CONTRASTING THOUGHTS ABOUT DECEPTIVE ORCHIDS: A RESPONSE TO SOBEL AND RANDLE

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Sobel and Randle (2009) challenge several methodological choices in the comparative study of the evolution of reproductive isolation in Mediterranean deceptive orchids of Scopece et al. (2007) including the species concept used and the selection of taxa, together with the perceived comparison of clades of different ages. They further criticize that pollinator information was taken from the literature and that two different methods were used to estimate pollinator specificity in food-deceptive and sexually deceptive orchids, respectively. Here we reply to these challenges.

Definition and Selection of Taxa

The problem of species sampling and delimitation is a critical issue in comparative studies and has been discussed in several previous studies on patterns of reproductive isolation, both in animals (Presgraves 2002) and plants (Moyle et al. 2004). Sobel and Randle (2009) question the species sampling and delimitation used in Scopece et al. (2007). For studies of reproductive isolation, the problem of species delimitation is of particular importance, especially in groups that are considered problematic, such as the genus Ophrys. The taxonomy of European orchids in general, and of the genus Ophrys in particular, is plagued by the “amateur species concept,” which means that species are often described by orchid enthusiasts without careful assessment of the validity of species status and without specifying the species concept that was applied to distinguish new species (Pillon and Chase 2007). This inflation of species designations has led to an increase of “species” in Ophrys from 16 (Sundermann 1980) to 252 (Delforge 2005) in just 25 years. When performing comparative analyses among species pairs in such groups, authors inevitably have to specify how taxa were selected for inclusion in a study, because the results of comparative studies may be affected by species sampling, especially when taxa of different taxonomic rank are included. Therefore, Scopece et al. (2007) used a conservative approach in their selection of taxa and only included representatives of morphologically and phylogenetically distinct groups (p. 2625, last paragraph). We refrained, however, from defining species in Ophrys, as advocated by Sobel and Randle (2009). Furthermore, we were wrongly cited by Sobel and Randle (2009) with respect to our phrase “and differing in their known (or suspected) pollinators,” which refers to species groups.
identified in a recent phylogenetic analysis (Devey et al. 2008), and not to our criteria for species selection as implied by Sobel and Randle (2009).

The approach chosen in Scopece et al. (2007) represents a pragmatic approach to a complex problem that defies any simple or ideal solution. If anything, however, our approach reduced the risk of estimating within species isolation in Ophrys, rather than the intended between species isolation. Otherwise, differences between orchids pollinated by sexual deceit and food deceit would be overestimated, and the weak postmating isolation found among Ophrys species could be a consequence of comparing conspecific entities. Thus, our choice of Ophrys species was conservative with respect to our estimates of postmating isolation, which were the aim of the study.

Admittedly, there are easier plant groups than orchids, and in many instances it may be possible to apply the recommendations of Sobel and Randle (2009). If, however, one decides not to simply ignore more complicated—and interesting—plant groups, in principle, a thorough and consistent species selection (even if subjective) needs to be established and applied. Potential biases introduced by such a selection need to be both acknowledged as a caveat to the study as well as investigated as to whether the selection criteria have affected the outcome of the study, as done by Scopece et al. (2007), who found that their results were not influenced by their choice of species.

Finally, Sobel and Randle (2009) criticize our choice to only include species that “occur in sympatry and have overlapping flower phenologies” and infer that “this study... does not necessarily provide information about general patterns of reproductive isolation evolution. Habitat and geographical isolation, for example, may play a prominent role in speciation.” We agree with Sobel and Randle (2009) on the importance of ecogeographic isolation in speciation even if, by virtue of disturbance, its historical importance in the Mediterranean region is difficult to work out. However, we were not primarily interested in identifying which components of reproductive isolation may play a prominent role in speciation but rather investigated which components of isolation are most important when there is no habitat or temporal isolation, because it is only in these situations, in which gene flow may occur among related species, that postmating barriers are important for the maintenance of species boundaries. For this, we specifically only selected species pairs with overlapping habitats and floral phenologies.

**Clade Ages**

Sobel and Randle (2009) find that “an additional difficulty is that these two deceptive strategies are in clades of vastly different ages.” First of all, a clade refers to a group of related organisms descended from a common ancestor. Although the sexually deceptive orchids used in Scopece et al. (2007) all belong to the genus Ophrys and conform to a clade, food deception occurs in several different genera that do not form a monophyletic clade (Bateman et al. 1997; Aceto et al. 1999). Scopece et al. (2007) were thus not comparing clades in the first place. Second, all genera included in Scopece et al. (2007) are closely related and belong to orchid subtribe Orchidinae. Relationships among major lineages are poorly resolved in available phylogenetic analyses, possibly because of similar ages (as suggested by a comparable number of autoapo- morphies leading to similar branch lengths of genera) and because some of the predominantly food-deceptive genera form a sister group to Ophrys (Bateman et al. 1997; Aceto et al. 1999). Sister groups by definition have the same age. What Sobel and Randle (2009) may want to say instead is that interspecific differences among species in each genus of food- versus sexually deceptive genera may be different (for a discussion see also Cozzolino and Widmer 2005). The reason for this pattern is unclear, but it may be a consequence of differences in the rate of speciation and extinction. Notably, a low level of genetic divergence among species, as reported in Ophrys, has been found also in the Australian sexually deceptive genus Chiloglottis (Mant et al. 2002). A consequence of this phenomenon is that only species pairs falling in a common interval of genetic distance should be compared, as done by Coyne and Orr (1989) and by Scopece et al. (2007). As was already noted by Scopece et al. (2007), the number of food-deceptive species pairs that falls into a common interval of genetic distance with the sexually deceptive species pairs is small. Yet, despite this small number, differences between groups were still marked and statistically significant.

We also recognize that our analysis includes a single clade of sexually deceptive orchids. Although food deception has arisen multiple times throughout all the orchid family, sexual deception, excluding few sporadic reports, (reviewed in Schiestl 2005) has only evolved once in the Mediterranean genus Ophrys and several times in the Australian orchids. Unfortunately, for these latter, with the exclusion of the above said genus Chiloglottis, very few phylogenetic data are already available and only few pollination studies have been done, thus not allowing a large comparative study as done by Scopece et al. (2007) with Mediterranean orchids.

**Pollinator Isolation**

Studies that have the aim to compare pollinator isolation between species should rely on pollinator observations and be performed experimentally by collecting data on pollen removal and deposition by each pollinator species, as advocated by Sobel and Randle (2009) and typically done in studies of individual species pairs (see e.g., Ramsey et al. 2003; Kay 2006). Unfortunately, this approach may not always be feasible in comparative studies, in
which large numbers of species are compared and direct observations on all species may not be feasible. The situation is aggravated in deceptive orchids, where pollination success is often low (Neiland and Wilcock 1995) and the number of observations of natural pollinations for each species included may preclude meaningful statistical analysis. As a consequence of this limitation, and because no direct comparisons of pollinator specificity between groups was intended, Scopece et al. (2007) compiled data on orchid pollinators from the literature.

As Sobel and Randle (2009) rightly point out, it is difficult to extract valid estimates of pollinator specificity purely from literature reports, but we feel that the approach chosen in Scopece et al. (2007) was justified for the following reasons. First, the comparison of pollinator specificity in the two orchid groups was not the primary aim of the study but was done in an attempt to quantify the well-known differences in pollinator specificity that exist between sexually deceptive orchids on the one hand, and food-deceptive orchids on the other hand, to compare these estimates of pollinator isolation to other components of reproductive isolation. Thus, the well-known difference in pollinator specificity between the two groups was a prerequisite for the study and known beforehand. This was expressed at the end of the introduction in Scopece et al. (2007), where we stated that “we tested the hypothesis that differences in specificity of orchid—pollinator associations (i.e. premating isolation) affect the insurgence of post-mating reproductive isolation. For this purpose, we comparatively investigated two closely related groups of orchids with different pollination specificity: the more specialized sexually deceptive species (genus Ophrys) and the less specialized food-deceptive species (genera Anacamptis, Dactylorhiza, Neotinea, Orchis).” Second, our choice to use two different approaches to estimate pollinator isolation in the two orchid groups may not have been ideal from a purist’s perspective, but was warranted given the biological differences between the two groups. Sexual-deceit pollination in the investigated Ophrys species relies on the plant’s ability to emit floral cues that mimic the visual and olfactory signals of females of a single insect species (in most cases solitary bees) and thus allows these orchids to specifically attract the males of this pollinator species that attempt copulation with the flowers, which leads to pollinia removal or deposition, and thus to pollination (reviewed in Schiestl 2005). Sexually deceptive orchids are therefore highly specialized to attract a single pollinator species and are often cited as prime examples of floral specialization (Grant 1994; Alcock 2005). In contrast, food-deceptive orchids exploit the innate association in naïve nectar-seeking insects between large and showy flowers and food reward (nectar in the case of Mediterranean food-deceptive orchids) (Dafni 1984). Pollinators duped by these orchids are pollinators foraging for nectar in spring, including bumblebees (Bombus), solitary bees, butterflies, and hover flies (Van der Cingel 1995). Because pollinators in spring may be scarce and some quickly learn to avoid the nectarless orchid flowers, food-deceptive orchids have a highly promiscuous pollination system and thus a very broad spectrum of pollinators (Dafni 1984; Jersák et al. 2006). As a consequence of the lack of specialization on one or few pollinator species, food-deceptive orchids that have overlapping flowering phenologies and grow in sympathy may share not only the same pollinator species, but even the same individual pollinators, which may lead to pollen transfer between species (Cozzolino et al. 2005).

These marked biological differences between the two investigated orchid groups led us to use two different estimates of pollinator isolation. For sexually deceptive orchids, typically pollinated by a single pollinator species, we defined pollinator isolation by species. In contrast, for food-deceptive species we grouped pollinators into functional classes, as proposed by Fenster et al. (2004). For both groups, pollinator information was gathered from literature data. This choice may not seem ideal, as it is not based on direct and personal observations, but it has the great advantage that it is based on decades of observations of orchid pollination biology and is thus less likely to be flawed by small numbers of observations that are carried out on a limited geographic and temporal scale, as is often the case in individual studies of pollinator isolation. This is of particular importance for food-deceptive orchids, as it is well established that pollinator densities and the composition of pollinator assemblages often differ substantially across years and in different geographic areas of a plant’s distribution range (Herrera et al. 2006). For instance, for some widely distributed food-deceptive species, for which pollinators have been investigated in independent studies (Nilsson 1980, 1983, 1984; Cozzolino et al. 2005) in geographically distant localities (northern and southern Europe), strong differences in pollinator identity have been recorded. For example, of 15 pollinators recorded on Anacamptis morio in southern Europe by Cozzolino et al. (2005), only two species were also found by Nilsson (1984) in Scandinavia, as a consequence of the differences in the insect fauna of these areas. When data from different geographic regions are then used for comparisons between those species adopting several pollinators as the food-deceptive orchids, grouping pollinators by species would bias any comparison. This problem can be overcome when grouping pollinators into functional groups, as suggested for studies of plant–pollinator associations (Fenster et al. 2004). The pollination syndrome concept implies that specialization onto functional groups (rather than on pollinator species groups) may represent an indication of floral specialization, and thus of floral isolation. It has been suggested by Fenster et al. (2004) that, for those species pollinated by more than one pollinator species (as is the case for food-deceptive orchids), functional groups are more relevant for estimating specialization than are species lists. Thus, our decision
to use two different estimates is well justified by the inherent biological differences between the studied orchid groups.

As a consequence of our choosing two different approaches for the two groups, we never directly compared the quantitative estimates of pollinator isolation between the two orchid groups and also did not use these estimates for subsequent calculations of partial or total isolation indices (as done for instance in Ramsey et al. 2003). Finally, an indirect support for the different levels of pollinator isolation in food- and sexually deceptive orchids comes from a recent meta-analysis (Schießl and Schütter 2009) carried out on representatives of different orchid tribes (including some species analyzed by Scopece et al. 2007) and estimating the level of pollinator sharing (a measurement of pollinator isolation) by using available data on pollinator species lists. These authors, comparing congeneric orchid species and their pollinator species, found that food-deceptive orchids had higher pollinator sharing (and thus less floral isolation) than sexual.deceptive orchids.

Another source of criticism was our admittedly arbitrary but clearly reasoned choice of setting the value of premating reproductive isolation in sexually deceptive Ophrys to 0.9 instead of 1. This choice did not affect the conclusions of Scopece et al. (2007) because differences in pollinator isolation were known to exist a priori and were calculated exclusively for illustrative purposes. Our choice reflects a conservative approach and takes into account recent studies that have found that some Ophrys species hybridize and thus may sometimes share pollinators (i.e., minor responders) whereas others are fully reproductively isolated (Schütter et al. 2007; Stökl et al. 2008). Thus, our isolation coefficient of 0.9 may provide a balanced estimate of what is known about reproductive isolation in this sexually deceptive orchid group in which many species are completely reproductively isolated by their different pollinators (Paulus and Gack 1990), whereas others have some pollinator overlap (isolation coefficient of 0.8 calculated from Stökl et al. 2008) that may lead to hybridization and introgression (Soliva and Widmer 2003; Devey et al. 2008; Cortis et al. 2009).

The discussion about how to estimate premating isolation in sexually and food-deceptive orchids led us to realize that one aspect may not have been sufficiently discussed in Scopece et al. (2007). They found that postmating barriers have higher relative importance in food-deceptive species than in sexually deceptive species but did not argue against premating isolation being the strongest isolation barrier among species of both groups. As pointed out by Jerry Coyne (pers. comm.), at low genetic distance, in both orchid groups, premating isolation is the most important component of species isolation when compared to later isolation stages, even when pollinators are combined into functional groups as done in Scopece et al. (2007). This is simply a consequence of the fact that pollinator isolation operates at a very early stage of isolation and thus contributes disproportionally to total reproductive isolation. Thus, premating isolation does act as a major filter of interspecific gene flow even in those plant groups with little pollinator specialization, such as the food-deceptive orchids (Fig. 1). However, it is well known that in case of breakdown of premating barriers and absence of postmating barriers, interspecific gene flow can lead to an erosion of accumulating divergence and ultimately lead to the collapse of species differences (Taylor et al. 2006). In contrast to the situation in sexually deceptive orchids, in food.deceptive orchids pollinator isolation alone is thus not sufficient for reproductive isolation, which is only achieved by the contribution of later acting barriers.

**Conclusion**

We agree with Sobel and Randle that one faces a number of difficulties when performing comparative analyses of reproductive isolation and that all possible measures should be taken to avoid biases due to species sampling or data collection. However, datasets for comparative analyses are never ideal and it is therefore of particular importance that authors clearly state how data were collected and species are sampled. Although comparative studies may suffer from such minor limitations, they provide a perspective on general evolutionary trends (rough portraits, Presgraves 2002) that single studies based on individual species pairs cannot provide. Ideally, comparative studies such as the one of Scopece et al. (2007) provoke discussions and therefore stimulate further investigations of plant reproductive isolation.
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LITERATURE CITED


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