A contribution to the pollination biology of *Ophrys scolopax* Cavanilles (Orchidaceae) in southern France

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MANUSCRIPT

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Abstract. We here provide records on the pollination of *Ophrys scolopax* Cavanilles by patrolling males of *Eucera (Hetereucera) elongatula* Vachal (Hymenoptera, Apidae) in southern France. A the time of our observations, the only pollinator species known for *O. scolopax* was *Eucera (Eucera) nigrescens* Pérez, a species not found on the study site. Fresh inflorescences of *O. scolopax* were also tested for their attractiveness to males of another sympatric *Eucera* species, namely *E. (Eucera) nigrilabris*, but no approaching flights or contacts with the flowers were observed, suggesting that *E. elongatula* alone acts as pollen vector for *O. scolopax* on the site of observations. These investigations are discussed in light of other *Ophrys-Eucera* interactions and an account on the ecology and reproductive biology of *E. elongatula* is provided.

Résumé. Nous fournissons ici des observations relatives à la pollinisation de l'*Ophrys scolopax* Cavanilles par les mâles d'*Eucera (Hetereucera) elongatula* Vachal (Hymenoptera, Apidae) dans le sud de la France. A ce jour, les seules observations de pollinisation de l'*Ophrys scolopax* dans la région étudiée concernaient les mâles d'*Eucera (Eucera) nigrescens* Pérez, une espèce non présente sur le site d'étude. Nous avons également testé des inflorescences fraîches d'*O. scolopax* pour leur attractivité vis-à-vis des mâles d'une espèce d'*Eucera* observée en sympatrie, *E. (Eucera) nigrilabris*, mais aucune approche ou aucun contact des mâles avec les fleurs n'a pu être observé. Ces informations sont synthétisées et discutées à la lumière d'autres interactions *Ophrys-Eucera* et nous fournissons également un aperçu de l'écologie et de la biologie reproductive d'*E. elongatula*.

Key-words. *Eucera elongatula*, *Eucera nigrescens*, *Eucera nigrilabris*, Hymenoptera, *Ophrys scolopax*, pollination by sexual deception, pseudocopulation.

Introduction

Orchid pollination holds some of the most illustrative examples of how pollinator-mediated selection can act to generate a bewildering diversity of floral shapes, colours and scents (Darwin 1862; Van der Pijl & Dodson 1966; Van der Cingel 1995; Alcock 2006). Although most orchids provide floral "rewards" to their pollen vectors, an estimated one third of all orchid species known to science (i.e, ca. 10.000 taxa) are pollinated without offering any reward to their visiting insects (Cozzolino & Widmer 2005). Pollination in these "deceptive" orchids is usually brought by spectacular instances of mimicry of rewarding plants, oviposition sites or even signals emitted by mating partners. The latter case is usually referred to as "sexual deception", and involves hundreds of species belonging to different genera across Australia, South America, South Africa and Europe. Hence sexual deception is best viewed as a pollination strategy that has evolved independently in different orchid lineages across different regions in the world (Schiestl 2005).

The West-Palaearctic (comprising Europe, north Africa and the Middle East) is home to the orchid genus *Ophrys*, which encompasses a spectacularly diverse assemblage of species that are all, at a few exceptions such as *O. apifera* and *O. helenae*, pollinated by sexual deception (Paulus 2005, 2006). In this system, patrolling male bees, wasps and even beetles, attempt mating (i.e., pseudocopulate) on the female decoys of the flowers that mimic the mating signals (shape, colour and scent) of receptive female insects (Correvon & Pouyanne 1916, 1923; Kullenberg 1961, Schiestl et al. 1999). Since the signals mimicked by the orchids, especially the sex pheromones of the female insects, are usually described as species-specific, most *Ophrys* species are usually pollinated by one or a few closely-related insect taxa (Paulus & Gack 1990; but see e.g. Tyteca et al. 2005).

Ophrys scolopax Cavanilles has a widespread distribution in the Mediterranean Basin, ranging from the Iberian Peninsula to North Africa (Delforge 2005). The flowers of *O. scolopax* are usually medium-sized (< 26mm) and the colour of the sepals is quite variable within populations, varying from deep pink to virtually white. The labellum is narrow and trilobed, with strong and ciliate gibbosities. The labellum appendix is usually yellowish to greenish and points forward (Figure 1). In France, this species is only present in the southern half of the country where it blooms from March to June in garrigues, grasslands, heathlands and open pine forests (Souche 2004, Bournérias & Prat 2005, Delforge 2007). To date, all pollinator records available for this species in France involve patrolling males of *Eucera (Eucera) nigrescens* Pérez (Bournérias & Prat 2005; Delforge 2005, 2007) and probably *E. (Eucera) interrupta* Baer (Delforge 2005, 2007), two species that emerge from mid-March until early May. However, the paucity of *Ophrys* pollination records raises the question whether all populations of *O. scolopax* are pollinated by these *Eucera* species, or if different pollinator taxa could act as pollen vectors in different populations.

In this study we investigated the pollination biology of *O. scolopax* in southern France, in the region of Perpignan-Narbonne. Specifically, we examined whether early-flowering populations of *O. scolopax* are pollinated by males of one of the aforementioned *Eucera* species or if any other species could act as pollen vector in the population under study.

Materials and Methods

Study site

All observations have been carried out in March 2007 in a natural population at Rivesaltes (Pyrénées-Orientales, France). The study site is a military area that extends over hectares of sandy/rocky soil. At the time of our observations, the first plants of *O. scolopax* had just started blooming. The following plant species have also been observed:

Asparagus actutifolius L. (Liliaceae)
Calicotome spinosa (L.) (Fabaceae)
Euphorbia characias L. (Euphorbiaceae)
Juniperus oxycedrus L. (Cupressaceae)
Muscari neglectum Guss. (Liliaceae)
Olea europaea L. (Oleaceae)
Ophrys arachnitiformis Grenier & Philippe (Orchidaceae)
Ophrys bilunulata Risso (Orchidaceae)
Ophrys lupercalis Devillers-Terschuren & Devillers (Orchidaceae)
Ophrys lupercalis x *lutea* (Orchidaceae)
Phillyrea angustifolia L. (Oleaceae)
Rosmarinus officinalis L. (Lamiaceae)
Thymus vulgaris L. (Lamiaceae)

At the time of our observations, only very few species of solitary bees were active. The most abundant species that we have recorded in the study site were *Andrena (Melandrena) nigroaenea* (Kirby) (Andrenidae), *Anthidium (Rhodanthidium) sticticum* Fabricius (Megachilidae), *Anthophora (Anthophora) plumipes* (Pallas) (Apidae), *Eucera (Pareucera) caspica* Morawitz, *E. (Hetereucera) elongatula* Vachal and *E. (Eucera) nigrilabris* Lepeletier (Apidae), as well as *Nomada agrestis* Fabricius (Apidae), the presumed cuckoo bee of the latter *Eucera* species (Vereecken et al., in prep).

Pollinator observations

Our observations took place around noon (local time) on the 11.III.2007 and the 15.III.2007. The insect specimens have been observed attempting copulation during ca. 10 seconds on flowers of *O. scolopax* that had just started flowering. Five photographs have been taken during these pseudocopulations, of which one is reproduced herein (Figure 2). The specimens were caught before the end of their pseudocopulatory activity, stored in a 1.5mL Eppendorf capsule, killed by freezing, set on an insect pin and prepared for identification. All comments concerning the phenology and biogeography of *Eucera* species are drawn from the "eucdat" database which contains some 45.000 records of Palaearctic Eucerine bees from various entomological collections. This database was developed and is currently managed by SR who also identified the *Eucera* specimens collected during this study.

Results and Discussion

Eucera males and *Ophrys scolopax*

Our results indicate that the medium-flowered and early flowering *O. scolopax* in southern France is pollinated by patrolling males of *Eucera (Hetereucera) elongatula* Vachal (= *E. trivittata* auctt.) (Figure 2). Although several *Eucera* species have been recorded as pollinators of *O. scolopax* over the latter's geographic range (see Table 1), the only pollinator species observed for *O. scolopax* in southern France was *Eucera (Eucera) nigrescens* Pérez (Bournérias & Prat 2005), which usually emerges in late March/early April until early May (Westrich 1990, Müller et al.

1997). In Mediterranean France the males of *E. nigrescens* are generally active from the beginning of April to the end of May with the peak of activity in the last decade of April and the first decade of May.

Our observations therefore represent the first account of a second pollinator species for *O. scolopax* in southern France. During our experiments, we have tested fresh inflorescences of this orchid species for its attractiveness to patrolling males of a sympatric *Eucera* species, namely *E. (Eucera) nigrilabris*, which is known to pollinate *O. tenthredinifera*. Virtually no inspecting flights or pounces were triggered with the flowers, suggesting that *E. elongatula* is probably the only pollen vector for *O. scolopax* on the site of observations.

Several other *Eucera* species have already been mentioned as pollinators of *O. scolopax* (see Table 1). Only the males of *E. (Pteneucera) nigrifacies* Lepeletier are similar in size an appearance to *E. elongatula*, but according to the available data, its flight season in France is delayed from early May onwards (the earliest record being a male caught on the 4th of May at Vias, 35km SE of Montpellier) until the beginning of July. The females of *E. nigrifacies* are usually active in June and July and are regarded as pollen oligoleges on Asteraceae. The other species mentioned as pollinators of *O. scolopax* are not closely related and differ considerably. *Eucera barbiventris* and *E. notata* also belong to the subgenus *Hetereucera* Tkalcu but these are much larger species. Both have also a restricted west-Mediterranean distribution: *E. notata* is common in northwestern Africa and in the Iberian Peninsula. The only record from France, a specimen labelled "Marseille" (without additional information) held in the collection of the Oberösterichisches Landesmuseum in Linz (Austria), is of doubtful origin and should therefore not be taken into account until additional records can be provided. *E. barbiventris* Pérez is most probably an Iberian endemic. On the other hand, *E. interrupta* Baer, *E. longicornis* (L.) and *E. nigrescens* Pérez all belong to the subgenus *Eucera* Scop. which includes several species oligolectic on Fabaceae. All three species are common and widely distributed in southern and central Europe but absent in North Africa, where *E. nigrescens* is replaced by the closly related vicariant *E. vidua* Lepeletier.

Table 1. Records of *Eucera* males as pollinators of *Ophrys scolopax*

Pollinator observed	**Reference**
Eucera (Eucera) interrupta Baer	Delforge (2005, 2007)
Eucera (Eucera) longicornis (L.)	Borg-Karlson (1990)
Eucera (Eucera) nigrescens Pérez	Bournérias & Prat (2005)
Eucera (Hetereucera) barbiventris Pérez	Paulus et al. (1983)
Eucera (Hetereucera) elongatula Vachal	Present study
Eucera (Hetereucera) notata Lepeletier	Delforge (2005)
Eucera (Pteneucera) nigrifacies Lepeletier	Delforge (2005)

Eucera elongatula - species profile

Eucera elongatula was originally described from Algeria and Morocco. The species is widely distributed in North-Western Africa (Tunisia, Algeria, Morocco) as well as in the Iberian Peninsula, and can be locally abundant. Its presence in the Baleares (visiting *Asteraceae*), as well as from Sicily (Italy) reported by Friese (1896) remains dubious and would require original records for confirmation. In France, the species is obviously rare and was only recently recorded from the departement Aude (Banaszak & Rasmont 1994). At the study site, both the males and the females have been observed foraging on inflorescences of *Muscari neglectum* Guss.

(Liliaceae) and *Salvia* sp. (Lamiaceae). The females of this species have also been observed foraging on *Rosmarinus officinalis* L. (Lamiaceae) at the Massif de la Clape (departement Aude, France: Gruissan, 18.III.2007, 2m and 2f, *leg. NJ Vereecken*), close to the locality where it was first recorded from France back in 1988 (Banaszak & Rasmont 1994). In addition to the records mentioned above, another two specimens have been collected in southern France (departement Bouches-du-Rhone: St.-Martin-de Crau, 24.V.1987, 1m, *leg. P Rasmont*; departement Pyrenées-Orientales: St.-Hippolyte, 20.VI.1994, 1f, *leg. P Rasmont*).

Patrolling males of *E. elongatula* can be observed establishing a territory in the near vicinity of flower patches and shrubs where virgin females forage after their emergence. At the study site, males were flying a few centimetres above the ground in a typical "patrolling" behaviour, occasionally defending their territory against intruders (whether conspecific males or any other insect passing by), and regularly landing back on bare and elevated stones within their territory (Figure 3). Several males have been observed flying away from their observation spot for a little while, and coming back on the same stone, which presumably provides a good outlook over their reproductive territory. Whether these observation spots change from one day to the other or even during the day requires further investigations. After copulation, the females start building an nest in the ground, mostly in sandy/rocky soils (Figure 4) where they provide their brood cells with pollen and nectar collected on the neighbouring plants.

Acknowledgements

We are grateful to Rémy Souche (Saint-Martin-de-Londres, France) and Alain Bonny (Perpignan, France) for guiding us on the study site in Rivesaltes (France). The manuscript has benefited from a proofreading by Denis Michez (Université de Mons-Hainaut, Belgium). This study has benefited of financial support by the Belgian Academy of Sciences ("Agathon de Potter" studentship 2007 to NJV) as well as by the Belgian "Fonds pour la formation à la Recherche dans l'Industrie et l'Agriculture" (F.R.I.A. grant to NJV).

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Figures

Figure 1. Floral detail of *Ophrys scolopax* in the population investigated. Rivesaltes (F), 15.III.2007 (Photo NJ Vereecken).

Figure 2. A male of *Eucera elongatula* Vachal (Hymenoptera, Apidae) pseudocopulating on the flower labellum of *Ophrys scolopax*. Rivesaltes (F), 15.III.2007 (Photo NJ Vereecken).

Figure 3. A male of *Eucera elongatula* Vachal (Hymenoptera, Apidae) resting on a piece of rock in its reproductive territory. Rivesaltes (F), 13.III.2007 (Photo NJ Vereecken).

Figure 4. A female of *Eucera elongatula* Vachal (Hymenoptera, Apidae) close to its nest entrance. Rivesaltes (F), 13.III.2007 (Photo NJ Vereecken).