

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

III. Volatile compounds of species in the *Ophrys* sections *Fuciflorae* and *Bombyliflorae* as insect mimetic attractants/excitants

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Abstract

Volatile compounds from the flowers of *Ophrys scolopax attica*, *O. s. cornuta*, *O. s. scolopax*, *O. bombyliflora*, and *O. tenthredinifera* belonging to the sections *Fuciflorae* and *Bombyliflorae* and from two pollinator species of the genus *Eucera* were analyzed by gas chromatography and mass spectrometry. The volatiles were enriched through solvent extraction or sorption techniques. Preliminary behavioural tests in the field with various odouriferous samples were made using mainly *Eucera longicornis* males. A few volatile compounds, such as geranial, geraniol and linalool, were found in certain species of the *Ophrys* and the *Eucera* genera. Possible excitants/attractants in the pollination relationship, taxonomical classification and hybridization based on the present chemical findings are discussed.

Introduction

Orchids of the genus *Ophrys* L. are mainly pollinated by hymenopteran males (Hymenoptera Aculeata: Apoidea, Sphecidae and Scoliidae). The *Ophrys* flowers have no nectar or pollen to reward the visiting insects, but attract/excite the males by their scents. The excited males approach, alight, and perform movements resembling copulation on the flower labellum and the pollinaria may then become attached to the head or, in the section [1] *Fusci-Luteae*, the tip of the abdomen of the insect. Pollination may ensure when the insect visits another flower. According to Kullenberg [2], the odour, together with visual and tactile stimuli is important for releasing the whole behaviour chain from the approach flight to the attempted copulation with the labellum.

Based on observations and on odour attraction experiments in the field the genus *Ophrys* can be divided into at least six groups with regard to their main pollinators [2,3]: *Ophrys insectifera* – *Argogorytes* spp.; *O. speculum* – *Campsoscolia* sp.; the section *Fusci-Luteae* – *Andrena* spp.; the sections *Arachnitiformes* and *Araneiferae* – *Andrena* spp. and *Colletes* spp.; the sections *Fuciflorae* and *Bombyliflorae* – *Eucera* and *Tetralonia* spp.; and the section *Orientalis* – *Melecta* spp.

Support for the hypothesis of chemical mimetism has been obtained in the *Fusci-Luteae* group and their pollinators of the genus *Andrena* [4]. Earlier studies on the role of volatile compounds in the *Ophrys* pollination relationship have been made by Kullenberg [2, 5–7], Kullenberg *et al.* [8], Kullenberg and Bergström [3], Tengö [9], Borg-Karlson *et al.* [10]. Electro-

physiological studies on *Ophrys* pollinators have been made by Priesner [11] and Ågren and Borg-Karlson [12].

This paper is the third of a series reporting on the chemical basis for the pollination relationship between *Ophrys* orchids and their pollinators. The aim of the project is to chemically – and regarding behavioural performance – identify the volatile compounds of five species/forms in the sections *Fuciflorae* and *Bombyliflorae* that attract/excite the *Eucera* males to pollinate the flowers. The composition of the volatile compounds will then be used as a tool to improve the chemotaxonomy within the section *Fuciflorae*. Finally, the volatiles from the orchids and the pollinators will be examined in terms of chemical mimetism and hybridization.

Materials and methods

The biological material was collected in the years 1978–85 (Table I). Solvent extracts were made of different parts of the *Ophrys* flowers (labella, sepals, petals, and gynostemium) and different parts of the *Eucera* bees (mandibular glands, heads with the mandibles detached, toraces, abdomina, Dufour glands of females, and whole bodies). The solvents used were principally pentane (P), hexane (H) and methanol (M). The compounds in the methanol extract were transferred to pentane using a methanol/water/pentane distribution [4]. Most of the identifications and separations were made on labella and sorption extracts of *O. s. scolopax*.

Fractionation of *Ophrys* and *Eucera* extracts

(1) TLC fractions were freshly made for each field test season in the years 1978–84. Totally about 1800 *O. scolopax* labella, 2000 *O. bombyliflora* labella, 4 *Eucera* female abdomina and 5 heads of males respective females were used for preparation of various TLC and RP (reversed phase: octyl-chain-bounded silica gel, Merck) fractions [4].

(2) Methanol (MWP) extracts of ca. 200 *O. s. scolopax* labella were fractionated using column chromatography. Stationary phase was Woelm Silica gel 60–200 mesh and a gradient (0–50%) of diethyl ether in hexane was used as eluent.

(3) Hexane extracts of ca. 200 *O. s. scolopax* labella were