# Understanding Evolution and Complexity of Species Interactions Using Orchids as a Model System

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Orchids have been a subject of fascination to biologists for a few hundred years, and to humankind no doubt much longer. By the time Charles Darwin wrote his volume on orchids in 1862, many of the mysteries surrounding these plants, including the origins and functions of their spectacularly diverse and complex floral forms, were already well-articulated. In May 2013, the 31st New Phytologist Symposium focused on some of the most intriguing new enigmas surrounding orchids. Entitled "Orchid Symbioses: Models for Evolutionary Ecology" and held at the University of Calabria (Italy), this symposium focused on two sets of interactions upon which orchids critically depend: those with pollinators and with mycorrhizae.

Generous support from the New Phytologist Trust made it possible to welcome an audience from six continents, including international experts but also young scientists who represent the future of orchid biology. We would like to recognize the

winner of the student poster prize, Florent Martos of the University of Kwazulu-Natal, South Africa, for his poster, entitled 'Evidence for extreme specialization in both above- and belowground symbioses in *Gastrodia* (Orchidaceae)'. Three outstanding runners-up for this award also deserve congratulations: Karin Gross (University of Zurich, Switzerland; 'Floral signal evolution in the rewarding orchid genus *Gymnadenia* is influenced by pollinators and ploidy level'), Ursula Jaros (University of Salzburg, Austria; 'Reproductive and population genetic consequences of remote island colonization in *Bulbophyllum occultum* THOUARS (Orchidaceae) from Madagascar and La Réunion'); and Rafael Valadares (Universidade de São Paulo, Brazil; 'Differential protein accumulation in mycorrhizal and non-mycorrhizal roots of *Oeceoclades maculate*').

As the titles of these posters indicate, the presentations focused on a wide array of enigmatic above- and below-ground phenomena in orchids worldwide. Here, rather than attempting to summarize the many scientific highlights, we wish to expand upon the second part of the symposium title. Can the study of such an unusual plant family hosting such an unusual set of interspecific interactions really serve as a model system for addressing fundamental questions in evolutionary ecology? We are convinced that it can. Below, we elaborate on three ways in which orchids can take us well beyond the rapidly increasing base of knowledge we heard about at this meeting.

**Orchids and the Mutualism-Parasitism Continuum**

A model system can be one that showcases variations on a single ecological and evolutionary theme, and that can therefore offer raw material for comparative studies. Recent years have seen a growing interest in the conditions that foster evolutionary transitions between interaction outcomes (mutualistic, antagonistic, and competitive), as well as the realization that a single interaction can exhibit different outcomes when placed into different ecological contexts. Talks at this New Phytologist Symposium made it abundantly clear that orchids can offer an exceptional laboratory in which to study this continuum of species interactions.

Why orchids? Beyond the sheer number of orchid species lies the prime importance of two groups of associates, pollinators and mycorrhizae, without which almost no orchid can persist. What makes orchids really special for studying species interactions, though, is that within these associations are fascinating “variations on a theme”: associations with pollinators and mycorrhizal fungi usually benefit orchids, but the effects of those associations range from beneficial to antagonistic to their partners.

Talks at this symposium provided ample evidence of this exceptional range of interaction outcomes. James Ackerman of the University of Puerto Rico reviewed pollination systems in which insects are attracted to orchid flowers by deceit; that is, rewards are promised but not delivered. Deceptive pollination is widespread in orchids, particularly in species-rich genera, suggesting that a shift from rewarding to cheating pollinators may be key to understanding orchid diversification. Left unanswered, however, is the question of why floral rewards remain as common as they are within orchids, given the economic advantage of nectarlessness and the fact that insects do in fact visit nectarless flowers often enough to lead to high fruit set. Some orchid species, we learned at this meeting, are polymorphic for nectar production. These should be particularly interesting systems for research into the costs and benefits of reward vs. deception.

The spectrum from mutualism to parasitism can also be seen below ground. For example, Martin Bidartondo of Imperial College London provided examples in which plants parasitically tap into mycorrhizal networks that benefit their neighbors, rather than establishing mycorrhizal mutualisms themselves. As in the case of pollinator deception, this phenomenon has evolved many times within the orchids. There are also fungi that exploit rather than benefit the orchids upon which they depend. In his presentation, Bidartondo resurrected a “symbiotic continuum” first proposed by de Bary in 1879, ranging from mycorrhizae that exploit plants, though mutually beneficial mycorrhizal/plant associations, to plants that exploit mycorrhizae. Orchids offer the opportunity to study this entire, generally overlooked continuum.

We now need to step back to ask how these fascinating spectra of outcomes have arisen and how they are maintained. What are the conditions that favor reward and hence mutualism in some pairwise interactions, yet deception and antagonism in other, closely related ones? How beneficial is it save resources that would otherwise be channeled into reward production? Might the benefit of reduced geitonogamy (pollinator movement between flowers on the same plant) in rewardless orchids compensate for the cost of lower visitation rates? Conversely, how costly is it for floral visitors to be deceived? If the cost is significant, why haven't organisms evolved mechanisms to prevent being duped by their partners? Finally, are orchids unusual in exhibiting such a wide range of outcomes in their two critical interspecific associations, or are we simply more aware of it because of the intrinsic fascination that orchids hold for pollination and mycorrhizal biologists?

**Interactions among Species Interactions**

The talks at this symposium centered on orchid interactions with either mycorrhizae or pollinators. However, there was very limited reference to relationships between these two kinds of interactions. Bringing together the effects of such disparate interactors is potentially an exciting area of future research, perhaps leading to insights into causes of evolutionary transitions, key innovations, and evolutionary novelty.

Plants probably integrate all positive and negative interactions physiologically, but we can also ask whether the interaction of interactions has evolutionary consequences. Here too, orchids may prove to be a good model system. Understanding evolutionary interactions among orchid interaction systems may reveal factors playing key roles in the evolution of ecological novelty. For example, some tropical orchids (*Maxillaria* and relatives) attract pollinators with chemically unusual rewards, waxes and resins collected by pollinating bees for nest construction (Davies et al. 2004; Davies and Stpiczynska 2012). How did these novel relationships originate? Could random mutations have assembled the chemical and morphological traits needed to establish a new mutualism or are other evolutionary mechanisms, such as "exaptative borrowing" (preadaptations) from other interaction systems, more likely?

Evolutionary studies of organisms as diverse as yuccas, birds, and dinosaurs suggest that complex relationships and functions are usually assembled by chance from pre-existing complex features that serve other functions, a process called "exaptation" (or "preadaptation") (Pellmyr 1997; Pellmyr and Lebens-Mack 2000; Prum 2005; Balanoff et al. 2013). This might lead us to predict that the origin of a wax reward in orchids was predicated on previous chemical adaptations for defense against disease or small herbivores, or reduction of water loss by production of cuticular waxes in flowers and/or leaves. Because protective cuticular waxes are nearly ubiquitous in plants, we are left wondering why it is only in the orchids in which wax rewards have been thus far discovered (see below). A similar case has been made for the origin of resins as pollinator rewards, although this transition has been discovered in three or more lineages in addition to orchids, generally in species that secrete resins or latex elsewhere for defense of flowers and/or leaves. Orchids are not known for defending themselves with resin, although production of prenylated flavonoids have been described (Liu et al. 2013), and these could be constituents or precursors. In fact, one puzzling thing about orchids, as reinforced by this meeting, is that there very few studies of their herbivory. Is this because they are so well defended that herbivory is negligible, or have researchers simply not been drawn towards studying it? Clearly, much remains to be learned about the chemical ecology of orchids, as well as evolutionary origins of non-nutritive rewards in this group.

Similar evolutionary feedbacks between interactions are possible in orchids, between mycorrhizal and pollinator interactors. Some investigations in this direction have been conducted. For example Waterman et al. (2011) found in a clade of South African orchids that pollinator shifts were important both in orchid speciation and in promoting coexistence in sympatry. However, shifts in mycorrhizal partners were not important in orchid speciation but were for coexistence of species in sympatry. Of course, not all interactions necessarily interact with each other. Determining the factors that promote linkages between interactions and what factors promote autonomy of interactions are areas yet to be explored.

**The Extremes of Evolution**

Orchids have been viewed both as models of the evolutionary process and as intriguing extremes of the traits favored in plants by natural selection. Darwin used orchids as an extreme model to show, with great elegance, evidence of descent with modification. It was a brilliant choice, because it showed that even the most intricate adaptations could be traced, part by part, to preexisting structures that had been modified time and again. Darwin showed that a model does not need to be representative of the patterns found in nature in order to be useful. A model can helpful because it shows the extreme limits of the underlying processes.

For reasons that are still not clear, natural selection on orchids has been unusually effective at pushing the limits of what we often consider normal in the life histories and morphologies of plants and their interactions with other species. Many non-orchid plant lineages include species that are extreme in some trait or interaction, but orchids stand out by the number of ways in which they have pushed the limits. Their dustlike seeds, their reliance of fungi for germination, their complicated interactions with mycorrhizal fungi throughout their lifetimes, and the many highly specialized pollination systems they have evolved are the most obvious extremes. These extremes are sometimes viewed by non-orchid biologists as wonderfully interesting curiosities, but they are much more. They are clear evidence of how far natural selection can push a suite of traits, a life history, or a form of interaction. They are like observing the outcomes of mathematical models of evolution with the parameter values set to the outer boundaries of what would be considered tenable.

At these extremes, it can become challenging to decipher how natural selection has shaped a trait or interaction in the past and how it is acting currently. As researchers probe more deeply into orchid biology, they are revisiting Darwin's problem of descent with modification in extreme orchid flowers on even more complicated suites of traits. Some talks at this meeting grappled with the problem how best to understand the biochemical interactions between orchids and fungi along the continuum of parasitism to mutualism. The interplay of carbon, nitrogen, and other chemical elements in biosynthetic interactions between orchid and fungal physiology now seems to be much more intricate than previously supposed.

The increasing focus on these difficult problems shows how far we have come in trying to understand the process of natural selection. It is no longer about understanding the evolution of single traits, small suites or traits, or simple interactions. It is about the broader problem of how natural selection manages to integrate the many selection pressures acting on populations and produce, despite all the apparently conflicting selection pressures, relatively extreme traits and life histories rather than general-purpose solutions. In that respect, orchids are a useful window into why the world is made up of millions of evolutionary solutions (i.e., species) with billions of smaller solutions (i.e., locally adapted populations) rather than a few general solutions.

**Conclusions**

Well-understood model systems offer exciting opportunities for integrative approaches to studying interspecific interactions. This New Phytologist Symposium made clear that enough is now known about interactions between orchids and other species to make them ideal subjects of further physiological, ecological, and evolutionary study. Orchids exhibit interactions of varying strength and specificity with both mycorrhizal fungi and pollinators. Pollination is an above-ground process, while interactions with mycorrhizae take place either below-ground (in terrestrial orchids) or above-ground (in tropical epiphytic orchids, including the majority of orchid species). Relationships with diseases and herbivores are less studied, but are likely to be important both below and above ground in some orchid systems. Here we have highlighted three promising research foci that would build on the rapidly expanding knowledge highlighted so effectively at this symposium. Others can certainly be envisioned as well.

In closing, we wish to stress a point that Tupac Otero of the National University of Colombia made during the meeting: most of our ecological understanding emerges from studies of temperate zone orchids. Relatively few of the highly diverse tropical orchids have yet been studied in an ecological or evolutionary context; many remain undescribed. Tropical orchids provide opportunities to test hypotheses that have developed over decades of studies of temperate systems. Indeed, orchids have probably pushed the limits in many more ways than we currently know. It is incumbent to assure that the speciose, yet fragile habitats in which these fascinating plants and their associates occur be preserved for future generations of study, enjoyment, and evolution.

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