

Responses of *Argogorytes* (Hymenoptera: Sphecidae) Males to Odor Signals from *Ophrys insectifera* (Orchidaceae). Preliminary EAG and Chemical Investigation

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ABSTRACT

Ågren, L. & A.-K. Borg-Karlson (Ecological Station of Uppsala University, S-386 00 Färjestaden, Sweden, and Department of Organic Chemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden.) Responses of *Argogorytes* (Hymenoptera: Sphecidae) males to odor signals from *Ophrys insectifera* (Orchidaceae). Preliminary EAG and chemical investigation.

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Extracts, sorption extracts, thin layer chromatography (TLC) fractions, authentic compounds, and odors from living labella of *Ophrys insectifera* were tested electrophysiologically on whole antenna of different species of *Argogorytes* and *Gorytes* males and females.

The extracts were analysed by gas chromatography (GC), TLC and mass spectrometry (MS). The labellar extracts and sorption extracts differed widely. Quantitatively dominating compounds found in the extracts were homologous series of hydrocarbons, aldehydes and methyl esters, whereas the sorption extracts contained the hydrocarbons tridecane, pentadecane, heptadecane, a heptadecene, nonadecane, and the terpenes cyclosativene and linalool.

The composition of components in the Dufour gland of *Argogorytes* closely resembled that of the sorption extract of *O. insectifera*, except for the terpenes, that were lacking in the insects.

The EAG responses followed the ethological findings closely, in that the observed pollinators of *O. insectifera* responded stronger than the non-pollinators to the odor of the flower and its main components.

Key words: aliphatic hydrocarbons, cyclosativene, Dufour gland, electroantennogram, gas chromatography, *Gorytes*, linalool, mass spectrometry, sorption, terpenes.

INTRODUCTION

The aim of the present work is to further elucidate the intricate ecological association between the orchid *Ophrys insectifera* and its main pollinators, male sphecid wasps of the genus *Argogorytes*. These relations have been thoroughly described by Kullenberg (1961), that showed the coexistence to depend largely on signals mediated from

the flower to the insect, signals that seem to be evolved by the orchid to secure its genetic propagation by a form of mimetism. Priesner (1973) investigated the chemical portion of the signalling by analysing electrophysiologically the receptor responses from male *Andrena* and *Eucera* bees to natural and synthetic odors of different *Ophrys* species. New findings (Borg-Karlson, to be published) concerning the chemical composition of these odors have recently been made using TLC (thin layer chromatography) and GC-MS (gas chromatography/mass spectrometry). This justified a further investigation of the receptor sensitivity, now with another pollinator, wasps of the genus *Argogorytes*, to odors emitted by *Ophrys* species, and authentic substances related to these. It is also of special interest to compare the receptor responses to substances present in both female, male and/or flower.

Ophrys insectifera's way of utilizing *Argogorytes* males as pollen vectors is described in detail by Kullenberg (1961), but is briefly recapitulated here:

The principal occupation of a male is, when not feeding or sleeping, to search for females. The sense organs appear to be tuned to signals from the opposite sex. Depending on weather factors and/or inborn circumstances, the threshold for approaching an object that fulfills the correct stimulus configuration, is higher or lower. The males have a set of genetically programmed triggers to initiate and to stimulate the instinct chain at different excitation levels, being the accumulated sensations from receptors of several modalities. One provoking agent is for instance a combination of chemicals emitted by the females. A similar spectrum of substances could also be expected in *O. insectifera*. Being lured by such a "female" scent from a flower during a flight, the male is enticed to approach in a mainly upwind direction. At that state of arousal, the male is further excited by the flower with visual stimuli. The labellum has a dark color, reflects ultraviolet light of similar wavelengths to those of female wings, and has the size and proportions of a female.

At that level of excitation, the *Argogorytes* male alights on the labellum. There, tactile stimuli add to the accumulated olfactory and optical stimuli and make the male attempt to copulate with the labellum, whereby the

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