RESEARCH PROJECT

# Testing speciation mechanisms in orchids with molecular and ecological methods

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## Summary

1 It is often assumed that the high species-diversity of orchids is a result of adaptations to pollinators, and that pollinators are a driving force for floral divergence and speciation. This assumption, however, has rarely been confirmed by investigations, and the view that pollinators may drive plant speciation has recently been challenged by the notion that most plant–pollinator interactions are opportunistic, rather than specific.

2 The pollination systems of European orchids are highly diverse, ranging from opportunistic to highly specific plant–pollinator interactions. *Gymnadenia* spp. are pollinated by a wide variety of butterflies, moths and hawkmoths which are attracted by floral scents and copious nectar offered in spurs (reward-based pollination). Orchids of the genus *Ophrys* on the other hand attract male Hymenoptera by mimicking female insects leading to pseudocopulation (sexually deceptive model-mimic pollination).

3 To determine whether pollinators may drive orchid speciation, we use Thompson's geographic mosaic theory of coevolution as conceptual framework. We will investigate whether the interactions between orchids and pollinators vary geographically, whether this geographic mosaic leads to differential selection pressures among populations, whether it may cause floral divergence, and how floral divergence relates to speciation.

4 Ecological experiments will be designed to identify floral traits under selection and to investigate the causes of floral variation within and among populations. To study the genetic structure of populations within and among taxa, molecular markers such as cpDNA sequences and microsatellites will be used.

**Keywords:** adaptive radiation, geographic mosaic theory, pollinator specificity, reproductive isolation

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## Introduction

There is wide consensus among evolutionary biologists and botanists that the adoption of animal pollination was a critical step in the evolutionary history of flowering plants. Over

the last forty years, pollination biologists have shown that pollinator specificity may mediate plant reproductive isolation, and that pollinator-mediated selection may affect floral traits (Grant 1948, 1994). This is consistent with the view that plant–pollinator systems are coevolved and tend towards increasing pollinator specialisation, supporting the idea that pollinators may drive plant speciation. However, this hypothesis has rarely been tested. Moreover, recent progress in pollination biology has challenged the view that plant–pollinator systems are generally specific and suggests rather that opportunism, and geographic and temporal variation of the interaction between plants and pollinators are more typical (Waser *et al.* 1996). The conditions under which geographic and temporal variation either prevent or drive floral divergence and ultimately speciation are not clear. The geographic mosaic theory of coevolution, originally developed by Thompson (1994) for the study of coevolving organisms, provides the conceptual framework to take spatial and temporal pollinator variation explicitly into account, and to assess its evolutionary consequences for plant speciation.

Speciation in plants can best be studied in groups that have diverged recently, or are currently in the process of speciation (Gill 1989). Such systems may also allow one to investigate the importance of floral divergence and the role of pollinators in plant speciation. As noted by Ollerton (1996), speciation (e.g. allopatric speciation) may precede floral divergence. It is generally assumed that floral divergence is often a by-product of allopatric speciation and is maintained through geographic separation, ecological differences, incompatibility mechanisms or reproductive isolation prior to pollination through pollinator specificity. The importance of pollination syndromes for the maintenance of floral

isolation has been confirmed by evidence in two coevolved systems, the hawkmoth pollinated orchids *Platanthera bifolia* and *P. chlorantha* (Nilsson 1983) and the columbines *Aquilegia formosa* and *A. pubescens* (Hodges & Arnold 1994; for a review see Grant 1994). In both *Aquilegia* and *Platanthera,* the plants provide nectar in spurs. Acquisition of spurs and simple changes in spur morphology may influence reproductive isolation and are indeed correlated with increased plant species diversity (Hodges & Arnold 1995).

Floral divergence, however, is not necessarily a by-product of allopatric speciation. Pollinator-mediated selection on floral traits, either within or among populations, may drive floral divergence, provided that sufficient quantitative genetic variation for floral traits exists (Galen 1996a, 1996b), that geneflow does not counter-balance selection (Hartl & Clark 1989), that variation in pollinator abundance and efficiency are weak (for discussion see Herrera 1996), or that directional pollinator-mediated selection pressure is not balanced by opposing selection pressure through alternative pollinators or pathogens (e.g. Shykoff *et al.* 1997). If none of these counter conditions exist, then floral divergence can evolve either within populations through disruptive selection or among populations through different directional selection pressures between populations. Speciation may then be a consequence of floral divergence. Disruptive selection has rarely been searched for. In a study of the two bumblebee-pollinated plant species *Impatiens pallida* and *Erythronium grandiflorum*, Wilson & Thomson (1996) found no evidence for disruptive selection within populations that might ultimately lead to speciation. However, they admit that disruptive selection may be more likely to occur among rather than within populations, especially in plant–polli-

nator systems where pollinator communities differ geographically. It is currently not clear how pollinator specificity and geographical and temporal pollinator variation affect divergence, and ultimately speciation. The geographic mosaic theory of coevolution (Thompson 1994) provides a conceptual framework to explicitly account for spatial and temporal pollinator variation and its evolutionary consequences for plant speciation.

## The geographic mosaic theory of coevolution

Evolutionary interactions among plants and their pollinators may vary considerably in time and space due to variation in pollinator guild composition, pollinator efficiency and abundance (Herrera 1996). This variation has led to the view that coevolution between plants and their pollinators is diffuse. Thompson (1994), however, argued that what appears to be diffuse is probably much more specific when one studies the variability in interactions among populations or in time. He suggested that the geographic mosaic in the interactions between species often drives the coevolutionary process and the evolution of interactions in general. The study of coevolutionary processes therefore should take into account the life histories, genetics, and the geographic structure of the interacting organisms (Thompson 1994). A detailed example of the application of the geographic mosaic theory to a plant–pollinator/herbivore system is given in Thompson (1997).

## Ophrys: sexually deceptive model-mimic pollination

Species diversity in the orchid genus Ophrys in the Mediterranean area has been regarded as the consequence of rapid adaptive radia-

tion (Paulus & Gack 1990). Pollination is often highly pollinator-specific but at the same time very dynamic. It is pollinator-specific in that flowers of the genus Ophrys attract male Hymenopterans by mimicking female scent and morphology (Kullenberg 1961). Males attempting to copulate with the mimic (pseudocopulation) pollinate the flower. The pollination syndrome can thus be described as a deceptive model-mimic system. Yet the system is also dynamic: different pollinators may be attracted by the same Ophrys taxon in different geographical areas while populations of the (presumably) same Ophrys taxon that differ in flowering phenology may attract different pollinators (Paulus & Gack 1990). Opposing views exist about the evolution of floral divergence in Ophrys. Grant (1994) assumes that floral divergence is a by-product of allopatric speciation, whereas Paulus & Gack (1990) state that "speciation in Ophrys is always a consequence of a change in pollinator". The diversity of Ophrys taxa form a continuum from randomly mating populations to genetically and morphologically separated species. Their interactions with the specific pollinators vary in time (within seasons and over years) and space (different pollinator species in geographically separated areas). These characteristics make Ophrys an ideal study system to investigate the role of pollinators in the evolution and maintenance of floral isolation.

## Gymnadenia: reward-based pollination

Pollination in the species-poor genus Gymnadenia is less pollinator-specific than Ophrys, and also unlike Ophrys, is rewarding to pollinators. Considerable variation exists both within and among Gymnadenia populations with respect to flower morphology (e.g. Reinhard et al. 1991; Soliva & Widmer,

unpubl. results), scent (Kaiser 1993) and flowering phenology (e.g. Reinhard *et al.* 1991; Soliva & Widmer, pers. observ.). *Gymnadenia conopea* is pollinated by a variety of diurnal and nocturnal pollinators. Pale red flowers and scent emitted towards the evening may suggest that *G. c.* ssp. *conopea* is pollinated preferentially by nocturnal pollinators, whereas *G. c.* ssp. *densiflora,* which has darker flowers and emits scent during the day, may be pollinated mainly by diurnal pollinators. Molecular data revealed extensive genetic differentiation between the morphs, suggesting that they are strongly separated (Soliva & Widmer, in prep.). Whether the divergence of these subspecies is the result of pollinator-mediated disruptive selection remains an open question. However, if pollinators choose plants within populations according to flower traits such as scent or spur length, disruptive selection may theoretically occur, thus making this an ideal group for studying this form of selection.

## Discussion

Patterns of species diversity in *Ophrys* and *Gymnadenia* do not conform to expectations from models about speciation through pollinator-mediated selection. Under disruptive selection where pollinators select plants with differing floral traits, such as scent (e.g. Wilson 1989), considerable radiation of species is expected. Although disruptive selection is conceivable for *Gymnadenia conopea,* it is a member of a species-poor genus. Under a system of deceptive pollination on the other hand, pollinators are expected to impose negative frequency-dependent selection on plant populations, thus leading to a rare-type advantage (Smithson & Macnair 1997). This form of selection is not likely to lead to speciation since morphs that increase in frequency are selected against; yet *Ophrys* is a species-rich genus. To what extent negative frequency-dependent selection is important in *Ophrys* populations is currently not clear and needs to be assessed both theoretically and empirically. *Ophrys* represents a special case of deceptive pollination since it is a model-mimic system, in contrast for example, to the genus *Dactylorhiza.* If morphs deviate too much from their particular model, they will be selected against since the male Hymenoptera are no longer attracted. However, pollinator observations and experiments by Paulus & Gack (1990) suggest that variation in flower morphology and scent within populations is necessary to prevent rapid habituation of male bees after initial pseudocopulations, suggesting that negative frequency-dependent selection may be operating also in the highly diverse genus *Ophrys.*

Comparative studies of the plant-pollinator interactions in these orchids may allow us (1) to assess how pollinator variation in both time and space affect floral divergence and speciation, and (2) to identify ecological and evolutionary conditions that either promote or prevent divergence and speciation.

## Funding of the project

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