

Pollination Efficiency and the Evolution of Specialized Deceptive Pollination Systems

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ABSTRACT: The ultimate causes of evolution of highly specialized pollination systems are little understood. We investigated the relationship between specialization and pollination efficiency, defined as the proportion of pollinated flowers relative to those that experienced pollen removal, using orchids with different pollination strategies as a model system. Rewarding orchids showed the highest pollination efficiency. Sexually deceptive orchids had comparably high pollination efficiency, but food-deceptive orchids had significantly lower efficiency. Values for pollinator sharing (a measure of the degree of generalization in pollination systems) showed the reverse pattern, in that groups with high pollination efficiency had low values of pollinator sharing. Low pollinator sharing may thus be the basis for efficient pollination. Population genetic data indicated that both food- and sexually deceptive species have higher degrees of among-population gene flow than do rewarding orchids. Thus, the shift from food to sexual deception may be driven by selection for more efficient pollination, without compromising the high levels of gene flow that are characteristic of deceptive species.

Keywords: food deception, pollination efficiency, pollination strategy, pollinia, sexual deception, specialized pollination.

Introduction

Most flowering plants rely on insects for pollination, and the extraordinary evolutionary success of this plant group is likely due to the efficiency of animals as pollen vectors, as compared with wind pollination (Pellmyr 2002). While it is often assumed that pollination efficiency (PE) is a key factor for the evolution of pollination systems, comparative data on pollen loss in different pollination systems are scarce.

The orchid family is renowned not only for its enormous diversity of pollination mechanisms (Darwin 1862; Van

der Pijl and Dodson 1966) but also for the unusually common occurrence of species with nonrewarding flowers (i.e., attracting pollinators by deception; Van der Pijl and Dodson 1966; Dressler 1981; Dafni 1984; Schiestl 2005; Jersáková et al. 2006): about one-third of all orchid species (~6,500–10,000; according to Ackerman 1986) are nonrewarding, suggesting an important adaptive advantage of this pollination strategy. Most nonrewarding orchids employ generalized food deception to attract their pollinators (Jersáková et al. 2006). These species are typically pollinated by a broad assemblage of flower visitors (Dafni 1987; Nilsson 1992; Van der Cingel 1995; Cozzolino et al. 2005). A different and less common mode of deception, exclusive to the orchid family, is based on attraction of male insects through mimicry of the visual and olfactory sexual signals of female insects (Kullenberg 1961; Schiestl et al. 1999; Ayasse et al. 2003; Schiestl 2005). An outstanding feature of sexually deceptive orchids is the high level of specificity in their pollination systems, as most species are pollinated by a single species of insect (Paulus and Gack 1990; Bower 1996).

Sexual deception seems to have evolved in different orchid clades (see Van der Cingel 2001; Jersáková et al. 2006); however, the best-documented evolutionary radiations involving transitions to sexual deception are those reported in the Orchidinae and Caladeniinae (Schiestl 2005). Phylogenetic analyses suggest that food deception is the likely ancestral pollination strategy in these groups (Cozzolino and Widmer 2005) and that some transitions have occurred between pollination systems. For example, sexual deception and nectar reward evolved from food deception in both the Orchidinae and the Diuridae (Cozzolino et al. 2001; Kores et al. 2001). Similarly, it has been shown for the South African genus *Disa* that nectar-rewarding and sexually deceptive species evolved from food-deceptive ancestors (Johnson et al. 1998). The independent occurrence of these shifts and the apparent concordance in their di-

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rection (from food to sexual deception) suggest a common fitness advantage underlying this change of pollination strategy. However, while a few studies have focused on the fitness implications of deceptive versus rewarding strategies (Johnson and Nilsson 1999; Smithson 2002; Johnson et al. 2004; Jersáková and Johnson 2006), there has been very little progress in uncovering the selective factors that have driven the shifts from food to sexual deception.

Male mating success in plants depends largely on the fates of pollen. These fates include overall pollen receipt by stigmas, rates of pollen removal, and the overall PE, which can be defined as a ratio of pollinated flowers to the flowers that experienced pollinaria removal (Johnson et al. 2004; Tremblay et al. 2005). In orchids, packaging of pollen in discrete pollinia makes it relatively easy to estimate pollen removal and deposition (Johnson and Edwards 2000). Pollen fates depend strongly on patterns of pollinator behavior, which differs among pollination systems. When visiting rewarding species, for example, pollinators tend to restrict their visits to flowers of a single species (Waser 1986). This so-called flower constancy represents short-term specialization (Grant 1950; Heinrich 1976; Pellmyr 2002) and leads to higher foraging efficiency by the pollinator and to a higher probability of intraspecific pollination for the plant (Free 1963; Waser 1986). However, when foraging on rewarding plants, insects also tend to visit more flowers per plant and spend considerable time on each flower (Ne'eman et al. 2006). This can lead to high levels of pollinator-mediated self-pollination both within and among flowers on a plant (Johnson and Nilsson 1999; Johnson et al. 2004; Jersáková and Johnson 2006). On the contrary, the tendency of pollinators to visit fewer flowers and fly longer distances after visiting food-deceptive orchids (Jersáková and Johnson 2006) greatly increases the chances of outcrossing (Johnson et al. 2004). However, in food-deceptive systems, this outcrossing advantage may be counterbalanced by low levels of pollinator visitation and low levels of fidelity by pollinators (Schiestl and Schlüter 2009). Low pollinator fidelity is evidenced by high values of pollinator sharing (Cozzolino et al. 2005), a form of generalization in pollination that likely leads to high losses during pollen transfer.

Sexually deceptive orchids exclusively attract male pollinators of a particular insect species (summarized in Schiestl 2005) and show lower values of pollinator sharing (Schiestl and Schlüter 2009). Pollen fates may therefore differ from those in food-deceptive species. Lower pollinator sharing suggests higher PE, but the extreme specialization in this system can lead to low overall levels of fruit set (Ayasse et al. 2000). On the positive side, mate-seeking behavior has been suggested to foster high levels of cross-pollination and long-distance pollen flow (Peakall 1990; Peakall and Beattie 1996; Peakall and Schiestl 2004).

As yet, comparative data on reproductive performance in these different pollination systems are lacking, thus making it difficult to infer the driving forces for the evolution of pollination system diversity. The aim of this study is to determine the influence of pollination strategy, and the associated levels of outcrossing, pollinator sharing, and specialization, on the fates of pollen by comparing PE in multiple populations of food-deceptive, rewarding, and sexually deceptive orchid species.

Methods

Data Collection

This study was conducted as part of a large survey of orchid populations of Italy (March–June 2006 and 2007) and Western Australia (September–October 2007). All the species included in our study belong to the subtribes Orchidinae and Caladeniinae and were selected in order to gain a representative sample of each of the three most widespread pollination strategies characterizing this group (i.e., reward pollination, food deception, and sexual deception). For the Orchidinae, we analyzed 37 Italian orchid populations representing 8 genera and 31 species, with 6 rewarding species (8 populations), 16 food-deceptive species (19 populations), and 9 sexually deceptive species (10 populations; table A1 in the online edition of the *American Naturalist*). Field observations were conducted on a total of 1,911 individuals (8,772 inspected flowers). For the Caladeniinae, in the southwestern part of Western Australia, we analyzed eight populations representing three genera and seven species, with two rewarding species (two populations), three food-deceptive species (four populations), and two sexually deceptive species (two populations; table A1). Field observations were conducted on a total of 419 individuals (483 inspected flowers).

Because pollinators influence the fitness of hermaphrodite plants through both male and female components, we collected data on pollen removal and pollen deposition. From a random sample, we recorded the number of flowers with at least one pollinium removed and the number of flowers with any amount of pollen on the stigma of flowers in each population.

Calculation of Reproductive Success and Pollination Efficiency

The male function of pollinaria removal (hereafter male reproductive success [MRS]) was calculated as $(F_r/F_{\text{tot}}) \times 100$, where F_r is the number of flowers found with one or both pollinia removed and F_{tot} is the total number of investigated flowers (calculated for each population as the mean number of open flowers per individual

multiplied by the number of individuals investigated). Female reproductive success (FRS) was calculated as $(F_p/F_{tot}) \times 100$, where F_p is the number of pollinated flowers (i.e., the number of flowers found with at least one pollen massula on the stigma).

Pollinaria removals in orchids usually exceed pollinations, so PE can be expressed as a ratio of the latter to the former: F_p/F_r . The value of PE potentially ranges between 0 and 1, with 1 representing the maximum and 0 the lowest efficiency.

To test whether PE was influenced by local environmental conditions, we sampled two different populations for each of seven species (two rewarding, four food deceptive, and one sexually deceptive). We collected our data when flowers were still open, and therefore our reproductive success values (both MRS and FRS) could represent an underestimation of the values at the end of flowering time. An underestimation of FRS and PE could also be due to the fact that for the Orchidinae data set, we did not record the few cases in which we found pollen on the stigma of flowers from which no pollinia were removed. Nevertheless, these caveats apply to all the sampled populations and therefore do not affect the reliability of our comparative study. Finally, we were unable to discriminate between conspecific and heterospecific pollen on stigmas, and this could result in an overestimation of PE and FRS.

In addition to the empirical data collection, among-population gene flow was estimated by comparing the level of mean genetic differentiation (G_{st}) between rewarding, food-deceptive, and sexually deceptive orchids, using data available in the literature (reviewed in Forrest et al. 2004; Tremblay et al. 2005).

Specialization in Pollination

Data on numbers of pollinators and pollinator-sharing indexes for the orchid genera investigated here are taken as a subsample of an earlier meta-analysis (for detailed methodology, see Schiestl and Schlüter 2009) and reanalyzed using a one-way ANOVA. Data for the following genera were available: *Gymnadenia*, *Platanthera* (rewarding), *Anacamptis*, *Orchis* (food deceptive), *Caladenia*, and *Ophrys* sect. *Euophrys* and sect. *Pseudophrys* (sexually deceptive). *Ophrys* was split into two subsections to avoid the recording of pollinator sharing enabled through different placement of pollinia on the pollinators (head and abdomen pollination; morphological isolation). *Platanthera* was not included in the analysis because in this genus, the placement of pollinia on different parts of an insect body may allow for higher pollinator sharing without pollen loss. Thus, for rewarding species, we used only *Gymnadenia*.

Data Analysis

Statistical analyses were performed using the SPSS 13.0 statistical package (SPSS, Chicago). Because our data were not normally distributed, we used the nonparametric Kruskal-Wallis test with Mann-Whitney U -tests for a posteriori multiple comparison, with the significance level set to 0.01 (Bonferroni correction). We compared MRS, FRS, PE, and G_{st} among the three pollination systems. All tests of significance were two tailed.

When data were available, the two components of reproductive success (MRS and FRS) and PE were calculated at the population level (by using the data of each population independently) and at the species level (by averaging the data from different populations of the same species). All statistical analyses were performed at both levels.

Phylogenetically Independent Comparisons

A potential limitation of multispecies comparative studies is that species descend from common ancestors in a hierarchical fashion and that species traits are therefore not independent (Felsenstein 1985). In order to place our comparisons in a phylogenetic framework, using published molecular phylogenies (Hopper and Brown 2001, 2004; Bateman et al. 2003; Devey et al. 2008), we selected a set of strictly independent species pairs (i.e., whose internal nodes do not overlap; see table A2 in the online edition of the *American Naturalist*; Felsenstein 1985). Independent species pairs were selected in order to maximize the number of possible comparisons. Then, we evaluated the PE differences of independent species pairs with the same pollination strategy (i.e., food deceptive vs. food deceptive, sexually deceptive vs. sexually deceptive, rewarding vs. rewarding), and we compared them with PE differences of independent species pairs with different pollination strategies (food deceptive vs. sexually deceptive, food deceptive vs. rewarding, sexually deceptive vs. rewarding). Because of the lack of a strong phylogenetic framework for most of the species included in the comparison of levels of G_{st} between rewarding, food-deceptive, and sexually deceptive species, we did not correct this particular analysis for phylogeny.

Results

We found significant differences in all investigated reproductive parameters among the three pollination systems studied at population level (MRS: $\chi^2 = 21.45$, $df = 2$, $P < .001$; FRS: $\chi^2 = 20.79$, $df = 2$, $P < .001$; PE: $\chi^2 = 27.76$, $df = 2$, $P < .001$) and species level (MRS: $\chi^2 = 17.70$, $df = 2$, $P < .001$; FRS: $\chi^2 = 16.71$, $df = 2$, $P < .001$; PE: $\chi^2 = 23.22$, $df = 2$, $P < .001$; fig. 1). Mean values

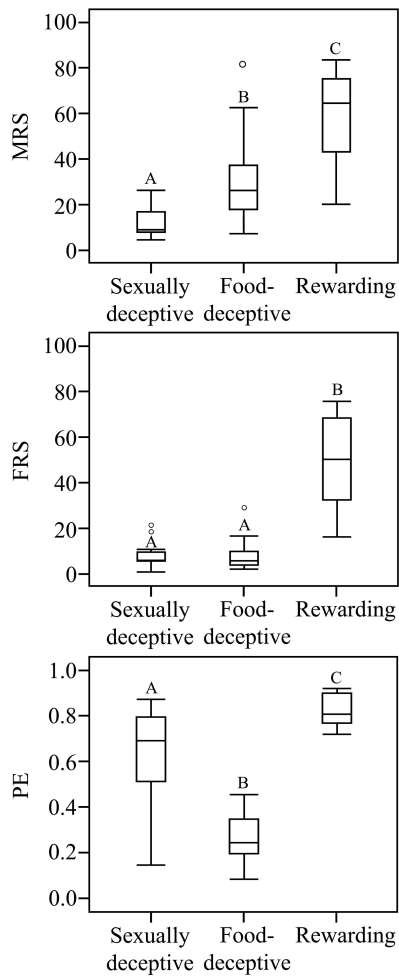


Figure 1: Comparison of measures of reproductive success among orchids with different pollination strategies (rewarding, food-deceptive, and sexually deceptive). MRS = male reproductive success; FRS = female reproductive success; PE = pollination efficiency. Different letters indicate significant differences (Mann-Whitney U -test, $P < .01$ after Bonferroni correction). Circles represent outliers.

of MRS, FRS, and PE for the three investigated pollination strategies are listed in table A1.

Male reproductive success. MRS was lower in sexually deceptive species than in food-deceptive species (population level: $U = 39.0$, $P < .001$; species level: $U = 35.0$, $P = .002$) and rewarding species (population level: $U = 3.0$, $P < .001$; species level: $U = 3.0$, $P < .001$). Food-deceptive species also had lower MRS than did rewarding species (population level: $U = 44.0$, $P = .004$; species level: $U = 25.0$, $P = .005$; fig. 1).

Female reproductive success. FRS was higher in rewarding species than in food-deceptive species (population level: $U = 6.0$, $P < .001$; species level: $U = 4.0$, $P < .001$) and

sexually deceptive species (population level: $U = 2.0$, $P < .001$; species level: $U = 2.0$, $P < .001$). Food-deceptive species and sexually deceptive species had similar values of FRS (population level: $U = 135.0$, $P = .932$; species level: $U = 97.5$, $P = .767$; fig. 1).

Pollination efficiency. PE was lower in food-deceptive species than in sexually deceptive species (population level: $U = 31.0$, $P < .001$; species level: $U = 27.0$, $P < .001$) and rewarding species (population level: $U = 0.0$, $P < .001$; species level: $U = 0.0$, $P < .001$). Rewarding species showed a higher value of PE than did sexually deceptive species, but the differences were significant only at the species level (population level: $U = 27.0$, $P = .030$; species level: $U = 13.0$, $P = .009$; fig. 1).

In the analysis of PE of multiple populations of the same species, we found similar values in six out of the seven comparisons (see table A1). Differences range from 0.02 in *Anacamptis papilionacea* to 0.12 in *Ophrys fusca*, with a mean of 0.06.

The G_{st} from literature data differed significantly among the three groups (fig. 2; $\chi^2 = 12.816$, $P = .002$). Food-deceptive and sexually deceptive orchids were not significantly different ($U = 330.5$, $P = .725$). Rewarding species' G_{st} values were higher than those of food-deceptive ($U = 292.5$, $P = .001$) and sexually deceptive ($U = 143.5$, $P = .01$) species.

Phylogenetically independent comparisons showed that the results were robust to statistical phylogenetic correction. In the comparison between food and sexual deception, the differences among independent species pairs with different pollination strategies were significantly higher than those among independent species pairs with the same pollination strategy ($U = 0.00$, $P = .003$); the same was

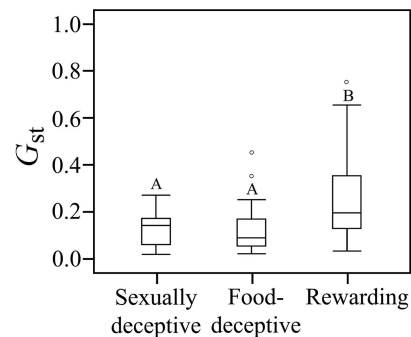


Figure 2: Comparison of levels of mean genetic differentiation (G_{st}) among orchids with different pollination strategies. Data from Forrest et al. (2004) and from Tremblay et al. (2005) are uncorrected for phylogeny; the data set includes all the species for which literature data were available. Different letters indicate significant differences (Mann-Whitney U -test, $P < .01$ after Bonferroni correction). Circles represent outliers.

true for the comparison between food deception and reward pollination ($U = 0.00$, $P < .001$) but not for the comparison between reward pollination and sexual deception ($U = 9.50$, $P = .188$).

The diversity of pollinators was lowest in sexually deceptive species ($F_{2,3} = 16.69$, $P = .02$; table 1). Pollinator sharing was similar in the rewarding orchid genus *Gymnadenia* and the sexually deceptive genera, but food-deceptive orchids had the highest value of pollinator sharing ($F_{2,3} = 29.44$, $P = .01$; table 1).

Discussion

Male and female components shape the total reproductive success of hermaphroditic plants and are thus of key importance for the evolution of pollination systems. Although local physical and biotic environmental factors are expected to influence pollen fate (Fritz and Nilsson 1996; Ackerman et al. 1997; Parra-Tabla et al. 2000; Maad and Alexandersson 2004), leading to population variation, our analysis of multiple populations (table A1) suggests that PE is mostly characteristic of species rather than populations.

Our results show that MRS and FRS differ strongly among the three groups of orchids with different pollination strategies (fig. 1). Food-deceptive species had high MRS (pollen removal) but low FRS (pollinated flowers) and thus low PE. In contrast, rewarding species showed high rates of MRS and FRS, leading to high PE. Sexually deceptive species, despite showing low MRS and FRS, have high PE, presumably because of low pollen loss in the transfer process. Thus, despite the similar values of FRS for food- and sexually deceptive orchids, these results suggest that specific pollen delivery arising from sexual deception could be the driving force explaining its relatively frequent independent evolution from food-deceptive ancestors. Indeed, variants that improve the probability of their pollen reaching a stigma would have the important male advantage of siring more offspring despite producing less pollen (and thus fewer flowers). This could be strongly beneficial for plants by relieving the trade-off between investment in flowers and other life-history traits or defense. Thus, in this regard, the efficiency of sexual deception may

have important implications for overall resource allocation and life histories of a species and can produce a selective advantage.

An explanation of the low PE in food-deceptive orchids may be their low specificity for particular pollinators and high degree of pollinator sharing with other plant species (Cozzolino et al. 2005; Schiestl and Schlüter 2009; table 1). The use of a diverse range of pollinators may lead to high pollen removal but cause more pollen to be lost in the transfer process or to be deposited on heterospecific stigmas. Providing a reward for the pollinators seems to improve pollination success. In accordance with earlier studies (reviewed in Tremblay et al. 2005), we found dramatically higher pollination success in rewarding orchids compared with food-deceptive species (fig. 1); in addition, PE was significantly higher in rewarding orchids (but see Ackerman et al. 1994). Our results were obtained by analyzing temperate terrestrial orchid species, whereas most orchids are tropical and epiphytic. However, by calculating PE data from a general survey of pollination success in orchids (table 3 in Tremblay et al. 2005) that includes tropical and epiphytic species, we found the same significant trend for PE to be higher in rewarding species than in deceptive ones ($U = 64.0$, $Z = -1.388$, $P < .001$, $n = 60$). This finding confirms that rewarding orchids export much more pollen to conspecific stigmas than do food-deceptive orchids, which may be a consequence of flower constancy of pollinators in the presence of a reward (Wells et al. 1983; Waser 1998; Chittka et al. 1999).

Given that rewarding orchid species have higher fruit set and higher PE than do deceptive species, it seems paradoxical that deceptive species are so common among orchid lineages, especially with respect to the other flowering plant families (Gill 1989). Rewarding orchids may, however, experience higher levels of pollen discounting and inbreeding depression in progeny as a consequence of geitonogamy (Johnson and Nilsson 1999). Nectar supplementation experiments with food-deceptive orchids have shown that pollinators tend to visit more flowers on an inflorescence in the presence of a reward, leading to higher levels of pollinator-mediated self-pollination (Johnson et al. 2004; Jersáková and Johnson 2006). In addition, rewarding plants are often characterized by a high propor-

Table 1: Mean numbers (\pm SD) of pollinator species and pollinator sharing index (\pm SD) between orchid species belonging to genera with different pollination strategies

	Reward ($n = 1$)	Food deception ($n = 2$)	Sexual deception ($n = 3$)	P
No. pollinator species	5.75	6.75 \pm 2.25	1.2 \pm .09	.02
Pollinator sharing	.01	.12 \pm .02	.05 \pm .02	.01

Note: n = number of genera included (see "Methods" for details).

tion of near-neighbor matings, which may restrict pollen dispersal distances and increase genetic differentiation among patches/populations (Turner et al. 1982). Our finding of higher G_{st} among different populations of rewarding orchids compared with deceptive species (fig. 2) is consistent with the expected higher levels of among-population gene flow in these latter plants. Therefore, it seems that nectariferous orchids trade off these negative factors of geitonogamy and short-distance pollen dispersal for higher pollination success and PE. The evolution of reward from food deception is thus expected in situations with low inbreeding depression and/or low pollinator abundance (Johnson et al. 2004).

Sexual deception is a particularly unusual pollination system, with high specificity and the exclusive recruitment of male insects as pollinators (Schiestl 2005), and it seems to have evolved multiple times independently from food deception, which suggests that there may be a common selective factor behind these transitions. However, the lack of a robust phylogenetical framework for a large part of the orchid family makes it difficult to infer how representative the transitions from food deception to sexual deception are in the wider context of the orchid family. An alternative pattern has been suggested, for instance, for the South American genus *Mormolyca* (Maxillariinae), where sexual deception could have evolved from nectariferous ancestors (Singer et al. 2004).

Our results, from analyses of both the full data set and the phylogenetically independent species pairs, show that sexual deception leads to higher PE compared with food deception because of improved pollen delivery (fig. 1). In contrast to food-deceptive species, sexually deceptive orchids are pollinated only by male insects through mating behavior, and, as a consequence, pollination is more specific, and pollinator sharing is lower (Schiestl and Schlüter 2009). Specialization and low pollinator sharing could be an advantage for reproductive success if they lead to a reduction of pollen loss through the high fidelity of the male pollinators that are unlikely to visit other sexually deceptive species and that also spend less time foraging on other flowers (Ne'eman et al. 2006; Schiestl and Schlüter 2009). As shown for food deception (e.g., Jersáková and Johnson 2006), high levels of cross-pollination and long-distance pollen dispersal mediated by pollinator behavior are also expected in sexual deception (Peakall 1990; Peakall and Beattie 1996; Peakall and Schiestl 2004). Our estimates of G_{st} are consistent with this assumption and suggest that sexually deceptive species retain the advantage of high levels of gene flow among populations after the evolutionary transition from food deception (fig. 2). This indicates that, in contrast to that in rewarding orchids, the high PE in sexually deceptive species is not counter-

balanced by higher levels of pollinator-mediated self-pollination and near-neighbor matings.

High levels of PE combined with extensive outcrossing may be a common advantage of pollination systems that involve reproductive behavior of their pollinators, as they are characterized by low values of pollinator sharing (Schiestl and Schlüter 2009). However, in spite of high PE, overall reproductive success is generally lower in sexually deceptive orchids than in rewarding orchids (Tremblay et al. 2005), which may constrain the evolution of this pollination system. Our findings suggest a prominent role for male function in the evolution of floral specialization. Further studies are needed to test whether this factor also drives evolutionary transitions among other pollination strategies in orchids and other plant groups.

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Sexually deceptive *Ophrys* orchid and its male pollinators. Photograph by Florian Schiestl.