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Chemical Basis for the Relationship Between *Ophrys* Orchids and their Pollinators

II. Volatile compounds of *O. insectifera* and *O. speculum* as insect mimetic attractants/excitants

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Abstract

Volatile compounds from the orchids *Ophrys insectifera* and *O. speculum* and their respective pollinators of the hymenopteran genera *Argogorytes* and *Campsoscolia* are investigated using gas-chromatography and mass spectrometry. The volatile compounds from the flowers and the solitary wasps are isolated by solvent extraction and sorption techniques. Six different isolation techniques of the fragrance from *O. insectifera* are compared. Chemical similarity consisting of a series of aliphatic straight chain hydrocarbons are found between the fragrance emitted by *O. insectifera* flower labella and the Dufour gland secretion of *Argogorytes mystaceus* and *A. fargei* females. 3-Hydroxy-fatty acid methyl esters and 2,5-dimethyl-3-isopentylpyrazine are found in the cephalic secretion of *Campsoscolia ciliata* males and females. Field tests using *Argogorytes* males and made with authentic and synthetic odour samples. The results of the chemical analyses and the field tests are discussed in terms of chemical mimesis, chemotaxonomy, and hybridization.

Introduction

Ophrys L. orchids are pollinated by hymenopteran males (Apoidea, Scoliidae and Sphecidae). The males are attracted to the nectarless flowers by chemical and visual cues, and fly towards the orchids guided by an odour that is sexually stimulating [13–15]. The pilosity and form of the flower labellum resembles that of the female and act as a tactile and proprioceptive stimulus [1, 15] guiding the excited male to perform initial copulatory movements on the labellum (Fig. 1). The male's head during this performance is directed towards the gynostemium and the pollinaria become attached to the head. Subsequent visits to conspecific flowers bring about pollination. Immediate auto-pollination may also occur.

This study is a part of a comprehensive investigation concerning the chemical basis for the relationship between *Ophrys* orchids and their pollinators. *O. insectifera* and *O. speculum* are regarded as closely related and taxonomically placed in the same section, *Ophrys* [22]. The two species and their pollinators are regarded as the oldest evolutionarily. We have earlier reported on chemical mimesis between the *Fusci-Luteae* section of *Ophrys* and their pollinators, which are solitary bees of the genus *Andrena* Fabr. [7, 8].

Preliminary isolations and identifications of the volatile compounds from the orchids [1, 19, 20] and the wasps [6], have previously been reported. Six different isolation/enrichment methods (solvent extracts, sorption, enfleurage, low-temperature trapping, steam distillation and dried labella) for volatiles from *Ophrys* orchids were described and the advantages/disadvantages of the methods discussed [2].

The present work deals with results of the chemical analyses, as well as giving a preliminary report on behaviour field tests, related to the two species of orchids, *Ophrys insectifera* and *O. speculum* and their respective pollinators *Argogorytes mystaceus*, *A. fargei* and *Campsoscolia ciliata*. The compositions of the volatile compounds from the different plant and insect species are compared and the similarities in chemical structures are discussed in terms of chemical mimesis, chemotaxonomy and hybrid formation.

Materials and methods

Biological material analysed

Ophrys insectifera L. flower labella, stalks with or without flowers and whole plants were collected in the years 1975–83 from several localities on Öland, one of the large islands in the Baltic Sea close to the mainland of south Sweden.

Ophrys speculum Link (*O. vernixia* Brot.) flower labella were collected on Majorca (Spain) in 1979. Sorption of volatiles released from stalks with flowers were performed in 1983 and 1984 using a few individuals cultivated in a greenhouse.

Argogorytes mystaceus (L.) and *A. fargei* Shuck. males and females were collected in 1975–83 on Öland (Sweden).