

POLLINATION WITHOUT REWARD: DECEPTION AS A POLLINATION SYSTEM

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Introduction

Pollination is one of the greatest examples of interaction between plants and animals resulting in evolution of specific traits. In the Early Cenozoic, angiosperm representation in the fossil record exploded from less than 10% in fossil abundance to nearly 75%. This relatively rapid change in representation can likely be attributed to pollination, a system that increased the importance of plant and animal interaction (Crane, Friis, & Pedersen, 2000). Typical pollination involves a system where animals transfer pollen from the anther of one flower to the stigma of another flower of the same species and receive a reward such as nectar. Contrary to the norm, pollination has evolved in many species of orchid to attract pollinators through deception as opposed to reward. An estimated 10,000 species of orchids attract pollinators deceptively (Ackerman, 1986). This number illustrates how orchids are extremely diverse. Two of the most common forms of deception are food deception and sexual deception. In food deception, there is often an interspecific model-mimicry system that involves a mimic without rewards imitating the phenology of a rewarding model plant in order to attract pollinators (Ackerman, 1986). Sexually deceptive orchids attract pollinators by mimicking mating signals of the female, with visual or chemical signals playing an important role (Cozzolino & Widmer, 2005). Among these, sexual deception may provide more answers on the evolution of deception, with many species using chemical signals to imitate female mating signals. Sexual deception appears to be a strategy almost unique to orchids, with only one daisy and one iris being the other known users of this strategy (Ellis & Johnson, 2010; Vereecken & McNeil, 2010). The great number of deceptive orchids and miniscule number of other plant species employing deception begs the question: why

are orchids so diverse and how have orchids evolved deceptive pollination? Tools for phylogenetic analysis are improving thanks to advances in DNA sequencing. These tools can be used to map the diversity of orchids. Observation and experimentation on orchid pollination strategies can then be mapped onto trees to understand their evolution, and to see how the evolution of such traits influence speciation. In the case of sexual deception through chemical imitation, spectrometry methods can be used observe what chemicals orchids are using that are identical or similar to those found in female insects. By tracing chemical composition and using phylogenies, we can explain trends not observable through morphology alone. Together with knowledge of ecological factors, these methods may allow us to better understand what causes high orchid diversity and to better understand what causes orchids to evolve a deceptive strategy from a rewarding strategy and vice-versa.

Study system: Orchidaceae

The orchid family contains nearly 19,500 species, with radiation occurring in a relatively short period starting in the Mid-Cretaceous (Jersáková, Johnson, & Kindlmann, 2006). Attention was brought to the orchid system by Charles Darwin and his book *On the various contrivances by which British and foreign orchids are fertilised by insects*, which was published in 1862. Darwin was skeptical about pollination by deceit, questioning observations by Christian Sprengel in 1793 that some orchids did not offer nectar as a reward (1877; 1793). However, later studies on orchid species around the world have confirmed that deceptive pollination occurs in nature, with studies in the early 1900s being some of the first examples of sexual deception in pollination (Coleman, 1927; Pouyanne, 1917).

Orchids have a unique morphology that affects their pollination efficiency. A structure located in the center of the flower called the gynostemium contains both male and female reproductive organs, and a structure called the rostellum separates the organs (Cozzolino & Widmer, 2005). The rostellum helps prevent self-pollination, which reduces the benefits of pollinator attraction. Benefits are reduced because self-pollination often results in inbreeding depression and inefficient exportation of pollen. A field study on non-rewarding plant *Anacamptis morio* has shown that prevention of self-pollination is a factor that may affect the evolution of deceptive pollination (Johnson, Peter, & Agren, 2004). Inflorescences were manipulated through artificial addition of nectar. To test pollinator behavior, they used the bee-interview technique, presenting the control or manipulated inflorescences on a 2 m long cane to foraging *B. lapidarius* queen bees. Results show that queen bees probed flowers with nectar 2.3 times more often in addition to spending 5.3 times more time on inflorescences. A second component of the experiment tested for bending of pollinaria, a mechanism that prevents self-pollination. Longer visits provided enough time for pollinaria to bend towards the stigma of the same plant, leading to self-pollination. These results support the hypothesis that deceptive pollination increases the efficiency of pollen export by causing pollinators to leave early upon discovery that there is no nectar reward. This is not true for all species however, and there exists the possibility that other ecological factors like flowering time affect whether this phenomenon occurs or not. In *Barlia robertiana*, which flowers earlier in the season than *A. morio*, self-pollinations do not increase when nectar is added to the flower (Cozzolino & Widmer, 2005). Further tests of more species at both the genetic and ecological levels would help confirm whether prevention of self-pollination is a driving force in the majority of deceptive orchids and help explain if there are certain ecological factors that affect self-pollination in orchids.

Chemical attraction in sexually deceptive pollination

Unlike typical cases of pollinator attraction where common compounds are used to attract pollinators, sexually deceptive orchids use chemicals identical to those used by female insects for male attraction (Schiestl et al., 2003). This allows orchids to target a specific species of pollinator for attraction. Once the pollinator is attracted, the insect will attempt to copulate with the flower, only to discover there is no female and no nectar reward either. The insect instead receives pollen, increasing the orchid's chances of reproduction.

What drives the evolution of sexually deceptive orchids? A study by Whitehead and Peakall on two sympatric *Chiloglottis* orchid species *C. valida* and *C. jeanesii* has helped to answer this question. Sympatric species are especially helpful because we can see that reproductive isolation has occurred despite the species being in close geographic proximity. For speciation to occur in sympatry, there must be some kind of selection that prevents gene flow between populations, leading to reproductive isolation (Whitehead & Peakall, 2014). The study used genetic analysis, chemical analysis, and crossing of plants to determine the importance of prepollination and postpollination barriers. A lack of postpollination barriers was found, and the two species were even able to form F1 hybrids. This left only prepollination barriers as possible causes for reproductive isolation, an observation also made by Ayasse et al. (2011). Within possible prepollination barriers, geography, habitat, and phenology were not found to be isolating mechanisms. Floral isolation was discovered to be the reproductive barrier with the greatest effect. This finding confirms that attracting specific pollinators through chemicals is a driving force for speciation of sexually deceptive orchids.

Chemical mimicry has been observed to be a powerful method of deceiving pollinators, which explains why speciation through pollinator specificity occurs in orchids. A study by

Schiestl et al. on the orchid *Chiloglottis trapeziformis* and thynnine wasp *Neozeleboria cryptoides* tested for chemical attraction by using labella extracts from the orchid and head extracts of female wasps. They combined methods of gas chromatography and electroantennographic detection with male antennae to look for biologically active components. One compound was found, 2-ethyl-5-propylcyclohexan-1,3-dione (chiloglottone), which was identical in both the orchid and female wasps. Next, they tested if chiloglottone attracted more male wasps in the field. Five different tests were compared to the odorless control: the orchid flower, orchid labellum extract, a pheromone emitting female wasp, female head extract, and synthetic chiloglottone. All five tests resulted in a significantly greater number of male copulation attempts (see Figure 1). The synthetic chiloglottone results show that the chemical alone can result in male copulation attempts without the presence of the orchid or female wasp.

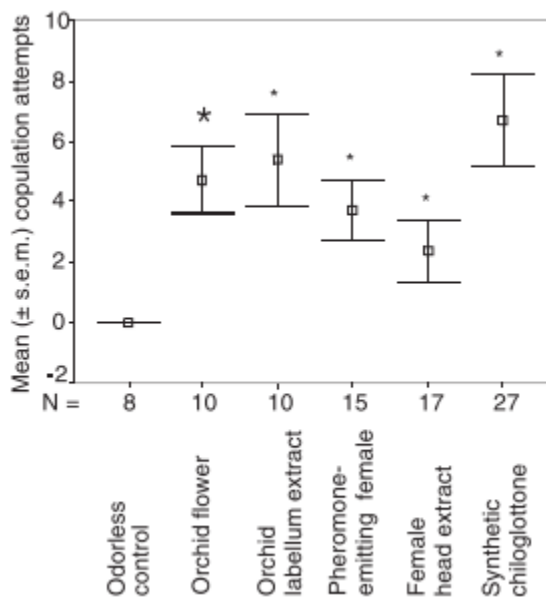


Figure 1. Data from Scheistl et al. (2003) showing the influence of the chemical chiloglottone.

Although visual cues are important in some species, they may not hold the same power that chemical cues have over pollinators. Unlike the chiloglottone case where wasps could not tell the difference between synthetic, orchid-produced, or wasp-produced chiloglottone, visual

cues offer a greater opportunity for pollinators to distinguish between females and mimics. For example, males of the bee fly *Megapalpus capensis* are attracted to fly-mimicking spots of the daisy *Gorteria diffusa* (de Jager & Ellis, 2014). Male bee flies have been found to learn through experience that they should avoid deceptive flowers. This could possibly lead to antagonistic coevolution, also known as an evolutionary arms race, where the bee fly evolves in response to deceptiveness in the plant. Later on evolution could occur in the plant in response to mimic recognition in the bee fly. Antagonistic coevolution is still possible when there is chemical exploitation, but what occurs is different. Looking at *C. trapeziformis* and *N. cryptoides* again, male wasps are unable to distinguish whether the orchid or the female produced the chemical, so rather than learning to avoid the orchid itself, they learn to avoid whole areas where the orchid is populated (Wong & Schiestl, 2002). Naturally, orchid fitness is reduced because the number of visits decreases. However, fitness of the wasp also decreases as a result of female wasps present in orchid-inhabited areas being unable to find mates. In Wong's experiment, females near orchids were approached less often and no copulation attempts were made by males. As orchid patch size increases, even fewer approaches are made by males. This example provides one explanation to why chemical deception is effective: first, chemicals can be made identically in orchids and female insects, and second, learning to avoid the orchid is difficult without a fitness cost.

A model for coevolution in sexual deception

Differentiating between coevolution and one-sided exploitation is important for understanding the evolution of deceptive plants and their pollinators. A model proposed by Lehtonen and Whitehead addresses this need. The model examines four components: the cost of

mimicry, the cost to the receiver for being fooled, the density of mimics, and the relative magnitude of the mimicry-independent component of fitness (Lehtonen & Whitehead, 2014). Such as discussed above, observed fitness costs to male wasps due to orchid avoidance could be used to predict the degree of selective pressure on the wasp. Other examples of costs that could exert selective pressure include: preference of mimics over females, unfinished copulation with a female from distraction by a mimic, sperm wastage, and pollinator death from being trapped in a flower (Lehtonen & Whitehead, 2014). In all these examples, males lose opportunities for real matings with a female, producing a larger fitness cost than simply being fooled by a mimic (Kokko & Heubel, 2011; Wiley, 2013). High fitness costs help keep receivers from being too choosy, explaining why the model predicts that mimicry is evolutionarily stable when mimicry is accurate and receivers have low choosiness. Differing costs also exist for mimics. Visual mimicry of female insects by altering floral morphology is thought to be more costly than mimicking chemical signals, in addition to being more difficult to imitate perfectly (Lehtonen & Whitehead, 2014). Combining the lower costs with the observed effectiveness of chemical mimicry makes it clear to see why this kind of deception is a viable strategy. Next, frequency and density of a mimic was found to be the best predictor of whether a coevolutionary arms race could result, while the costs to the mimic and the receiver decided the winner of an arms race. When costs to both the receiver and mimic were high, the model found that the receiver was the winner of the race. However, when mimicry is cheap, the model predicts that the mimic will be the winner. These predictions again emphasize the importance of costs towards development of effective mimicry.

In a comparison of the *Chiloglottis* orchid phylogeny with the *Neozeleboria* wasp phylogeny, coevolution seems to play a role. A pattern of congruence was observed, with related

orchids often using related wasps as pollinators (Mant, Schiestl, Peakall, & Weston, 2002). These patterns of chemical development through coevolution may be useful for identifying causes of speciation. This experiment is also one of many that demonstrates the effectiveness of combining biological information with phylogenetic data.

Phylogenetic analyses of orchids

Using phylogenetic analyses may become an important tool in tracing the evolution of deceptive pollination in orchids. By knowing the patterns of ancestry and descent of orchids, further research in morphology and chemical composition can tell us how evolution has occurred. Contemporary methods allow for construction of phylogenetic trees through sequencing of DNA. Some methods that have been used are allozyme analysis, RPLP analysis, plastid DNA sequencing, and nuclear DNA sequencing (Bateman et al., 2003). For instance, one study of the *Apostasioideae* subfamily of orchids used ITS (nuclear), trnL-F (RPLP), and matK (plastid) sequences to determine the sister taxa of *Apostasioideae* and the relationships of the species within *Apostasioideae* (Kocyan, Qiu, Endress, & Conti, 2004). They determined that *Apostasioideae* is monophyletic and is a sister group to the rest of the Orchidaceae through matK analysis (see Figure 2). The patterns for the *Neuwiedia* genus are different than expected through a solely morphological analysis. This demonstrates that factors other than morphology are important drivers in evolution of orchids.

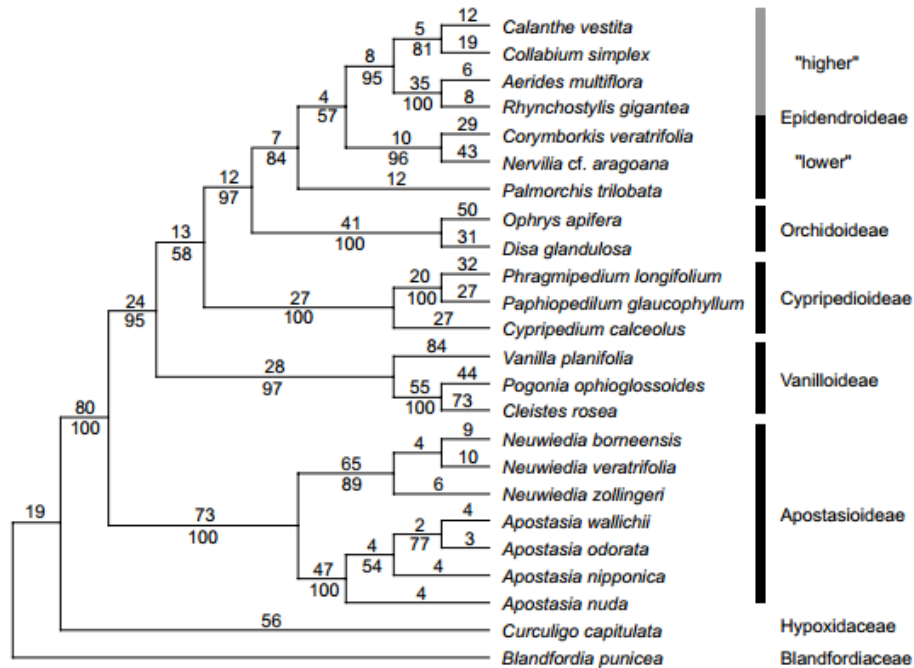


Figure 2. From Kocyan et al. (2004). The most parsimonious tree from matK analysis which supports that apostasioids form a monophyletic group.

Phylogenetic analysis has already indicated some general trends regarding speciation in orchids. Differences are apparent in divergence of food deceptive orchids and divergence in sexually deceptive orchids. ITS sequencing results have shown that genetic distances between species are smaller in sexually deceptive species (see Figure 3) (Bateman et al., 2003). The hypothesis that could explain this is that speciation occurs quickly in sexually deceptive orchids (Cozzolino & Widmer, 2005). The *Ophrys* clade typically uses visual cues, so fast speciation is difficult to imagine when cues need to be specific to gain fitness benefits. An alternate hypothesis is that hybridization allows for adaptive radiation, because hybridization creates genetic variation, facilitating colonization of populations into new environments (Seehausen, 2004). More research is needed to come to a conclusion, and addition of more clades could tell us more about speciation in different kinds of orchids. Being able to compare speciation speed of

sexually deceptive orchids that rely on chemical cues with those that rely on visual cues could also have some important revelations.

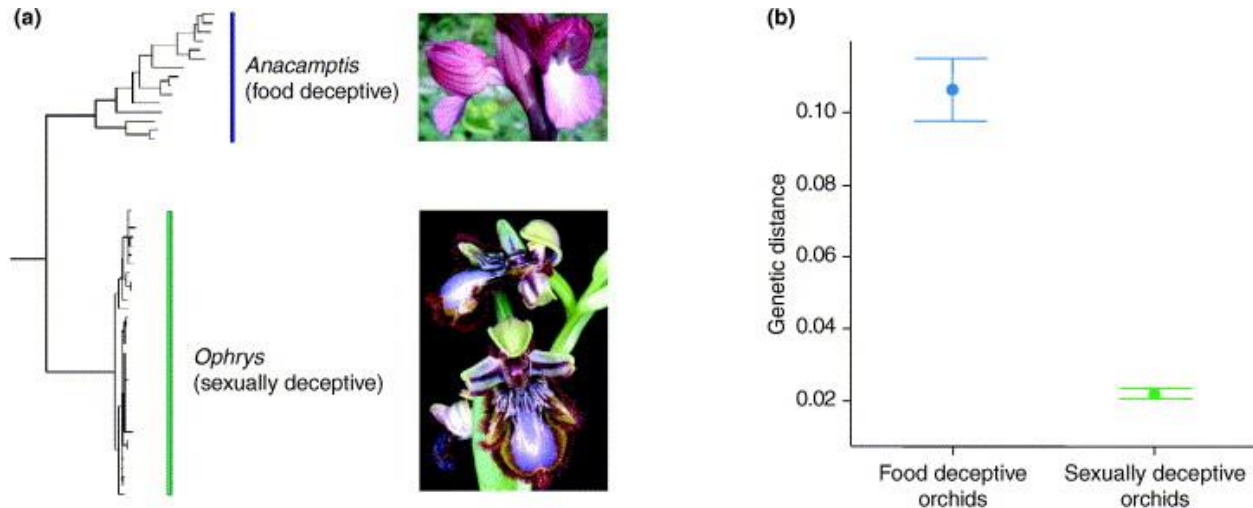


Figure 3. Comparison of the clades *Anacamptis* and *Ophrys*. Image from (Cuzzolino & Widmer, 2005), data from (Bateman et al., 2003).

Phylogenies have also proven to be a useful method to narrow down traits or interactions that are likely driving forces for orchid speciation. When Roche et al. discovered that the symbiotic fungus *Tulasnella* was associated with many species of the sexually deceptive orchid *Chiloglottis*, they used phylogenetic analyses of ITS sequences to test whether the symbiotic interaction could be an important factor in orchid diversification. The phylogeny would show whether orchid-fungus relationships were specific. Since sexual deception is a highly specific pollination strategy, findings of specificity with fungi would have implications for orchid diversity. (Roche et al., 2010). Results showed that six *Chiloglottis* species were associated with *Tulasnella* lineages, disproving the hypothesis that orchid-fungus interactions are a driving force for orchid speciation. Instead, specific pollination through chemical cues remains as the most likely driving force for orchid speciation.

Study of related species has added to our understanding of chemical mimicry. Chemical tests of the *Chiloglottis* genus has revealed that the majority of the species use one or more of the six variations of the chiloglottone chemical (Peakall et al., 2010). Peakall's team also found chiloglottone in *Arthrochilus prolixus* and *Paracaleana minor*. They suggest that this finding of chiloglottone in three orchid genera indicates possible widespread involvement of chiloglottones in orchid-wasp interactions. Phylogenetic analysis followed their chemical studies, and they mapped floral chemistry onto the tree to look for patterns. Doing this resulted in polyphyly of chiloglottones, because *Paracaleana* shared types 1, 2, 3, and 5 with *Chiloglottis* and *Arthrochilus* also shared type 2 with both groups. Other patterns noticed within *Chiloglottis* were that chemical types are shared both within closely related taxa and among distantly related taxa. Overall, even closely related species were found to have different chemical types. However, they did not look further into how this may translate biologically. It would be interesting to see further research that checks if there are changes in pollinator species in these orchids to see how changes in chiloglottone types may relate to changes in pollination strategy. Nevertheless, Peakall's study is a great start in demonstrating how a combination of chemical and phylogenetic analyses can deepen understanding on orchid speciation.

Conclusion

Orchids are a diverse family with a large number of species that employ deceptive pollination. Traditional pollination has typically involved some kind of reward, so orchids present many evolutionary questions with their high diversification through deceptive pollination. Deception through chemical cues is a highly specific method of pollination, with chemicals being identical in many cases. Modeling has shown that chemical cues are also cheaper and

easier to mimic compared to visual mimicry of female insect bodies which can be costly and tough to imitate. Many studies have also provided evidence for sexual deception through chemical cues as a driving force for evolution and speciation, including in sympatric sites. These discoveries make orchids an ideal study system for looking at diversification and speciation.

A greater number of tools are at our disposal in modern times to make this kind of study possible. Spectrometry methods are available to identify chemical structures in orchids and their pollinators, allowing us to see when orchids are using chemical mimicry. New techniques in DNA sequencing make phylogenetic analyses easier and more accurate. With phylogenetic trees showing relationships between species, mapping chemical data onto these trees could improve our ability to recognize patterns that lead to speciation. A few studies such as Peakall et al. in 2010 have begun to analyze both chemical and phylogenetic data in the same experiment. These studies should be taken further in biological analysis to determine whether the chemical patterns observed have any meaning in terms of pollinator relationships. If patterns between chemicals and pollinators correspond, we will be able to better explain what drives speciation in orchids. We could also partly explain why orchids diversify at a greater rate than other taxa. In the long term, deeper understanding of the role of chemicals in speciation could perhaps even be applied to systems unrelated to pollination.

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