

1 **Understanding Evolution and Complexity of Species Interactions Using**
2 **Orchids as a Model System**

3

4 Judith L. Bronstein¹, W. Scott Armbruster², John N. Thompson³

5

6 ¹Department of Ecology and Evolutionary Biology

7 University of Arizona

8 Tucson, Arizona, USA

9

10 ²School of Biological Sciences

11 University of Portsmouth

12 Portsmouth PO1 2DY

13 UK

14

15 ³Department of Ecology and Evolutionary Biology

16 University of California, Santa Cruz

17 Santa Cruz, CA 95060 USA

18

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

28

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

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

43

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

53

54 **Orchids and the Mutualism-Parasitism Continuum**

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

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

70

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

83

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

93 mycorrhizae. Orchids offer the opportunity to study this entire, generally
94 overlooked continuum.

95

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

108

109 **Interactions among Species Interactions**

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

116

117 Plants probably integrate all positive and negative interactions physiologically, but
118 we can also ask whether the interaction of interactions has evolutionary
119 consequences. Here too, orchids may prove to be a good model system.

120 Understanding evolutionary interactions among orchid interaction systems may
121 reveal factors playing key roles in the evolution of ecological novelty. For example,
122 some tropical orchids (*Maxillaria* and relatives) attract pollinators with chemically
123 unusual rewards, waxes and resins collected by pollinating bees for nest

124 construction (Davies et al. 2004; Davies and Stpiczynska 2012). How did these novel
125 relationships originate? Could random mutations have assembled the chemical and
126 morphological traits needed to establish a new mutualism or are other evolutionary
127 mechanisms, such as “exaptative borrowing” (preadaptations) from other
128 interaction systems, more likely?

129

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

151

152 Similar evolutionary feedbacks between interactions are possible in orchids,
153 between mycorrhizal and pollinator interactors. Some investigations in this
154 direction have been conducted. For example Waterman et al. (2011) found in a

155 clade of South African orchids that pollinator shifts were important both in orchid
156 speciation and in promoting coexistence in sympatry. However, shifts in
157 mycorrhizal partners were not important in orchid speciation but were for
158 coexistence of species in sympatry. Of course, not all interactions necessarily
159 interact with each other. Determining the factors that promote linkages between
160 interactions and what factors promote autonomy of interactions are areas yet to be
161 explored.

162

163 **The Extremes of Evolution**

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

172

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

186

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

196

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

207

208 **Conclusions**

209 Well-understood model systems offer exciting opportunities for integrative
210 approaches to studying interspecific interactions. This New Phytologist Symposium
211 made clear that enough is now known about interactions between orchids and other
212 species to make them ideal subjects of further physiological, ecological, and
213 evolutionary study. Orchids exhibit interactions of varying strength and specificity
214 with both mycorrhizal fungi and pollinators. Pollination is an above-ground process,
215 while interactions with mycorrhizae take place either below-ground (in terrestrial
216 orchids) or above-ground (in tropical epiphytic orchids, including the majority of

217 orchid species). Relationships with diseases and herbivores are less studied, but are
218 likely to be important both below and above ground in some orchid systems. Here
219 we have highlighted three promising research foci that would build on the rapidly
220 expanding knowledge highlighted so effectively at this symposium. Others can
221 certainly be envisioned as well.

222

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

233

234 **References Cited**

235

236 **Balanoff AM, Bever GS, Rowe TB, Norell MA. 2013.** Evolutionary origins of the
237 avian brain. *Nature* **501**: 93-96.

238

239 **Davies KL, Turner MP, Gregg A. 2003.** Lipoidal labellar secretions in *Maxillaria*
240 Ruiz & Pav. (Orchidaceae). *Annals of Botany* **91**: 439-446.

241

242 **Davies KL, Stpiczynska M. 2012.** Comparative labellar anatomy of resin-secreting
243 and putative resin-mimic species of *Maxillaria* s.l. (Orchidaceae: Maxillariinae).
244 *Botanical Journal of the Linnean Society* **170**: 405-435.

245

246 **Liu J, Li CY, Zhong YJ, Yang L, Li YF. 2013.** Chemical constituents from *Spiranthes*
247 *sinensis*. *Biochemical Systematics and Ecology* **47**: 108-110.

248

249 **Pellmyr O. 1997.** Pollinating seed eaters: Why is active pollination so rare? *Ecology*
250 **78:** 1655-1660.

251

252 **Pellmyr O, Leebens-Mack J. 2000.** Reversal of mutualism as a mechanism for
253 adaptive radiation in yucca moths. *American Naturalist* **156:** S62-S76.

254

255 **Prum RO. 2005.** The evolution of feather diversity and function: Exaptation,
256 functional redundancy and historical contingency. In, Briggs DEG (ed),
257 *Evolving Form and Function: Fossils and Development.* Pp 245-256.

258

259 **Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Gebauer G, Savolainen V,**
260 **Barraclough TG, Pauw A. 2011.** The effects of above- and belowground
261 mutualisms on orchid speciation and coexistence. *American Naturalist* **177:**
262 E54-E68.