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Key words: hydrophobin, lichen, fungus, symbiosis, *Xanthoria*, *Dictyonema*, morphology, hydrophobicity, *in situ* hybridisation.

An orchid–fungus marriage – physical promiscuity, conflict and cheating

The dazzling forms and colours of numerous orchid species have fascinated people for centuries. My own familiarity with these plants began in earliest childhood in southern

France, when I encountered *Ophrys apifera* for the first time – I still remember the truly magical moment and the exact place where I found this strangely shaped flower, which resembles a female bee. Many years later, studying botany, I learned that the exquisite shapes and colours that distinguish most orchid flowers are indeed a ‘sexual device’ that have evolved for the sole purpose of attracting male pollinators and maintaining species isolation. I also discovered the existence of that other puzzling and intimate association, the obligate dependency of orchids on fungi for at least part of their life cycle (demonstrated in the early part of the last century by the French scientist Noël Bernard (Bernard, 1909)).

Recent methodological advances in molecular fingerprinting, isotopic labelling and manipulation of orchid seeds now enable the systematic analysis of orchid–fungus interactions in the field. In this issue, McKendrick *et al.* (pp. 233–247) make a very important contribution to the emerging, detailed picture of the interactions between orchids and their fungal symbionts, providing a theoretical framework for thinking about the dynamic interactions in the early stages of symbiotic development. They have examined the ontogeny of the bird’s nest orchid (*Neottia nidus-avis*), an achlorophyllous species, and identified the fungal symbionts from both seedlings and adult plants. With a reliable chronology in place for *N. nidus-avis*, it can be used as a model system that is appropriate for addressing a wide array of ecological and evolutionary questions about cheaters in mycorrhizal mutualisms.

In the Neottia–Sebacina couple, we are now able to glimpse the real complexity of an intriguing marriage

Physical promiscuity starts early ... let’s get married!

The *Orchidaceae*, according to the classification of Dressler (1993), at an estimated 20 000 species, are one of the largest angiosperm families. They exhibit large variations in their floral features, life forms, habitat distributions and trophic patterns. In addition to autotrophic species, the *Orchidaceae* also contain a large number of achlorophyllous species which have, partially or completely, lost their ability to photosynthesize. Where the loss is total, the species are termed ‘myco-heterotrophic’ because their immediate source of reduced carbon is provided by associated fungi (Leake, 1994; Smith & Read, 1997). The bird’s nest orchid is a terrestrial leafless species that is clearly a myco-heterotrophic plant for all of its life cycle.

Minute seeds of autotrophic and heterotrophic orchids lack storage materials and their undifferentiated embryos do not develop further unless colonized by compatible fungal

endophytes. The fungus penetrates embryo host cells forming hyphal aggregates (coils or pelotons) that are later 'digested' by the orchid. Evidence of parasitism by the fungus has also been reported in seedling stages (Smith & Read, 1997). There is a vast of literature on the germination of orchids seeds and embryogeny in artificial conditions (Rasmussen, 1995; Smith & Read, 1997). However, such studies are rarely conducted in natural conditions because seeds are hard to manipulate and little is known about the factors that promote their germination.

The paper by Kendrick *et al.* provides just such valuable and detailed information for a myco-heterotrophic species. The authors provide the first definitive chronology for germination of *N. nidus-avis* seeds in the field and confirm the critical importance of *Sebacina*-like fungi in this developmental process. They also conducted a set of experiments under natural conditions to address the effects of plant population structure on the likelihood and direction of the plant–fungus interaction. This revealed that seed germination and development are correlated with the presence of flowering spikes of nearby adult orchids. A positive relationship was also observed between the growth of the largest seedlings and the number of seeds that germinated. On the basis of their observations, the author's hypothesised a strong effect of mycorrhiza-forming fungi on both seed germination and plant growth.

Adult orchids usually form typical mycorrhizal associations, although dependency on their fungal symbionts varies widely according to the autotrophic or heterotrophic life style of the orchid, plant phenology and habitat distribution (Smith & Read, 1997). During this stage, the fungus forms coils of clumped hyphae within cortical cells of the orchid root. These intracellular pelotons have a limited life, similarly to the picture obtained with the immature embryo.

Continuous conflict of interests ... cohabitation is difficult

Orchid–fungus relationships are particularly interesting because, in many respects, they are arranged in a continuum from parasitism to mutualism. In the early stages of embryogeny of both autotrophic and myco-heterotrophic orchids, the plant exploits the resources of its fungal symbiont without providing obvious benefits to it (Smith & Read, 1997). In addition to a major carbohydrate supply, the fungus may also facilitate free water absorption (Yoder *et al.*, 2000).

In adult orchids, whether or not they are photosynthetic, the fungus provides the plant with soil-derived mineral nutrients such as nitrogen or phosphorus. In achlorophyllous orchids, the absence of photosynthesis during most of the plant's life has resulted in selection pressures favouring a strong dependency on fungi, which, in addition to mineral nutrients, provide the source of reduced carbon (Smith & Read, 1997). The picture of carbon movement is still unclear for orchids with chlorophyll. It appears that C trans-

fer from the fungus to the plant is not always reversed when the orchid becomes photosynthetic in laboratory experiments (Smith & Read, 1997). The question as to whether or not the fungus gains carbon from the green plant clearly deserves further research under ecologically relevant conditions or by using a broad range of realistic plant–fungus combinations. Other fitness-related benefits such as hormone responses in the fungal partner should also be explored.

Je t'aime moins plus'

How to escape marriage costs? Cheating may be fun ...

In addition to benefits, mutualisms impose substantial costs for the partners in that the fitness of one species may occur at the expense of the other (Pellmyr & Huth, 1994). These costs (which are not necessarily parallel) are inflicted by mutualists themselves (e.g. rewarding the partner that confers benefits) as well as by extrinsic factors such as nonmutualistic species (Bronstein & Ziv, 1997; Anstett *et al.*, 1997). These conflicts have been seen as forces destabilising the mutualism with outcomes that range from commensalism to parasitism of the mutualist. In this respect, mycorrhizal associations may not differ from other mutualisms. Comparative and experimental studies conducted on mycorrhizas have highlighted the omnipresence of conflict of interests present at all stages of the symbiosis between plant and fungal partners (Smith & Read, 1997). It is likely that strong conflicts have led to cheating in numerous orchid–fungus interactions.

Thus, there is increasing evidence that achlorophyllous orchids are cheaters that obtain their carbohydrates from photosynthetic plants via a shared mycorrhizal fungus (Leake, 1994; Smith & Read, 1997). Conventional and modern approaches that have been used to throw light on tripartite associations encompass the following: isolation of the fungi, and use in mycorrhiza-synthesis experiments; phylogenetic placement of the fungal symbionts using molecular methods; and measurements of carbon transfer using isotopic labelling. Kendrick *et al.* (2000a) recently provided an experimental confirmation of carbon transfer from ectomycorrhizal trees to the fully myco-heterotrophic orchid, *Corallorhiza trifida*, through linked fungal mycelia, presumably of an ectomycorrhizal basidiomycete (Zelmer & Currah, 1995) and sequencing of the fungi (Taylor, 1997; Kendrick *et al.*, 2000b). In the report in this issue, Kendrick *et al.* used similar molecular methods to identify the fungal symbionts in *Neottia* seeds and roots from two geographical provenances. Analysis of nuclear ribosomal DNA sequences revealed the fungal victim of the bird's nest orchid to belong exclusively to the genus *Sebacina*, a heterobasidiomycete taxon of the polyphyletic '*Rhizoctonia*'

group. This molecular identification accords with previous ultrastructural and morphological observations of the fungi associated with *Neottia*. Unfortunately, because controversy does exist in the literature over the parasitic, saprophytic or ectomycorrhizal status of *Sebacina*, Kendrick *et al.* could not conclude with certainty as to the lifestyle of this orchid fungus and so on the nature of the association. However, convincing unpublished results from Selosse *et al.* confirm that *N. nidus-avis* is an epiparasitic species exploiting a highly specific clade of *Sebacina* that form typical ectomycorrhizas on neighbouring photosynthetic trees.

... but it's a risky strategy

Parasitism has been documented in other plant associations with mycorrhizal fungi, such as the achlorophyllous *Monotropoideae* on various ectomycorrhizal basidiomycete taxa (Cullings *et al.*, 1996; Bidartando & Bruns, 2001); in pollination mutualisms, as for both the yucca–yucca moth and the fig–fig wasp relationships (Anstett *et al.*, 1997; Bao & Addicott, 1998); and in ant–plant mutualisms (Gaume & McKey, 1999). Interlopers may be naturally selected or favoured under certain ecological conditions because they take advantage of mutualistic resources/services while avoiding the cost of mutualism (Gaume & McKey, 1999). However, they cannot be too successful because, if they are, they may expose themselves to retaliation from their victims and lose them (Gaume & McKey, 1999). Therefore, fundamental difficulties in the study of mutualism are the determination of those factors that regulate cheating and how interacting species prevent excessive exploitation by their partners without discouraging attention from them (Pellmyr & Huth, 1994; Bao & Addicott, 1998).

Returning to the *Neottia*–*Sebacina* couple, we have now been able to glimpse the real complexity of an intriguing marriage, which has become more of a challenge today than it was a century ago for Noël Bernard. Kendrick *et al.* and other authors have recently demonstrated that we have both the appropriate techniques and sufficient knowledge to ask how such myco-heterotroph orchids have appeared and to examine the persistence and stability of this parasitic strategy. A full understanding of this plant–fungus interaction will require a synthesis of evolutionary and ecological approaches across various taxa. Key questions include the following:

- What is the origin of myco-heterotrophy and pattern of evolutionary change in both orchids and fungi?
- What are the nutritional and nonnutritional rewards in the tripartite association?
- How does the ‘cheater strategy’ remain stable?
- What are the genetic and ecological determinants of the *Neottia*–fungus relationship?
- Is ‘monogamy’ (i.e. orchid specificity towards a narrow range of *Sebacina* isolates) the only choice or ‘the price to pay’ for functional efficiency?

Final comments

The results presented by Kendrick *et al.* add to a growing body of data on myco-heterotrophic plants indicating that there may be much more specificity between such plants and their fungal endophytes that was previously thought. Indeed, many surprises and exciting discoveries will be provided by entering deeper into this system. I believe, in addition, that orchids have a tremendous potential to yield novel insights into the ecological processes and evolutionary dynamics involved in plant–fungal mutualism because of their wide variety of life forms, habitats and interactions. Important areas for future research include:

- The establishment of reliable molecular phylogenies in orchids and their fungal partners in order to study the origin and evolution of myco-heterotrophy as well as convergence and divergence in life-history strategies.
- Measurements across orchid taxa of nutritional and nonnutritional benefits and costs.
- Detailed investigations of the genetic, developmental and ecological factors that might promote or constrain the establishment and maintenance of the symbiosis.

Many orchids are threatened with extinction. Compelling evidence now exists that research strategies for their conservation and management should devote substantial effort towards the understanding of the ecology and evolution of their microscopic fungal partners.

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Key words: Bird's nest orchid (*Neottia nidus-avis*), *Sebacina* spp., myco-heterotrophy, mycorrhizal mutualism, fitness, evolution.

Letters

Does hairiness matter in Harare? Resolving controversy in global comparisons of plant trait responses to ecosystem disturbance

Land use changes and their interaction with atmospheric and climatic changes represent a major challenge to humanity. However, despite the wealth of literature about plant traits in general, such as leaf size and texture or canopy height, we still know amazingly little about the links between these traits and responses to disturbance of the ecosystem. Most of the empirical work on functional traits has focused on plant responses to resources and climate (Chapin *et al.*, 1996; Grime *et al.*, 1997; Cunningham *et al.*, 1999; Fonseca *et al.*, 2000), rather than to disturbances, such as changing resources, substrate availability or the physical environment (Pickett & White, 1985). In addition, plant classifications used in large-scale models have deliberately restricted the numbers of functional types and traits used, in order to reflect broad responses to climate. What is to be done?

An important response has been through international scientific programmes, including the Global Change and Terrestrial Ecosystems (GCTE) programme of the International Geosphere-Biosphere Programme (IGBP), which have promoted work leading to the prediction of ecosystem response to these factors. Just because disturbance usually operates at spatial scales smaller than climate (Woodward & Diament, 1991) does not necessarily mean that global-scale questions about disturbance and land use cannot be addressed. Some scientists have taken up the search for plant biological traits that are associated with major disturbance and land-use factors, such as grazing, fire and agricultural land abandonment, focusing on a comparative approach at the global scale.

Three key issues are crucial in this global comparison of trait response to disturbance:

- First, the relationship between traits linked to plant responses to disturbance and those linked to plant functional effects on ecosystem properties, and the fact that some key plant traits are related both to plant responses to several disturbance types, climate, and *in situ* resource availability. This topic has been recently addressed in the literature (Chapin *et al.*, 2000; Lavorel & Garnier, 2001), but its implications are still far from being fully covered.

- Second, and especially important having been a source of confusion and controversy, and poorly addressed in the recent literature, the importance of ecosystem and regional context in determining what traits to focus on.