coyne and Orr, 2004). Recent work has increased our knowledge of which isolating barriers were likely to have initially caused divergence in specific groups of orchids. In particular, a recent study has compared reproductive barriers in two groups of orchids pollinated via different forms of deception (Scopece *et al.*, 2007), as described later.

The frequency of pollination by deception is an unusual aspect of orchid biology. The ability to attract pollinators without offering them a reward has evolved independently in many angiosperm lineages, but usually in only a few species per family (Renner, 2006). In contrast, it has been estimated that around one-third of orchid species are pollinated by ‘food-deception’ (Ackerman, 1986; Nilsson, 1992). This is the attraction of pollinators by signalling the presence of a food reward, such as nectar or pollen, without ever providing the reward. These orchid species may resemble a specific co-occurring rewarding species (Johnson, 2000). More often, food-deceivers rely on a general resemblance to food-rewarding species with large bright flowers that exploit innate searching preferences of pollinators (Nilsson, 1992; Schiestl, 2005). A less common, but fascinating, form of deception occurs in sexually-deceptive species. Here, flowers mimic the mating signals of female insects, primarily using floral odours that mimic female insect pheromones, and also visual or tactile cues (Cozzolino and Widmer, 2005; Schiestl, 2005). The plant is pollinated by deceived male insects as they attempt to copulate with the flower. This strategy is known exclusively from orchids, and is thought to have evolved independently on multiple occasions

within the family (Cozzolino and Widmer, 2005). At least 400 species of sexually deceptive orchids are known in several genera, including *Ophrys* in Europe (Borg-Karlson, 1990), *Disa* in southern Africa (Steiner *et al.*, 1994), nine genera of Australian orchids (Kores *et al.*, 2001), and several South American genera (Singer, 2002; Singer *et al.*, 2004; Blanco and Barboza, 2005).

In a recent study, Scopece *et al.* (2007) assessed pre-mating and post-mating isolation barriers between pairs of European food-deceptive orchids from the genera *Anacamptis*, *Dactylorhiza*, *Neotinea*, and *Orchis*; and between sexually-deceptive orchids from the genus *Ophrys*. Species with overlapping distributions and phenology were chosen such that pollinator specificity appeared to be the only likely pre-mating isolation barrier. They found weak pre-mating isolation and strong post-mating isolation among the food-deceptive orchids, and the opposite relationship in sexually-deceptive species. Examination of pollinator lists showed that food-deceptive orchids were pollinator generalists, all being pollinated by more than one insect species, and with substantial sharing of pollinators. In contrast, no pollinator overlap was found in the sexually-deceptive orchids, with the data suggesting that each species is pollinated by a single insect that does not pollinate any other species. However, the authors note that pollinator literature for these species is often unreliable, sometimes based on few observations. It should also be noted that sharing of pollinators does not necessarily indicate weak pre-mating isolation. In pollination guilds of oil-secreting (i.e., rewarding) orchids in southern Africa, numerous species share the same pollinator, but inter-species crosses are prevented by placement of pollinia on different parts of the pollinating bee’s body (Pauw, 2006). However, the differing levels of specificity among these orchids are well-documented and studies using these data can still provide valuable information. For instance, experimental orchid crosses were used to investigate post-mating isolation (Scopece *et al.*, 2007). This revealed that food-deceptive species had strong post-mating barriers with low fruit set and low viable seed production. In contrast, sexually-deceptive species were largely interfertile with relatively high proportions of viable seeds produced. A study of a sexually-deceptive species complex of *Chiloglottis* in Australia found a similar pattern of weak post-mating barriers and strong pre-mating isolation (Mant *et al.*, 2005a).

Scopece *et al.* (2007) also investigated correlations between reproductive isolation mechanisms and genetic distance between species. This showed that for food-deceptive orchids, but not sexually-deceptive orchids, post-zygotic isolation was correlated with genetic distance, indicating that post-zygotic isolation increased in a clock-like manner in the time since divergence. These results allow various inferences to be made about the evolution of diversity in these two groups. The genetics of

post-zygotic barriers can be complex, involving many genes (Wu, 2001), and their regular, clock-like evolution in food-deceptive orchids suggests a gradual accumulation of incompatibilities between incipient species. This is most likely to occur between populations separated by geographical barriers that preclude gene flow. If enough incompatibilities arise during a period of allopatry, then diverging populations may subsequently be able to co-occur in sympatry as reproductively isolated species. Cozzolino *et al.* (2004) demonstrated that co-existing Mediterranean orchids with generalist pollinator attraction are more likely to have divergent karyotypes than orchids with specific pollinators. Another recent study of European food-deceptive orchids supports this finding: Moccia *et al.* (2007) showed that although F₁ hybrids were readily formed between the sister species *Anacamptis morio* and *Anacamptis papilionacea*, there was no formation of F₂ or backcross hybrids in the wild. It is likely that this was primarily due to chromosome number differences between these two species ($2n=36$ and $2n=32$, respectively). In addition, lower than average pollinator visitation to hybrids was also detected; this may act to strengthen reproductive isolation. An informative area of future research may be to use experimental crosses to investigate post-zygotic barriers among geographically isolated populations of a species, or recently diverged species occurring in allopatry, to test whether a clock-like accumulation of post-zygotic incompatibilities can also be demonstrated.

The absence of post-mating barriers in sexually-deceptive orchids suggests a major role for pre-mating barriers in diversification. In *Ophrys*, differing levels of particular alkenes, which resemble female pollinator pheromones, appear to be responsible for pollinator specificity (Schiestl and Ayasse, 2002; Mant *et al.*, 2005b). In a sympatric population of *Ophrys* species, Mant *et al.* (2005d) revealed high levels of differentiation of active odour compounds corresponding to differences in specific pollinators. In contrast, differentiation of inactive odour compounds and neutral genetic markers between species was weak. This indicates that strong divergent selection for specific pollinator attraction has acted upon these active compounds, rather than simply genetic drift. A similar combination of well-differentiated active compounds and weak differentiation of inactive compounds was found in two sympatric species of *Gymnadenia* (Huber *et al.*, 2005). Here, both species were nectar-producing and attracted a range of Lepidopteran pollinator species (up to 37 species in *G. odoratissima*), but differentiation of active odour compounds still led to zero overlap of pollinator species between *G. conopsea* and *G. odoratissima*. In combination with other floral traits, such as spur length and colour, this has led to almost complete pre-mating isolation between these two species. In sexually-deceptive *Chiloglottis*, a single odour com-

pound is responsible for attracting male thynnine wasps (Schiestl *et al.*, 2003). Small changes in the functional group of this compound may be responsible for the species-specific attraction of wasps shown by *Chiloglottis* (Mant *et al.*, 2002). It has been shown in other plant groups that mutations in a few genes with major phenotypic effect can substantially change flower structure and attractiveness to different pollinators (Bradshaw *et al.*, 1998; Bradshaw and Schemske, 2003; Hoballah *et al.*, 2007). It is therefore easy to imagine that mutations causing small structural changes in active compounds or regulating expression levels of alkene genes could lead to the attraction of a new pollinator and rapid reproductive isolation. Field studies of *Chiloglottis* have found that wasps other than the specific pollinator are attracted by floral odours, but do not attempt copulation and so do not pollinate them (Bower, 1996). The occurrence of these 'minor responders' demonstrates the potential of *Chiloglottis* to use new pollinators. If selection is strong enough, it is possible that this can happen even in the presence of gene flow (Mant *et al.*, 2005d; Gegear and Burns, 2007). This has led some authors to suggest that sympatric speciation is likely among sexually-deceptive orchids (Borg-Karlson *et al.*, 1993; Schiestl and Ayasse, 2002; Mant *et al.*, 2005a; Schiestl, 2005). In the genera *Ophrys*, *Chiloglottis*, and *Caladenia*, species appear to be reproductively isolated due to specific preferences for different pollinators, and phylogenetic studies of all three genera show very little morphological or molecular differentiation between species (Kores *et al.*, 2001; Soliva *et al.*, 2001; Mant *et al.*, 2002; Bateman *et al.*, 2003). This could be due to rapid, recent speciation in these clades. In food-deceptive orchids, greater genetic distance has been found between species (Aceto *et al.*, 1999; Bateman *et al.*, 2003). However, even micro-satellite and AFLP markers appear to be shared among taxa in *Ophrys* (Mant *et al.*, 2005d) and *Chiloglottis* (Mant *et al.*, 2005e), respectively. This may be a consequence of extremely recent diversification with the sharing of molecular variation attributed to lasting ancestral polymorphisms or it could be evidence of ongoing gene flow across species boundaries (Mant *et al.*, 2005e; Schiestl, 2005). New variable micro-satellite loci have recently been described for *Chiloglottis* (Flanagan *et al.*, 2006) and further population genetics studies using these may help to resolve these issues. Studies comparing modes of reproductive isolation, such as Scopece *et al.* (2007), can assess patterns of speciation in large related groups and the role of pollinator specificity in plant speciation. Comparisons with other groups of orchids will show whether this is a general pattern.

Pollinator deception has evolved on multiple occasions in the Orchidaceae (Johnson *et al.*, 1998; Cameron *et al.*, 1999; Kores *et al.*, 2001; Bateman *et al.*, 2003) where it appears to be a highly successful evolutionary strategy.

However, both food and sexual deceit are associated with low fruit set [66.5% fruiting failure in deceptive orchid species compared to 29.1% fruiting failure in rewarding species (Tremblay *et al.*, 2005)] and populations often appear to be extremely pollinator-limited (Neiland and Wilcock, 1998; Tremblay *et al.*, 2005). This paradox can be explained by improved out-crossing and longer distance pollen flow. Insects often spend a longer time on rewarding plants and tend to visit more flowers from the same plant and neighbouring plants in a patch. In deceptive orchids, the pollinator often immediately leaves the patch upon discovering the lack of reward (Peakall and Beattie, 1996; Ayasse *et al.*, 2000; Johnson, 2000). This effect is seen even more clearly in sexually-deceptive orchids (Peakall and Beattie, 1996; Peakall and Schiestl, 2004). This behaviour increases the chances of out-crossing and long-distance pollen flow as pollinators are more likely to transfer pollen to an orchid in a different patch (Peakall and Beattie, 1996). Experiments in which nectar was artificially added to the food-deceptive *Anacamptis morio* showed an increase in pollinator visits, but also an increase in the amount of geitonogamous mating, where flowers are pollinated by pollen from another flower of the same plant (Smithson, 2002; Johnson *et al.*, 2004). This effect is likely to be particularly strong among orchids as their pollen is packaged into bundles called pollinia. Once pollinia become attached to a pollinator, fertilization can be very efficient, making numerous geitonogamous pollinations more likely. Geitonogamous mating not only causes inbreeding but affects male fitness by reducing the amount of pollen available for out-crossing (Johnson and Nilsson, 1999; Johnson *et al.*, 2004). Orchids show many adaptations to avoid inbreeding, and the advantages of out-crossing may compensate for the severe pollinator limitation and low fruit-set commonly found in deceptive orchids (Gill, 1989). Out-crossed plants have been shown to produce greater quantities of viable seeds than self-crossed plants (Aceto *et al.*, 1999; Ellis and Johnson, 1999; Borba *et al.*, 2001; Bowles *et al.*, 2002; Wallace, 2003).

Pollinator limitation appears to be extremely common among orchids (Ackerman, 1989; Calvo, 1993; Tremblay *et al.*, 2005; Pauw, 2007). In a thorough survey of orchid reproductive biology, Tremblay *et al.* (2005) showed that although resource constraints can sometimes affect orchids (Montalvo and Ackerman, 1987) the majority of evidence pointed to pollinator limitation as the dominant limiting factor for reproductive success. A thorough literature survey of studies in which flowers were subject to supplementary cross-pollination showed an increase in fruit-set compared with natural populations in 56 out of 57 orchid species (Tremblay *et al.*, 2005). Tremblay *et al.* (2005) argued for a drift–selection model of orchid speciation, as a consequence of general reproductive patterns in Orchidaceae. Reproductive success in orchids

is highly skewed: very often it is found that few individuals produce fruit, and complete fruiting failure in a reproductive season can be fairly common. Coupled with long-distance dispersal (facilitated by wind-dispersed dust seeds that can potentially travel hundreds of kilometres (Gandawijaja and Arditto, 1983)) this could lead to the formation of many separate founder populations. Genetic drift alone could lead to substantial differences between populations. Tremblay *et al.* (2005) argue that effective population sizes (N_e) in orchid populations are generally low. In such a scenario, genetic drift between populations coupled with intense directional selection could lead to speciation. This hypothesis assumes that populations have low N_e , low or infrequent gene flow, heritable variation, and high or consistent natural selection within populations. These assumptions should be testable among different groups of orchids to investigate whether this is a common pattern of speciation. Small effective population size and low genetic drift has been demonstrated in some tropical epiphytic species of *Lepanthes* (Tremblay and Ackerman, 2001). Among sexually-deceptive orchids, Mant *et al.* (2002) showed highly congruent phylogenies between *Chiloglottis* and its thynine wasp pollinators, suggesting that these orchids had undergone episodic selection for different pollinators leading to speciation.

Critics of Tremblay *et al.*'s hypothesis argue that although pollinator limitation does seem to be a general rule in Orchidaceae, the same may not be true of small effective population sizes. Large effective population sizes and high gene flow have been found between populations of European and Australian orchids (Peakall and Beattie, 1996; Soliva and Widmer, 2003; Forrest *et al.*, 2004; Mant *et al.*, 2005d). These studies are primarily on deceptive orchids of Europe and Australia, and deceptive pollination may act to increase out-crossing and gene flow. Here it seems unlikely that populations would diverge enough through genetic drift and local adaptation for cladogenesis to occur, in the face of high gene flow. However, the drift–selection theory may still be relevant to large groups of orchids and warrants further testing. Terrestrial orchid groups with high levels of clonality, such as some species of *Microtis* in Australia (Peakall and Beattie, 1991) and Coryciinae in southern Africa, may harbour isolated founder populations with low migration. Further studies using molecular markers specifically to test for conditions such as low gene flow and strong founder effects, and investigating the selection pressures for pollination acting on different populations would be useful.

Are epiphytes different? Epiphytic orchid species may represent an ideal group on which to test the assumptions of this drift–selection model. Epiphytes represent an understudied group considering that two-thirds of Orchidaceae are presumed to live in tree canopies (Gravendeel *et al.*,

2004). This high epiphytic diversity is shared by other groups of epiphytes such as aroids and bromeliads. Benzing (1990) considered that the fragmented nature of the epiphytic environment allowed allopatric speciation to drive this diversity. Others have considered pollinator specialization to be the main driver of epiphyte diversity (Tremblay, 1992); from available pollination information for epiphytic orchids it appears that two-thirds are pollinated by a single pollinator and less than 5% could be considered generalists. However, as noted earlier, pollinator information indicating specialization can often be unreliable when based on casual observations of a few individuals (Ollerton and Cranmer, 2002). A drift–selection model of speciation would appear to be highly plausible for epiphytic species. The literature suggests that effective population sizes are small and gene flow potentially low among epiphytes (Ackerman and Galarza-Perez, 1991; Tremblay, 1997; Tremblay and Ackerman, 2001; Tremblay *et al.*, 2005). Vasquez *et al.* (2003) noted that the long-distance dispersal of orchid dust seeds led to the formation of numerous widely separated founder populations. However, Kessler (2002) found that bromeliad taxa with long-distance dispersal were less diverse than plant taxa with short-distance dispersal, which he attributed to greater gene flow among taxa that rapidly colonize local areas. It may be that unique aspects of orchid biology promote the formation of founder populations with low effective population size. Most orchids are self-compatible and geitonogamous pollination is often highly likely in small populations; these factors may facilitate the establishment of new lineages. It has been suggested that the epiphytic condition itself could be the driver of speciation, perhaps due to the high number of variable niches. Gravendeel *et al.* (2004) used a phylogenetic analysis of epiphytes and non-epiphytes to show that diversity was higher in epiphytic than non-epiphytic species. They also used pollinator information to show that pollinator specialization was not associated with species richness. However, again, the pollinator information was basic, and studies focused on clades of epiphytes with well-known pollinator interactions are needed. Detailed pollinator studies, combined with phylogenetic and population genetic analyses, as in studies of European and Australian deceptive species, are needed to investigate diversification of other large groups of orchids such as epiphytes.

Fungi and orchids

Mycorrhizas are widespread intimate symbioses between members of three fungal phyla and the vast majority of plants. The symbiosis is generally a mutualistic one, with fungi providing the plant with soil nutrients in exchange for organic carbon assimilated by photosynthesis (Smith

and Read, 1997). The most widespread of these symbioses are the ancestral arbuscular mycorrhizas involving members of the Glomeromycota and the more recent and repeatedly evolved ectomycorrhizas involving members of the Ascomycota, Basidiomycota, and many woody plants. In nature, the formation of a mycorrhizal symbiosis is typically an obligate step in the completion of the fungal and plant life cycles. Orchids have a unique mycorrhizal relationship that was first documented over a century ago (Bernard, 1902), but difficulties associated with studying the fungi forming orchid mycorrhizas hampered research over the subsequent decades. Orchid mycorrhizal fungi have generally been classified as belonging to rhizoctonia-forming fungi, a polyphyletic group of fungi from three basidiomycete families (Sebacinaceae, Ceratobasidiaceae, and Tulasnellaceae) (Roberts, 1999). Fungal taxonomy is largely based on the morphology of sexual structures, but rhizoctonia-forming fungi rarely fruit in axenic culture and are difficult to identify using vegetative characteristics alone. Furthermore, most mycorrhizal fungi have proven unculturable in the absence of a plant host. Hence, questions relating to the identities of many orchid mycorrhizal fungi and the levels of specificity for fungal partners among orchids have long been controversial (Curtis, 1939; Hadley and Purves, 1974; Warcup, 1981; Clements, 1988; Masuhara and Katsuya, 1994). The advent of fungal molecular systematics and ecology revolutionized the study of mycorrhizas by allowing direct identification of fungi without axenic isolation.

Rhizoctonia-forming fungi are often saprophytes or plant pathogens so, unlike the fungi that form arbuscular mycorrhizas and ectomycorrhizas, they are not obligately mycorrhizal. The distributions of orchid mycorrhizal fungi have been shown to be independent of orchids (Brundrett *et al.*, 2003; Feuerherdt *et al.*, 2005); the ability to utilize otherwise free-living fungi in mycorrhizal symbioses appears to be a unique characteristic of orchids. Another characteristic trait of the orchid family is the exceptionally prolific production of tiny dust-like seeds (microspermy) that can engage in mycorrhizal interactions during the earliest stages of germination. Recruitment limitation is of paramount importance in orchid biology; in his book on orchid pollination, Charles Darwin (1887) estimated that if the germination of viable seeds went unchecked an orchid plant could ‘clothe with one uniform green carpet the entire surface of the land throughout the globe’ in only three generations. Each orchid seed is miniscule [lengths as small as 0.05 mm in *Anoectochilus imitans* (Arditti and Ghani, 2000)] and has minimal nutritional reserves; it is essential for the germinating seed to undergo mycorrhization with an appropriate fungal partner in order to grow. Otherwise, dormancy periods of up to several years can occur in some species (Whigham *et al.*, 2006). Upon germination, fungal hyphae penetrate the cell walls of the orchid and form characteristic coils, called pelotons,

within the cells. Growth of the fungus is restricted to cortical cells (Peterson *et al.*, 1998), probably by the deposition of phenolics (Beyrle *et al.*, 1995) and the production of anti-fungal compounds (Shimura *et al.*, 2007). Pelotons are subsequently 'digested', and through this process the orchid is thought to receive the essential nutrients and carbon that it needs to grow. Nutrient exchange may also, or instead, occur across intact cell membranes prior to 'digestion' as in other intracellular mycorrhizas. The germinated seed grows into a mass of differentiated cells called a protocorm, and remains in this form for a period that can extend up to several years, until leaves are produced. During this period of their life, many orchids are underground and rather than producing carbon through photosynthesis like most autotrophic plants, they obtain all of their energy from fungal pelotons. Therefore, before the production of leaves, all orchids go through a stage of their life-cycle in which they are myco-heterotrophs (Leake, 1994), rather than autotrophs.

Most adult orchids retain their mycorrhizal partnerships, and due to their characteristically poorly developed root systems, they are thought still to be heavily reliant on mycorrhizal fungi for mineral nutrition (Smith and Read, 1997). In contrast to other mycorrhizal symbioses, it has long been thought that orchid mycorrhizal fungi receive few benefits from the interaction (Hadley and Purves, 1974; Alexander and Hadley, 1985; Smith and Read, 1997). However, a recent study demonstrated bi-directional movement of carbon between adult *Goodyera repens* and its fungal partner (Cameron *et al.*, 2006). This study conducted on orchids growing in agar microcosms and the relevance of these results to orchids growing in natural conditions remains to be determined. The presence of fungi in albino variants of green orchids (Selosse *et al.*, 2004; Julou *et al.*, 2005) and the fact that some orchids undergo prolonged periods of underground dormancy (Shefferson *et al.*, 2007) suggests that the fungi are not reliant on carbon derived from orchid photosynthesis. The overall benefits and costs to fungi of associating with orchids remains debatable due to difficulties in quantifying fungal fitness in natural conditions.

Some orchid species have lost the ability to photosynthesize and remain entirely myco-heterotrophic as adults. This mode of nutrition has evolved in several plant families but is most common in Orchidaceae, with over 100 such species known, probably due to the obligate myco-heterotrophy of all orchid seedlings (Leake, 1994). Phylogenetic analyses have revealed that the loss of photosynthesis in adult plants may have occurred independently at least 20 times in the family (Molvray *et al.*, 2000). Our understanding of these intriguing plants has increased greatly in recent years due to the application of molecular techniques for identifying the fungi involved. This has revealed that many myco-heterotrophic orchids exhibit high specificity for their fungal partners, and rather

than free-living rhizoctonia-forming fungi, these are often fungi that form ectomycorrhizal relationships with trees. In this way, the orchid taps into and exploits ectomycorrhizal networks between the fungus and other neighbouring plant species, and they can therefore be considered epiparasites of those plants. Examples include *Neottia nidus-avis*, which is specialized on fungi in the Sebacinaceae known to be ectomycorrhizal with trees (McKendrick *et al.*, 2002; Selosse *et al.*, 2002) and *Corallorhiza maculata* and *C. mertensiana*, which are specialized on ectomycorrhizal members of the Russulaceae (Taylor and Bruns, 1999; Taylor *et al.*, 2004). In addition, other non-photosynthetic orchids specialise on free-living non-rhizoctonia fungi (Ogura-Tsujita and Yukawa, 2008).

Some green orchids capable of photosynthesis also exploit ectomycorrhizal mutualisms to supplement their carbon supply. This combined form of nutrition has been termed partial myco-heterotrophy or mixotrophy. Both green and albino varieties of *Epipactis microphylla* were found to associate with ectomycorrhizal fungi from the tree-ectomycorrhizal ascomycete genus *Tuber* (Selosse *et al.*, 2004). Stable isotopic compositions of photosynthetic orchids in the tribe Neottieae, that are subject to low light levels on forest floors, revealed that they obtain large amounts of their carbon from associations with ectomycorrhizal fungi (Gebauer and Meyer, 2003; Bidartondo *et al.*, 2004; Julou *et al.*, 2005). Girlanda *et al.* (2006) demonstrated that *Limodorum abortivum* has inefficient photosynthetic capabilities and supplements its carbon supply by associating with ectomycorrhizal *Russula* species. These findings indicate that a shift to exploiting ectomycorrhizal fungi precedes the loss of photosynthetic ability in some orchids. It has been hypothesized that the switch from rhizoctonia-forming fungi may have occurred because ectomycorrhizal fungi represent a more stable and reliable source of carbon for orchids with limited or no means of obtaining their own (Rasmussen, 2002).

High mycorrhizal specificity seems to be the rule in fully myco-heterotrophic species, but the specificity of photosynthetic orchids may vary. Some early research indicated that specificity was low as seeds were regularly able to germinate with a range of fungi *in vitro* (Curtis, 1939; Hadley, 1970). However, it appears that orchids may be capable of germinating with a much broader range of fungi under laboratory conditions than in the field (Masuhara and Katsuya, 1994). Several studies have found photosynthetic orchids associating with a narrow range of fungi over large geographic areas, indicating narrow specificity (Shefferson *et al.*, 2005, 2007; McCormick *et al.*, 2006; Bonnardeaux *et al.*, 2007; Irwin *et al.*, 2007). The photosynthetic orchid *Liparis lilifolia* was found to associate with fungi with extremely low genetic variability: only two bases in the highly variable ITS region differed between fungal associates throughout its range (McCormick *et al.*, 2004). Epiphytic orchids also appear

to be capable of high specificity (Otero *et al.*, 2002; Ma *et al.*, 2003; Suarez *et al.*, 2006). On the other hand, most of these studies have also identified orchids that are mycorrhizal generalists, associating with a wide range of fungi. Even closely related orchid species appear to have varying levels of specificity (Otero *et al.*, 2004; Shefferson *et al.*, 2007). The reasons for these different levels of specificity are not clear. There is some evidence that mycorrhizal generalist orchids appear to be capable of growing in a wider variety of habitats: two widespread weedy orchids studied in Australia were found to be compatible *in vitro* with a greater variety of fungi than orchids with narrower distributions (Bonnardeaux *et al.*, 2007). However, in a comparative study of *Cypripedium* species, *C. calceolus* had one of the narrowest ranges of fungi, but it was the most widespread species (Shefferson *et al.*, 2007).

The generally high mycorrhizal specificity of orchids towards fungi compared to other mycorrhizal symbioses, and of fully myco-heterotrophic orchids in particular, leads to the idea that mycorrhizal specificity is linked to the putatively one-sided nature of the orchid–fungus relationship. If the fungi receive little or no benefit from the symbiosis, as some evidence suggests, then the orchids might be considered parasites. Parasites often exhibit high specificity due to selection driven by evolutionary arms-races (Price, 1980; Taylor and Bruns, 1997), and it is conceivable that selection for the ability to recruit and manipulate otherwise free-living fungi into a mycorrhizal relationship may require specialization. Fungal specificity is a feature of other plant groups that exploit fungi for carbon (e.g. see Bidartondo *et al.*, 2002). Girlanda *et al.* (2006) speculated that the degree of specificity may be correlated with the degree of heterotrophy. Some orchids with narrow fungal specificity have been shown to have more efficient *in vitro* seed germination (Bonnardeaux *et al.*, 2007). Studies using stable carbon and nitrogen isotope signatures have greatly aided our understanding of myco-heterotrophic mycorrhizas (Gebauer and Meyer, 2003; Bidartondo *et al.*, 2004), and may provide insights into the reasons for differing levels of specificity in green orchids.

Although the degree of specificity varies among orchid mycorrhizas, narrow orchid specificity towards fungi (and no fungal specificity towards orchids) appears to be common, and it is likely to have major impacts on the ecology and distribution of orchids (Rasmussen and Whigham, 1998). It has also been suggested to affect orchid diversity (Otero and Flanagan, 2006; Shefferson *et al.*, 2007). Three closely related taxa in the myco-heterotrophic *Hexalectris spicata* complex were found to associate with closely related, but distinct fungi, suggesting that mycorrhizal specificity may have played a role in phylogenetic divergence (Taylor *et al.*, 2003). A similar result was found for sister species of *Corallorhiza*, each

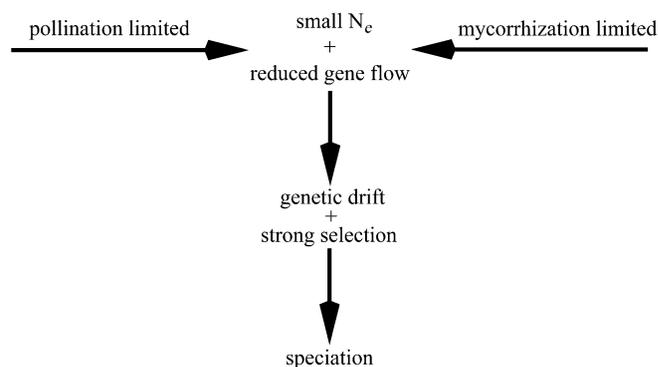


Fig. 1. A drift–selection model (sensu Tremblay *et al.*, 2005) postulates that orchids are primarily pollination limited. Thus, skewed reproductive success and small effective population sizes lead to rapid genetic drift and natural selection episodes. If orchids are mycorrhization limited, this could cause severe recruitment bottlenecks that exacerbate drift–selection episodes. For instance, specialization for pollinators (leading to limited pollination, reproductive skew, and low fruit production) and mycorrhizal fungi (leading to recruitment limitation, limited geographic distribution, and isolated hyper-dispersed populations) contribute to small effective population sizes in orchid species and reduced gene flow. This combination creates conditions suitable for genetic drift between orchid populations. Followed by strong, episodic selection, this can ultimately cause sufficient differentiation and incipient orchid speciation.

being found to associate with non-overlapping clades of Russulaceae (Taylor and Bruns, 1999). Perhaps the most remarkable example comes from a study of different genotypes of *C. maculata*, which detected sympatric orchid genotypes associating specifically with distinct subclades of fungi (Taylor *et al.*, 2004). In fact, this was the first demonstration of a geographic mosaic of co-evolution in mycorrhizas (Thompson 2005). Fitness was found to vary *in vitro* across different fungus–orchid combinations in *Tolumnia variegata*, suggesting potential for natural selection to act on these relationships (Otero *et al.*, 2005). These results certainly indicate that mycorrhizal specialization can evolve rapidly, but what role it plays in orchid diversification remains unclear. Unlike switches in specific pollinators, a direct link between mycorrhizal specificity and reproductive isolation is hard to envision. Local adaptation to different fungi does not appear to be occurring as closely related orchids adapted to different fungi have overlapping ranges and are found co-occurring (Taylor *et al.*, 2003, 2004; Otero *et al.*, 2004).

Mycorrhizal specificity may help drive diversity indirectly by determining patterns of orchid distribution. Patchy fungal distributions, together with high mycorrhizal specificity could be responsible for the hyper-dispersed populations common to many orchids (Otero *et al.*, 2005; Otero and Flanagan, 2006). Despite the production of tiny wind-borne seeds, orchid dispersal distances are generally small (Diez, 2007; Jacquemyn *et al.*, 2007), and long-range dispersal is likely to be a rare, albeit important

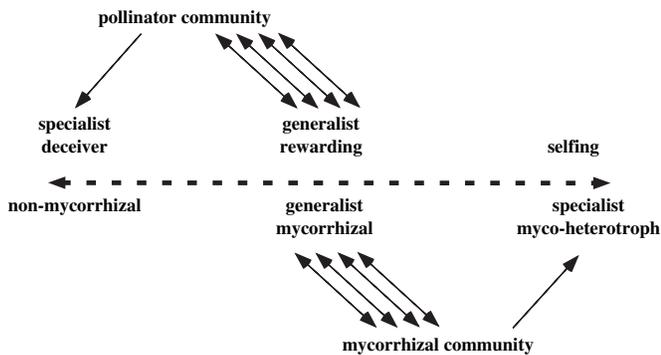


Fig. 2. A potential trade-off between above- and below-ground symbioses shown as a single symbiotic continuum (dashed). Specialization for both symbioses may limit ecological opportunities and be evolutionarily unstable, leading to a trade-off between specialization and generalization above- and below-ground. Most plant species are generalist mutualists towards both pollinators and mycorrhizal fungi (centre). Some plant species cheat their pollinators by withholding rewards and they are non-mycorrhizal (extreme left). Other plant species cheat their mycorrhizal fungi by failing to provide photosynthates and they are selfers (extreme right). Mutualists and cheater plants interact with subsets of the available community of symbionts.

event. This may result in small effective population sizes and reduced gene flow, creating ideal conditions for the drift–selection model of orchid speciation proposed by Tremblay *et al.* (2005). In this way, the orchid–fungi mycorrhizal relationship could increase the potential for rapid speciation in the Orchidaceae (Fig. 1).

Conclusions

Our knowledge of orchid mycorrhizal fungi and the role of pollinators in generating orchid diversity have increased greatly in recent years, although accurate assessments of pollinator and mycorrhizal ranges in nature remain challenging. New tools in molecular ecology have provided fresh insights into the diversity of orchid mycorrhizal fungi, the amount of differentiation between populations, and the specific scent preferences of insect pollinators. Aspects of orchid biology other than their remarkable symbiotic associations have undoubtedly played a role in orchid diversification. However, orchids do show a remarkable tendency towards specialized deceptive interactions both above- and below-ground that is likely to have had a major influence on the evolution of the group, warranting additional research in these fields. For example, are there trade-offs between specialization and deception between the two symbioses? (Fig. 2).

Some of the largest gaps in our knowledge of orchid biology relate to the animal and fungal partners. Information on the distributions of insect pollinators is often lacking for even the best studied systems (Mant *et al.*, 2005c). Evidence suggests that different populations of insect species pollinating sexually-deceptive orchids have

differing pheromone preferences, but studies examining intra-specific variation in insect pheromone chemistry are rare. Population genetics studies of both pollinators and orchids might be needed to understand fully the diversification among these orchids. Even less is known of the ecology of orchid mycorrhizal fungi. Their full distributions are currently difficult to detect, as are the fungal fitness benefits or costs of their associations with orchids. The molecular physiology of orchid mycorrhizal interactions has been the subject of relatively few studies compared with other types of mycorrhiza (Dearnaley, 2007). Further attention to insect and fungal ecology will be invaluable for studies of orchids.

The drift–selection pollination model proposed by Tremblay *et al.* (2005) encompasses aspects of both pollinator and mycorrhizal biology. Pollinator preferences are a probable cause of episodic selection and reproductive isolation. Specific mycorrhizal associations are likely to lead to isolated populations with small N_e and reduced gene flow where genetic drift will be an important factor. This theory remains to be tested. Studies combining analysis of both orchid symbiotic associations may provide the greatest insights into how symbioses drive diversification.

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References

- Aceto S, Caputo P, Cozzolino S, Gaudio L, Moretti A. 1999. Phylogeny and evolution of *Orchis* and allied genera based on ITS DNA variation: morphological gaps and molecular continuity. *Molecular Phylogenetics and Evolution* **13**, 67–76.
- Ackerman JD. 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* **1**, 108–113.
- Ackerman JD. 1989. Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). *Systematic Botany* **14**, 101–109.
- Ackerman JD, Galarza-Perez M. 1991. Patterns and maintenance of extraordinary variation in the Caribbean orchid, *Tolumnia (Oncidium) variegata*. *Systematic Botany* **16**, 182–194.
- Alexander C, Hadley G. 1985. Carbon movement between host and mycorrhizal endophyte during the development of the orchid *Goodyera repens* Br. *New Phytologist* **101**, 657–665.
- Arditti J, Ghani AKA. 2000. Tansley review No. 110: Numerical and physical properties of orchid seeds and their biological implications. *New Phytologist* **145**, 367–421.
- Ayasse M, Schiestl FP, Paulus HF, Lofstedt C, Hansson B, Ibarra F, Francke W. 2000. Evolution of reproductive strategies in the sexually deceptive orchid *Ophrys sphegodes*: how does flower-specific variation of odor signals influence reproductive success? *Evolution* **54**, 1995–2006.
- Bateman RM, Hollingsworth PM, Preston J, Yi-Bo L, Pridgeon AM, Chase MW. 2003. Molecular phylogenetics and

- evolution of Orchidinae and selected Habenariinae (Orchidaceae). *Botanical Journal of the Linnean Society* **142**, 1–40.
- Benzing DH.** 1981. Why is Orchidaceae so large, its seeds so small, and its seedlings mycotrophic? *Selbyana* **5**, 241–242.
- Benzing DH.** 1990. Epiphytism: a preliminary overview. In: Ashton PS, Hubbell SP, Janzen DH, Marshall AG, Raven PH, Tomlinson PB, eds. *Vascular epiphytes. General biology and related biota*. Cambridge University Press, 1–42.
- Bernard N.** 1902. Études sur la tubérisation. *Revue Générale de Botanique* **14**, 1–92.
- Beyrle HF, Smith SE, Peterson RL, Franco CMM.** 1995. Colonization of *Orchis morio* protocorms by a mycorrhizal fungus: effects of nitrogen nutrition and glyphosate in modifying the responses. *Canadian Journal of Botany* **73**, 1128–1140.
- Bidartondo MI, Redecker D, Hijri I, Wiemken A, Bruns TD, Domínguez L, Sérsic A, Leake JR, Read DJ.** 2002. Epiparasitic plants specialized on arbuscular mycorrhizal fungi. *Nature* **419**, 389–392.
- Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ.** 2004. Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 1799–1806.
- Blanco MA, Barboza G.** 2005. Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: Pleurothallidinae) by fungus gnats. *Annals of Botany* **95**, 763–772.
- Bonnardeaux Y, Brundrett M, Batty A, Dixon K, Koch J, Sivasithamparam K.** 2007. Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. *Mycological Research* **111**, 51–61.
- Borba EL, Semir J, Shepherd GJ.** 2001. Self-incompatibility, inbreeding depression and crossing potential in five Brazilian *Pleurothallis* (Orchidaceae) species. *Annals of Botany* **88**, 89–99.
- Borg-Karolson AK.** 1990. Chemical and ethological studies of pollination in the genus *Ophrys* (Orchidaceae). *Phytochemistry* **29**, 1359–1387.
- Borg-Karolson A, Groth I, Ågren L, Kullenberg B.** 1993. Form-specific fragrances from *Ophrys insectifera* L. (Orchidaceae) attract species of different pollinator genera. Evidence of sympatric speciation? *Chemoecology* **4**, 39–45.
- Bower CC.** 1996. Demonstration of pollinator-mediated reproductive isolation in sexually deceptive species of *Chiloglottis* (Orchidaceae: Caladeniinae). *Australian Journal of Botany* **44**, 15–33.
- Bowles ML, Jacobs KA, Zettler LW, Delaney TW.** 2002. Crossing effects on seed viability and experimental germination of the federal threatened *Platanthera leucophaea* (Orchidaceae). *Rhodora* **104**, 14–30.
- Bradshaw HD, Otto KG, Frewen BE, McKay JK, Schemske DW.** 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (*Mimulus*). *Genetics* **149**, 367–382.
- Bradshaw HD, Schemske DW.** 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**, 176–178.
- Brundrett MC, Scade A, Batty AL, Dixon KW, 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.
- Calvo RN.** 1993. Evolutionary demography of orchids: intensity and frequency of pollination and the cost of fruiting. *Ecology* **74**, 1033–1042.
- Cameron DD, Leake JR, Read DJ.** 2006. Mutualistic mycorrhiza in orchids: evidence from plant–fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. *New Phytologist* **171**, 405–416.
- Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Yukawa T, Hills HG, Goldman DH.** 1999. A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. *American Journal of Botany* **86**, 208–224.
- Clements MA.** 1988. Orchid mycorrhizal associations. *Lindleyana* **3**, 73–86.
- Coyne JA, Orr HA.** 2004. *Speciation*. Sunderland: Sinauer Associates.
- Cozzolino S, D’Emerico S, Widmer A.** 2004. Evidence for reproductive isolate selection in Mediterranean orchids: karyotype differences compensate for the lack of pollinator specificity. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, S259–S262.
- Cozzolino S, Widmer A.** 2005. Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology and Evolution* **20**, 487–494.
- Curtis JT.** 1939. The relation of specificity of orchid mycorrhizal fungi to the problem of symbiosis. *American Journal of Botany* **26**, 390–399.
- Darwin C.** 1876. *On the effects of cross and self fertilization in the vegetable kingdom*. London: John Murray.
- Darwin C.** 1877. *On the various contrivances by which orchids are fertilized by insects*. London: John Murray.
- Dearnaley JDW.** 2007. Further advances in orchid mycorrhizal research. *Mycorrhiza* **17**, 475–486.
- Diez JM.** 2007. Hierarchical patterns of symbiotic orchid germination linked to adult proximity and environmental gradients. *Journal of Ecology* **95**, 159–170.
- Dressler RL.** 1981. *The orchids: natural history and classification*. Cambridge, Massachusetts and London: Harvard University Press.
- Dressler RL.** 2005. How many orchid species? *Selbyana* **26**, 155–158.
- Ellis AG, Johnson SD.** 1999. Do pollinators determine hybridization patterns in sympatric *Satyrium* (Orchidaceae) species? *Plant Systematics and Evolution* **219**, 137–150.
- Feuerherdt L, Petit S, Jusaitis M.** 2005. Distribution of mycorrhizal fungus associated with the endangered pink-lipped spider orchid [*Arachnorchis* (syn. *Caladenia*) *behrii*] at Warren Conservation Park in South Australia. *New Zealand Journal of Botany* **43**, 367–371.
- Flanagan NS, Ebert D, Porter C, Rossetto M, Peakall R.** 2006. Microsatellite markers for evolutionary studies in the sexually deceptive orchid genus *Chiloglottis*. *Molecular Ecology Notes* **6**, 123–126.
- Forrest AD, Hollingsworth ML, Hollingsworth PM, Sydes C, Bateman RM.** 2004. Population genetic structure in European populations of *Spiranthes romanzoffiana* set in the context of other genetic studies on orchids. *Heredity* **92**, 218–227.
- Gandawijaja D, Arditti J.** 1983. The orchids of Krakatau: evidence for a mode of transport. *Annals of Botany* **52**, 127–130.
- Gebauer G, Meyer M.** 2003. N-15 and C-13 natural abundance of autotrophic and myco-heterotrophic orchids provides insight into nitrogen and carbon gain from fungal association. *New Phytologist* **160**, 209–223.
- Gegear RJ, Burns JG.** 2007. The birds, the bees, and the virtual flowers: can pollinator behavior drive ecological speciation in flowering plants? *American Naturalist* **170**, 551–566.
- Gill DE.** 1989. Fruiting failure, pollination inefficiency, and speciation in orchids. In: Otte D, Endlers JA, eds. *Speciation and its consequences*. Philadelphia: Academy of Natural Sciences Publications, 458–481.

- Girlanda M, Selosse MA, Cafasso D, et al.** 2006. Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. *Molecular Ecology* **15**, 491–504.
- Grant V.** 1949. Pollination systems as isolation mechanisms in angiosperms. *Evolution* **3**, 82–97.
- Gravendeel B, Smithson A, Slik FJW, Schuiteman A.** 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 1523–1535.
- Hadley G.** 1970. Non-specificity of symbiotic infection in orchid mycorrhiza. *New Phytologist* **69**, 1015–1023.
- Hadley G, Purves S.** 1974. Movement of 14 carbon from host to fungus in orchid mycorrhiza. *New Phytologist* **73**, 475–482.
- Hoballah ME, Gubitz T, Stuurman J, Broger L, Barone M, Mandel T, Dell’Olivo A, Arnold M, Kuhlemeier C.** 2007. Single gene-mediated shift in pollinator attraction in *Petunia*. *The Plant Cell* **19**, 779–790.
- Huber FK, Kaiser R, Sauter W, Schiestl FP.** 2005. Floral scent emission and pollinator attraction in two species of *Gymnadenia* (Orchidaceae). *Oecologia* **142**, 564–575.
- Irwin MK, Bougoure JJ, Dearnaley JDW.** 2007. *Pterostylis nutans* (Orchidaceae) has a specific association with two *Ceratobasidium* root-associated fungi across its range in eastern Australia. *Mycoscience* **48**, 231–239.
- Jacquemyn H, Brys R, Vandepitte K, Honnay O, Roldán-Ruiz I, Wiegand T.** 2007. A spatially explicit analysis of seedling recruitment in the terrestrial orchid *Orchis purpurea*. *New Phytologist* **176**, 448–459.
- Johnson SD.** 2000. Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biological Journal of the Linnean Society* **71**, 119–132.
- Johnson SD, Linder HP, Steiner KE.** 1998. Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *American Journal of Botany* **85**, 402–411.
- Johnson SD, Nilsson LA.** 1999. Pollen carryover, geitonogamy, and the evolution of deceptive pollination systems in orchids. *Ecology* **80**, 2607–2619.
- Johnson SD, Peter CI, Agren J.** 2004. The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 803–809.
- Jolou T, Burghardt B, Gebauer G, Berveiller D, Damesin C, Selosse MA.** 2005. Mixotrophy in orchids: insights from a comparative study of green individuals and nonphotosynthetic individuals of *Cephalanthera damasonium*. *New Phytologist* **166**, 639–653.
- Kessler M.** 2002. Environmental patterns and ecological correlates of range size among bromeliad communities of Andean forests in Bolivia. *Botanical Review* **68**, 100–127.
- Kiester AR, Lande R, Schemske DW.** 1984. Models of coevolution and speciation in plants and their pollinators. *American Naturalist* **124**, 220–243.
- Kores PJ, Molvray M, Weston PH, Hopper SD, Brown AP, Cameron KM, Chase MW.** 2001. A phylogenetic analysis of Diurideae (Orchidaceae) based on plastid DNA sequence data. *American Journal of Botany* **88**, 1903–1914.
- Leake JR.** 1994. The biology of myco-heterotrophic (saprophytic) plants. *New Phytologist* **127**, 171–216.
- Ma M, Tan TK, Wong SM.** 2003. Identification and molecular phylogeny of *Epulorhiza* isolates from tropical orchids. *Mycological Research* **107**, 1041–1049.
- Mant JG, Schiestl FP, Peakall R, Weston PH.** 2002. A phylogenetic study of pollinator conservatism among sexually deceptive orchids. *Evolution* **56**, 888–898.
- Mant J, Bower CC, Weston PH, Peakall R.** 2005a. Phylogeography of pollinator-specific sexually deceptive *Chiloglottis* taxa (Orchidaceae): evidence for sympatric divergence? *Molecular Ecology* **14**, 3067–3076.
- Mant J, Brandli C, Vereecken NJ, Schulz CM, Francke W, Schiestl FP.** 2005b. Cuticular hydrocarbons as sex pheromone of the bee *Colletes cunicularius* and the key to its mimicry by the sexually deceptive orchid, *Ophrys exaltata*. *Journal of Chemical Ecology* **31**, 1765–1787.
- Mant J, Brown GR, Weston PH.** 2005c. Opportunistic pollinator shifts among sexually deceptive orchids indicated by a phylogeny of pollinating and non-pollinating thynnine wasps (Tiphidae). *Biological Journal of the Linnean Society* **86**, 381–395.
- Mant J, Peakall R, Schiestl FP.** 2005d. Does selection on floral odor promote differentiation among populations and species of the sexually deceptive orchid genus *Ophrys*? *Evolution* **59**, 1449–1463.
- Mant J, Peakall R, Weston PH.** 2005e. Specific pollinator attraction and the diversification of sexually deceptive *Chiloglottis* (Orchidaceae). *Plant Systematics and Evolution* **253**, 185–200.
- Masuhara G, Katsuya K.** 1994. *In situ* and *in vitro* specificity between *Rhizoctonia* spp. and *Spiranthes sinensis* (Persoon) Ames var *Amoena* (M. Bieberstein) Hara (Orchidaceae). *New Phytologist* **127**, 711–718.
- McCormick MK, Whigham DF, O’Neill J.** 2004. Mycorrhizal diversity in photosynthetic terrestrial orchids. *New Phytologist* **163**, 425–438.
- McCormick MK, Whigham DF, Sloan D, O’Malley K, Hodkinson B.** 2006. Orchid–fungus fidelity: a marriage meant to last? *Ecology* **87**, 903–911.
- McKendrick SL, Leake JR, Taylor DL, Read DJ.** 2002. Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp. *New Phytologist* **154**, 233–247.
- Moccia MD, Widmer A, Cozzolino S.** 2007. The strength of reproductive isolation in two hybridizing food-deceptive orchid species. *Molecular Ecology* **16**, 2855–2866.
- Molvray M, Kores P, Chase MW.** 2000. Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. In: Wilson KL, Morrison DA, eds. *Monocots: systematics and evolution*. Melbourne: CSIRO.
- Montalvo AM, Ackerman JD.** 1987. Limitations to fruit production in *Ionopsis utricularioides*. *Biotropica* **19**, 24–31.
- Neiland MRM, Wilcock CC.** 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* **85**, 1657–1671.
- Nilsson LA.** 1992. Orchid pollination biology. *Trends in Ecology and Evolution* **7**, 255–259.
- Ogura-Tsujita Y, Yukawa T.** 2008. High mycorrhizal specificity in a widespread mycoheterotrophic plant, *Eulophia zollingeri* (Orchidaceae). *American Journal of Botany* **95**, 93–97.
- Ollerton J, Cranmer L.** 2002. Latitudinal trends in plant–pollinator interactions: are tropical plants more specialised? *Oikos* **98**, 340–350.
- Otero JT, Ackerman JD, Bayman P.** 2002. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. *American Journal of Botany* **89**, 1852–1858.
- Otero JT, Ackerman JD, Bayman P.** 2004. Differences in mycorrhizal preferences between two tropical orchids. *Molecular Ecology* **13**, 2393–2404.
- Otero JT, Bayman P, Ackerman JD.** 2005. Variation in mycorrhizal performance in the epiphytic orchid *Tolumnia*

- variegata* in vitro: the potential for natural selection. *Evolutionary Ecology* **19**, 29–43.
- Otero JT, Flanagan NS. 2006. Orchid diversity: beyond deception. *Trends in Ecology and Evolution* **21**, 64–65.
- Paulus HF, Gack C. 1990. Pollinators as pre-pollinating isolation factors: evolution and speciation in *Ophrys* (Orchidaceae). *Israel Journal of Botany* **39**, 43–79.
- Pauw A. 2006. Floral syndromes accurately predict pollination by a specialized oil-collecting bee (*Rediviva peringueyi*, Melittidae) in a guild of South African orchids (Coryciinae). *American Journal of Botany* **93**, 917–926.
- Pauw A. 2007. Collapse of a pollination web in small conservation areas. *Ecology* **88**, 1759–1769.
- Peakall R. 2007. Speciation in the Orchidaceae: confronting the challenges. *Molecular Ecology* **16**, 2834–2837.
- Peakall R, Beattie AJ. 1991. The genetic consequences of worker ant pollination in a self-compatible, clonal orchid. *Evolution* **45**, 1837–1848.
- Peakall R, Beattie AJ. 1996. Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentaculata*. *Evolution* **50**, 2207–2220.
- Peakall R, Schiestl FP. 2004. A mark-recapture study of male *Colletes cunicularius* bees: implications for pollination by sexual deception. *Behavioral Ecology and Sociobiology* **56**, 579–584.
- Peterson RL, Uetake Y, Zelmer C. 1998. Fungal symbioses with orchid protocorms. *Symbiosis* **25**, 29–55.
- Price PW. 1980. *Evolutionary biology of parasites*. Princeton NJ: Princeton University Press.
- Rasmussen HN. 2002. Recent developments in the study of orchid mycorrhiza. *Plant and Soil* **244**, 149–163.
- Rasmussen HN, Whigham DF. 1998. Importance of woody debris in seed germination of *Tipularia discolor* (Orchidaceae). *American Journal of Botany* **85**, 829–834.
- Renner SS. 2006. Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. In: Waser NM, Ollerton J, eds. *Plant-pollinator interactions: from specialization to generalization*. Chicago, IL, USA: University of Chicago Press, 123–144.
- Roberts P. 1999. *Rhizoctonia-forming fungi. A taxonomic guide*. Royal Botanic Gardens, Kew.
- Schiestl FP. 2005. On the success of a swindle: pollination by deception in orchids. *Naturwissenschaften* **92**, 255–264.
- Schiestl FP, Ayasse M. 2002. Do changes in floral odor cause speciation in sexually deceptive orchids? *Plant Systematics and Evolution* **234**, 111–119.
- Schiestl FP, Peakall R, Mant JG, Ibarra F, Schulz C, Franke S, Franke W. 2003. The chemistry of sexual deception in an orchid-wasp pollination system. *Science* **302**, 437–438.
- Scopce G, Musacchio A, Widmer A, Cozzolino S. 2007. Patterns of reproductive isolation in Mediterranean deceptive orchids. *Evolution* **61**, 2623–2342.
- Selosse MA, Weiss M, Jany JL, Tillier A. 2002. Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid *Neottia nidus-avis* (L.) LCM Rich. and neighbouring tree ectomycorrhizae. *Molecular Ecology* **11**, 1831–1844.
- Selosse MA, Faccio A, Scappaticci G, Bonfante P. 2004. Chlorophyllous and achlorophyllous specimens of *Epipactis microphylla* (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. *Microbial Ecology* **47**, 416–426.
- Shefferson RP, Weiss M, Kull T, Taylor DL. 2005. High specificity generally characterizes mycorrhizal association in rare lady's slipper orchids, genus *Cypripedium*. *Molecular Ecology* **14**, 613–626.
- Shefferson RP, Taylor DL, Weiss M, et al. 2007. The evolutionary history of mycorrhizal specificity among lady's slipper orchids. *Evolution* **61**, 1380–1390.
- Shimura H, Matsuura M, Takada N, Koda Y. 2007. An antifungal compound involved in symbiotic germination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae). *Phytochemistry* **68**, 1442–1447.
- Singer RB. 2002. The pollination mechanism in *Trigonidium obtusum* Lindl (Orchidaceae: Maxillariinae): sexual mimicry and trap-flowers. *Annals of Botany* **89**, 157–163.
- Singer RB, Flach A, Koehler S, Marsaioli AJ, Amaral MDE. 2004. Sexual mimicry in *Mormolyca ringens* (Lindl.) Schltr. (Orchidaceae: Maxillariinae). *Annals of Botany* **93**, 755–762.
- Smith SE, Read DJ. 1997. *Mycorrhizal symbiosis*. New York: Academic Press.
- Smithson A. 2002. The consequences of rewardlessness in orchids: reward-supplementation experiments with *Anacamptis morio* (Orchidaceae). *American Journal of Botany* **89**, 1579–1587.
- Soliva M, Kocyan A, Widmer A. 2001. Molecular phylogenetics of the sexually deceptive orchid genus *Ophrys* (Orchidaceae) based on nuclear and chloroplast DNA sequences. *Molecular Phylogenetics and Evolution* **20**, 78–88.
- Soliva M, Widmer A. 2003. Gene flow across species boundaries in sympatric, sexually deceptive *Ophrys* (Orchidaceae) species. *Evolution* **57**, 2252–2261.
- Stebbins GL. 1970. Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics* **1**, 307–326.
- Steiner KE, Whitehead VB, Johnson SD. 1994. Floral and pollinator divergence in 2 sexually deceptive South African orchids. *American Journal of Botany* **81**, 185–194.
- Suarez JP, Weiss M, Abele A, Garnica S, Oberwinkler F, Kottke I. 2006. Diverse tulasnelloid fungi form mycorrhizas with epiphytic orchids in an Andean cloud forest. *Mycological Research* **110**, 1257–1270.
- Taylor DL, Bruns TD. 1997. Independent, specialized invasions of ectomycorrhizal mutualism by two nonphotosynthetic orchids. *Proceedings of the National Academy of Sciences, USA* **94**, 4510–4515.
- Taylor DL, Bruns TD. 1999. Population, habitat and genetic correlates of mycorrhizal specialization in the 'cheating' orchids *Corallorhiza maculata* and *C. mertensiana*. *Molecular Ecology* **8**, 1719–1732.
- Taylor DL, Bruns TD, Hodges SA. 2004. Evidence for mycorrhizal races in a cheating orchid. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 35–43.
- Taylor DL, Bruns TD, Szaro TM, Hodges SA. 2003. Divergence in mycorrhizal specialization within *Hexalectris spicata* (Orchidaceae), a nonphotosynthetic desert orchid. *American Journal of Botany* **90**, 1168–1179.
- Thompson JN. 2005. *The geographic mosaic theory of coevolution*. Chicago: University of Chicago Press.
- Tremblay RL. 1992. Trends in the pollination ecology of the Orchidaceae: evolution and systematics. *Canadian Journal of Botany-Revue Canadienne De Botanique* **70**, 642–650.
- Tremblay RL. 1997. Morphological variance among populations of three tropical orchids with restricted gene flow. *Plant Species Biology* **12**, 85–96.
- Tremblay RL, Ackerman JD. 2001. Gene flow and effective population size in *Lepanthes* (Orchidaceae): a case for genetic drift. *Biological Journal of the Linnean Society* **72**, 47–62.
- Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* **84**, 1–54.

- van der Pijl L, Dodson CH.** 1966. *Orchid flowers: their pollination and evolution*. Coral Gables: University of Miami Press.
- Vasquez R, Ibisch PL, Gerkmann B.** 2003. Diversity of Bolivian Orchidaceae: a challenge for taxonomic, floristic and conservation research. *Organisms Diversity and Evolution* **3**, 93–102.
- Wallace LE.** 2003. The cost of inbreeding in *Platanthera leucophaea* (Orchidaceae). *American Journal of Botany* **90**, 235–242.
- Warcup JH.** 1981. The mycorrhizal relationships of Australian orchids. *New Phytologist* **87**, 371–381.
- Whigham DF, O'Neill JP, Rasmussen HN, Caldwell BA, McCormick MK.** 2006. Seed longevity in terrestrial orchids: potential for persistent *in situ* seed banks. *Biological Conservation* **129**, 24–30.
- Wu CI.** 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* **14**, 851–865.