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RESEARCH PROJECT

Odor communication and the evolution of pollination syndromes in orchids

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Summary

1 Whereas most orchid species offer a food-reward for their pollinators, some, without offering any reward, mimic the signals of rewarding flowers (food deception) or females of the pollinator species (sexual deception). Floral fragrances are important for attracting pollinators in both rewarding and deceptive species and are therefore significant for the reproductive success of a plant.
2 This project will investigate, in a comparative approach, the role of floral odor in orchid pollination ecology in species representing the pollination syndromes of food reward, food deception, and sexual deception. It will use a powerful combination of analytical chemistry, electrophysiology, and behavioral experiments.
3 The following aims will be targeted: (i) Identify the floral odor compounds that are detected by the pollinator insects. (ii) Determine the variability in active and non-active odor compounds within orchid populations. (iii) Investigate the attractiveness of specific odor compounds for pollinators and the consequence of their variability for influencing pollinator behavior. (iiii) Investigate the changes in odor communication in relation to the evolution of various orchid pollination syndromes.

Keywords: electroantennographic detection, floral odor, gas chromatography, orchid pollination, pollinator behavior

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Introduction

Orchid pollination mechanisms range among the most intriguing insect-plant relationships. The majority of species offer a reward, typically nectar, which is exploited by a number of insect pollinators e.g. bees, butterflies and moths (Dressler 1981; Nilsson 1992; van der Cingel 1995). However, a great number of orchids are deceptive, i.e. they do not offer any food for their pollinators. So-called food-deceptive species imitate a range of floral attractants, i.e. floral shape, color, and scent that are associated by the pollinators with an edible reward (Nilsson 1983, 1992; Dafni 1987, 1984, Vogel 1983; Roy & Widmer 1999). Most food-deceptive orchids are pollinated by social or solitary bees (Nilsson 1983, 1992; van der Cingel 1995). Sexually deceptive orchids mimic female sex pheromones and attract only male insects, which pollinate the flowers in an attempted copulation or precopulatory routine (Pouyanne 1917; Kullenberg 1961; Peakall & Beattie 1996; Schiestl *et al.* 1999).

Floral odor is an important cue for many plants to attract their pollinators (Vogel 1983). Most rewarding and some food-deceptive orchids show obvious floral odor emission (Nilsson 1983; Tollsten & Bergström 1989; Knudsen *et al.* 1993; Kaiser 1993). In sexually-deceptive orchids, the odor, although not detectable for humans, is the most important cue to attract pollinator males and release mating behavior (Kullenberg 1961, Schiestl *et al.* 2000a). Although numerous studies have identified floral odor compounds in various plant species (e.g. Knudsen *et al.* 1993; Kaiser 1995), we know very little about which compounds a pollinator may actually detect (Schiestl & Marion-Poll 2001).

Floral odor influences the behavior of pollinators in multiple ways. Pollinators are attracted to flowers by their scent and color over long distances (Vogel 1983). More subtle odor cues may also be used by insects at close range to decide which flower to visit (Dobson *et al.* 1999; Schiestl & Ayasse 2001). Bees learn to associate floral cues with food reward and scent is learned faster and is more prominent than color (Bogdany 1978). Since odor enables bees to discriminate species of food-providing plants it may also play a role in maintaining flower constancy of pollinators (Waser 1996). Assortative mating in plant populations may be mediated by preferential pollinator visitation of certain odor types (Pellmyr 1996). In food-deceptive orchids, high degrees of odor variability have been found, which was interpreted as an adaptive trait for reducing the learning and avoidance of flowers by a pollinator (Ackerman 1986; Ackerman *et al.* 1997; Ferdy *et al.* 1998; Roy & Widmer 1999). Odor variability may be maintained by negative frequency dependent selection, since pollinators will avoid a common phenotype more readily than a rare one. In rewarding orchid species, however, odor variability has also been found to be high (Patt *et al.* 1989; Tollsten & Bergström 1989; Kaiser 1993). Both in rewarding and in food-deceptive species behavioral consequences for pollinators are unknown. In the sexually-deceptive *Ophrys sphegodes* it has been demonstrated, by focusing on biologically active compounds, that intra-individual variability leads to visitation of multiple flowers of one plant by a single pollinator hence enhancing reproductive success of a plant (Ayasse *et al.* 2000).

Food reward is thought to be the ancestral mode of pollination within the orchids and food deception has probably evolved from it (Dressler 1981; Dafni 1987; Aceto *et al.* 1999). Since very little is known about functions of odor in pollinator attraction in rewarding and food-deceptive orchids, the consequences of this shift for odor communication are unknown. Sexual deception has most likely evolved from food deception (Dafni 1984; Kores *et al.* 2001). Whereas food-deceptive orchids may attract their pollinators with general floral odor compounds, a switch to sexual deception requires the emission of few specific sex pheromone compounds, like in the sexually-deceptive genus *Chiloglottis* (Schiestl *et al.* 2000b,c) or specific blends of common compounds, like in *Ophrys sphegodes* (Schiestl *et al.* 1999, 2000a). The Australian orchid genus *Caladenia* contains both food and sexually-deceptive species, which may share pollinators, which is the optimal prerequisite to investigate the changes in odor communication that accompany a switch in pollination syndrome.

Goals

This project will investigate the evolutionary ecology of odor communication within different orchid pollination syndromes by focusing on the following goals:

(1) Identification of biologically active and non-active floral odor compounds

To identify volatiles that are smelled by the pollinator insect, odor will be sampled from flowers and biologically active compounds, detected and identified. Biologically active compounds are here referred to as the ones that elicit electroantennographic responses in the olfactory receptors of an insect antenna. The following specific questions will be asked:

- Which compounds emitted from the flowers are detected by olfactory receptors in the pollinators and which are not detected?
- Is there a difference in detection by different pollinator species of one orchid species?

(2) Determination of odor variability

All samples will be analysed quantitatively to answer the following specific questions:

- What degree of odor variability can be found within a plant population?
- How does the variability differ between active and non-active compounds?
- Is there a difference in variability between orchids with different pollination syndromes?

(3) Pollinator behavior

Behavioral tests will be done to investigate the role of biologically active odor compounds in attracting orchid pollinators. Further it will be investigated how odor variability influences the pollinators' behavior when visiting rewarding or deceptive flowers. The following questions will be asked:

- Which compounds attract the pollinator?
- How is the visitation behavior of the pollinator influenced by odor variability?

(4) Evolutionary changes in odor communication

Since orchids either reward or cheat their pollinators, thereby exploiting food-seeking or mating behavior, different specialisation for attracting pollinators are likely to have evolved. However, the availability of biosynthetic pathways for odor compounds as a pre-adaptation may have greatly influenced the evolution of different pollination modes. The comparative approach will help to test the following questions:

- Is there an evolutionary trend in odor communication between different pollination syndromes, i.e. do rewarding and deceptive species attract their pollinators with different compounds or different amounts of compounds, and are patterns of variability different between rewarding and deceptive species?
- Is there an indication that energetic costs of odor emission influenced the evolution of pollination syndromes?
- Are there any pre-adaptations for the evolution of sexual deception (i.e. the synthesis of compounds similar to sex pheromones) in food-deceptive species?

Methods

STUDY SPECIES

Gymnadenia conopsea

This rewarding species is widely distributed and easily accessible around Zürich. Its flowers show strong odor emission, which has been shown to vary considerably between individuals (Kaiser 1993). Most emitted odor compound have been identified by Kaiser (1993), which will speed up our investigations. This orchid is visited by a number of insects, but only long-tongued lepidoptera are able to access the nectar in the flower spur (van der Cingel 1995).

Orchis mascula
This species is fairly common around Zürich. Like most species of the genus *Orchis* (Dafni 1987), it is a food-deceptive orchid, which does not provide any reward for its pollinators. The most important pollinators of most *Orchis* spp. are bumble bees (*Bombus* spp.) and honey bees (van der Cingel 1995). Little is known about *Orchis* floral odor and its influence on pollinators (Nilsson 1983).

Caladenia tentaculata, C. arenaria, C. gracilis
These species grow sympatrically in the surrounding of Canberra/Australia. *C. tentaculata* (Fig. 1) is a sexually-deceptive, whereas *C. arenaria* and *C. gracilis* are food-deceptive species. Hybridisation between *C. tentaculata* and *C. arenaria* has been recorded (Bower, pers. comm.) and indicates that the two species share pollinators. Pollinators of these orchids occur in the surrounding of Canberra.

Fig. 1. Caladenia tentaculata *(Orchidaceae), one of the study species (Canberra, November 2000).*

ANALYTICAL TECHNIQUES

Collection of odor samples
To collect highly volatile odor compounds, single flowers are placed in glass tubes. Air is pulled over the flowers by a battery-operated membrane pump. Air coming into the glass chamber is cleaned by an adsorbent tube filled with 120 mg activated charcoal (SKC). The air exits through a cartridge that contains a small amount of Porapak Q or activated charcoal (CLSA Filter), onto which the volatiles are trapped. The volatiles are eluted using pentane or carbon disulphide according to the technique described in Grob *et al.* (1984) and Schiestl *et al.* (1997). These samples are subsequently analysed on a gas chromatograph.

Low volatile odor compounds of orchid-labella will be sampled by extracting these flower parts in pentane for 12 h, according to Schiestl *et al.* (2000). Again, gas chromatographic analyses will be performed.

Detection of biologically active compounds
In the electroantennogram (EAG) technique, developed by Schneider (1957), slow olfactory receptor potentials can be recorded from an isolated insect antenna positioned between two glass capillary micro-electrodes. By blowing puffs of various odor fractions over the antenna, their potential to elicit electroantennographic responses can be determined (Fig. 2). Combined gas chromatography and electroantennogram recording (GC-EAD) allows the analysis of multi-component volatile samples and enables the importance of single compounds to be tested (Arn *et al.* 1975; Wadhams 1990). It allows an extremely sensitive and selective detection of physiologically active compounds within a multi-component sample.

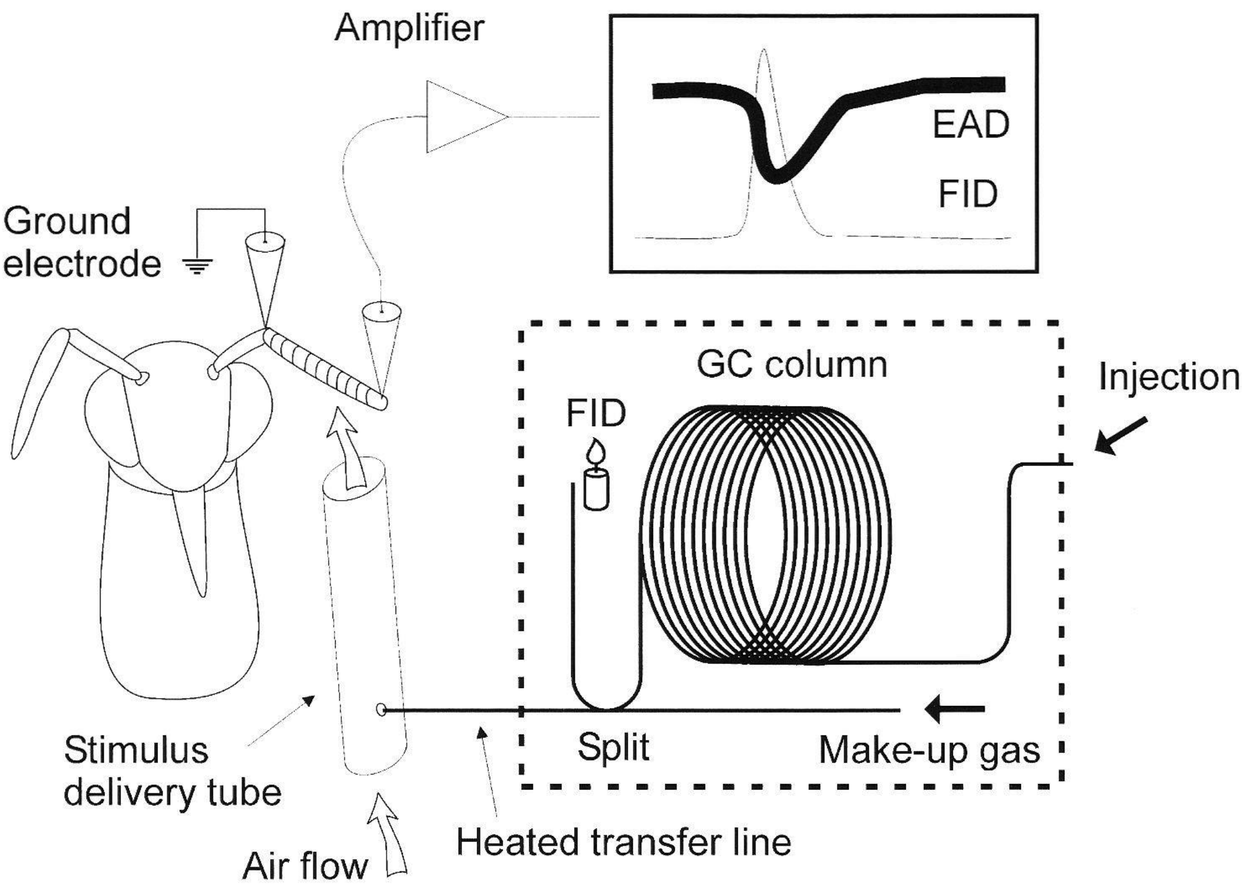

Fig. 2. Schematic represenation of the GC-EAD method used to detect and identify biologically active compounds in odor samples (Schiestl & Marion-Poll 2002). Odour samples are first separated into single compounds by a gas chromatograph (GC). The effluent from the GC column is then split: half the amount of each compound is directed to a flame ionisation detector (FID), and the other half into a stimulus delivery tube. Compounds in the stimulus delivery tube remain airborne and are blown over an insect antenna. Reactions from olfactory receptors in the insect antenna (electroantennographic detector EAD), i.e. differences in electric potential between the base and the tip of the antenna, are recorded with micro-electrodes and amplified. The simultaneous recording of EAD and FID traces makes it possible to judge which compound can be detected by an insect.

Chemical identification of odor compounds
The identification of semiochemicals by means of gas chromatography – mass spectrometry (GC-MS) will be done in collaboration with Dr. Roman Kaiser (Givaudan Dübendorf).

Relevance of the project

Although floral odor has fascinated scientists for centuries (Sprengel 1793; Darwin 1862; Vogel 1990), and recent investigations have accumulated a reasonable amount of data on its chemical composition in different plant species (e.g. Knudsen *et al.* 1993; Kaiser 1993), our knowledge of its ecological significance remains small. One problem for linking floral odor to pollination ecology is our limited knowledge about the ability of pollinators to smell specific compounds emitted by a flower, and the potential of these compounds to influence the pollinators' behavior. The proposed study is an interdisciplinary approach and will use modern analytical methods combined with electrophysiology and behavioral experiments. This combination has proven to be a powerful approach to investigate floral odor and its link to pollinator behavior (summarised in Schiestl & Marion-Poll 2002). Since odor communication is important for pollinator attraction, we need to understand its mechanisms to learn about the evolution of different pollination modes, which make the orchids one of the most fascinating plant families.

Orchids are ideal for studying the evolution of floral traits (Darwin 1862) because many species have pollinia, show a high degree of specialisation in their utilisation of pollinators, and are often pollinator limited in their reproductive success (Nilsson 1992). Further, the evolution of different pollination syndromes within the family allows a comparison of floral specialisations in response to different pollinators and their behavioral patterns. The Australian genus *Caladenia* is unique in its containing both food - and sexual deceptive species, which provides the exclusive opportunity to investigate odor communication and its evolutionary transition between these two pollination syndromes within closely related species. The family of orchids also contains many threatened species, therefore, the expected data on odor variability and pollinator behavior will be useful for question related to conservation biology.

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