Journal of Insect Science (2016) 16(1): 104; 1–7
doi: 10.1093/jisesa/iew088
Short Communication

Insect Visitors and Potential Pollinators of *Orchis militaris* (Orchidaceae) in Southern Belgium

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Subject Editor: Dr. Hongmei Li-Byarlay

Received 14 June 2016; Accepted 24 August 2016

Abstract

As part of a research project on the food deception strategy in *Orchis militaris* (L.), the objective of this study was to identify insect visitors and potential pollinators of this orchid species in Belgium. In 2013, insects were collected over 2 d per site in five localities distributed in Southern Belgium (Wallonia). A total of 104 insects belonging to 49 species were caught. Dipterans were the most abundant visitors (50% of total specimens), followed by Hymenopterans (32%). *Rhingia campestris* Meigen, *Bombylius venosus* Mikan, *Apis mellifera* (L.), and *Bombus lapidarius* (L.) were the most abundant species. Only five specimens bore one to more than 10 pollinia: four honeybees (*A. mellifera*) and one bumblebee worker (*B. lapidarius*). These two species should be considered as potential pollinators in the study area, but probably not confirmed ones.

Key words: *Orchis militaris*, pollinator, Apidae, *Apis mellifera*, *Bombus lapidarius*

Orchidaceae are one of the most diversified Angiosperm families, with more than 27,000 accepted names (The Plant List 2013). The majority of orchid species rely on biotic pollen vectors to reproduce (Nilsson 1992), so do 88% of flowering plant species (Ollerton et al. 2011). Usually, plants attract pollinators with floral signals that advertise rewards (Schiestl and Johnson 2013). However, some plant species also display attractive floral signals but don't provide any reward (or low or wrong reward) (Jersáková et al. 2009); they are called deceptive species (Renner 2006). Deceptive pollination occurs in about 30% of orchid species (Jersáková et al. 2006, Claessens and Kleynen 2011), and the commonest systems involve the exploitation of the food-seeking behavior of the pollinators (food deception—Jersáková et al. 2006).

Under natural conditions, insect visits on rewardless flowers are rare events difficult to record (Cozzolino et al. 2005, Claessens and Kleynen 2011), making direct observation time consuming (Widmer et al. 2000). Indirect approaches have been developed to study more easily orchid-pollinator relationships: pollinaria carried by insects (caught on rewarding plants growing in the vicinity of studied orchid populations) allow the identification, through molecular techniques, of the corresponding orchid species (Widmer et al. 2000); pollinaria found on insects can also be identified through specific morphological features (Singer et al. 2008, Micheneau et al. 2009); scales and hairs found on pollinated stigmas can allow the identification of the corresponding insect species (Micheneau et al. 2009). Videotape observations are another alternative to direct observations (Micheneau et al. 2009, Lortie et al. 2012, Nakase and Suetsugu 2016), but observation-based techniques don't allow accurate identification of numerous insects (especially dipterans and hymenopterans). The latter have to be collected and examined carefully in order to draw up accurately visitor and potential pollinator lists.

In the genus *Orchis* Tourn. ex L. (Orchidaceae), none of the species produce nectar and the majority depends on food deception to attract pollinators (Scopece et al. 2014). Visitor and pollinator guilds have been studied more or less extensively according to the species (e.g. van der Cingel 1995, Berger 2003, 2004; Claessens and Kleynen 2011). In *O. militaris* (L.), most data were collected in Austria by Vöth (1987) and in the United Kingdom by Farrell (1985). Other contributions come from Berger (2003, 2004), Ebert and Rennwald (1993), Petit (1998), Vöth (1999), etc. Among (altitudinal) regions, a plant species can be pollinated by different insect species. This spatial variation in pollinator identity is well known in various species, including orchids (e.g. Schatz et al. 2005, Gómez and Zamora 2006, González et al. 2014, Sun et al. 2014, Gross et al. 2016). The aim of this study was thus to determine the guilds of floral visitors and potential pollinators to *O. militaris* in a region that was not widely studied so far: Southern Belgium (Wallonia).

Materials and Methods

Study Species

O. militaris (Fig. 1) is a perennial herb with a Eurasian-Mediterranean distribution area (Kretzschmar et al. 2007). The species is confined to calcareous soils and occurs in sunny or partially shaded sites. It ranges from sea level to 2,200 m and it can grow in

Fig. 1. *O. militaris* visited by *A. mellifera* (Wijre-akkers, Netherlands, 1 June 2010, photographer: Jean Claessens).

chalk grasslands, sand dunes, wet meadows, abandoned cultivated lands, scrub communities or open woodlands. *O. militaris* appears as a moderately long stem, generally varying from 20 to 45 cm, with a single inflorescence bearing 10–40 hermaphroditic flowers (Farrell 1985, Delforge 2012). It blooms from April to June (-July) (Delforge 2012). *O. militaris* is assumed to be self-compatible (Neiland and Wilcock 1998, Metsare et al. 2015) and non-autogamous (Delforge 2012), and it depends on the food deception strategy to attract pollinators (Claessens and Kleynen 2011). Low levels of fruit set (<30%) are common (Tremblay et al. 2005, Claessens and Kleynen 2011).

Study Sites

The study was carried out in 2013 in five Belgian populations (Table 1). According to Alexandersson and Ågren (1996), a population was defined as a group of inflorescences separated from their closest conspecific by at least 100 m. All populations were at least 5 km apart, except those of Visé, which were 800 m apart (but separated by a canal 140 m wide). The biotopes were various: recolonized slag heaps, semi-dry calcareous grassland, mesic grassland, thickets and deciduous forestry plantations.

Insect Collection

Field work took place during sunny and warm (at least 18 °C) days with little or no wind, between 8.00 a.m. and 8.00 p.m. between 27 May and 17 June (Table 1). We made around 30 total hours of captures (180 10-min censuses), thus ∼6 h (36 10-min censuses) per population. Each population was studied during 2 consecutive days, and the 6 h were regularly distributed throughout the 2 days. During 10-min censuses, taking a standard route (100 m) across the population, we used a butterfly net to catch all insects that landed on *O. militaris* inflorescences. We put them in individual pill-boxes containing a piece of cotton wool soaked with ethyl acetate. Pill-boxes were conserved in a cool box (EDA Plastiques, Oyonnax, France) with cold accumulators (Kern Frio S.A., L'Hospitalet de Llobregat, Spain) and then in a freezer at −18 °C. When insects bore pollinia, we used morphological characters in order to distinguish the pollinia of *O. militaris* from those of other orchids found in the study sites and their surroundings [*Anacamptis pyramidalis* (L.) Rich., *Neottia ovata* (L.) Bluff & Fingerh., *Ophrys apifera* Huds., *O Ophrys insectifera* (L.), *Orchis anthropophora* (L.) All., and *Platanthera chlorantha* (Custer) Rchb.]. Insects were mounted and identified. Most of the specimens are kept at the Biodiversity Research Centre (Université catholique de Louvain); some dipterans are stored at the Royal Belgian Institute of Natural Sciences.

Results

We caught a total of 104 insects belonging to 49 species, 21 families and four orders (Table 2). Most of the specimens were dipterans (50%) and hymenopterans (32%). *Rhingia campestris* Meigen (14 individuals) and *Bombylius venosus* Mikan (10 individuals) were the most abundant dipterans; *Apis mellifera* (L.) (8 individuals) and *Bombus lapidarius* (L.) (6 individuals) were the most abundant hymenopterans. Some lepidopterans and coleopterans were also collected. Syrphids were the most diverse family with 12 different species. Pollinia (only from *O. militaris*) were only found in hymenopterans: four honeybee workers (*A. mellifera*) bore respectively 13, 12, 3 (attached to the clypeus) and 2 pollinia, and one bumblebee worker (*B. lapidarius*) bore 1 pollinium. *A. mellifera* was caught in three sites and *B. lapidarius* in four of the five sites. Because ethyl acetate fumes rapidly dissolve the viscidium glue (Peter and Johnson 2013), we failed to determine the position of pollinia on four of the five insects. A number of insects were also observed but not caught on *O. militaris* inflorescences: *Clytus arietis* (L.) (Coleoptera, Cerambycidae), *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae), *Eristalis* sp. (Diptera, Syrphidae), *Sarcophaga* sp. (Diptera, Sarcophagidae), *Pyrausta aurata* Scopoli (Lepidoptera, Crambidae), *Polyommatus icarus* Rottemburg (Lepidoptera, Lycaenidae), *Vanessa* sp. (Lepidoptera, Nymphalidae), and *Anthocharis cardamines* (L.) (Lepidoptera, Pieridae).

Discussion

In this study, *O. militaris* attracted a variety of visitors, mainly hoverflies (32 individuals), bumblebees (16 individuals) and bee flies (13 individuals), probably through the display of general floral signals (typical for rewarding plants) such as showy flower colors, presence of a spur, etc. Rewarding plants attract insects and non-rewarding *O. militaris* individuals flowering nearby benefit from chance visits (Jersáková et al. 2009). All caught dipterans and lepidopterans have to be considered as visitors (*sensu* Schatz et al. 2005) since they bore no pollinia. To our knowledge, dipterans and butterflies are never cited as pollinators in the literature. The former visit various orchid species for nectar (absent in deceptive species like

Table 1. Characteristics of *O. militaris* populations

Site no.	Municipality	Site name	Coordinates	Altitude (m)	Population size	Date of insect collections
A	Visé	Friche du canal Albert	50° 46′16″ N 5° 41′′04″ E	55	~10,000	4–5 June 2013
B	Visé	Oseraie de Lanaye	50° 46′45″ N 5° 40′59″ E	65	~1,500	6–7 June 2013
C	Virton	Contournement de Virton	49° 34′38″ N 5° 30′58″ E	250	~150	16–17 June 2013
D	Musson	Crassier de Musson	49° 33′09″ N 5° 43′04″ E	290	~230	27–28 May 2013
E	Rouvroy	Nature reserve ‘Raymond Mayné’	49° 30′34″ N 5° 28′53″ E	295	~80	11–12 June 2013

The population size is given in terms of number of inflorescences.

Table 2. Identity and number of insects caught on *O. militaris* inflorescences

Order	Family	Taxon	A	B	C	D	E
Coleoptera	Elateridae	Indet.			1		
	Scarabaeidae	*Phyllopertha horticola* (L. 1758)		2	2		
		Valgus hemipterus (L. 1758)		2			
Diptera	Agromyzidae	Indet.		1			
	Asilidae	*Neoitamus* cf. *socius* (Loew 1871)					1
	Bombyliidae	*Bombylius major* (L. 1758)				3	
		B. venosus (Mikan 1796)			3	1	6
	Empididae	*Empis discolor* (Loew 1856)					1
		Empis femorata (F. 1798)	1				
		Empis livida (L. 1758)		1			
	Hybotidae	*Hybos* sp.				1	
	Scathophagidae	*Scathophaga stercoraria* (L. 1758)			1		
	Syrphidae	*Chrysotoxum cautum* (Harris 1776)	3	1			
		Episyrphus balteatus (De Geer 1776)	1				
		Eupeodes nitens (Zetterstedt 1843)					1
		Helophilus pendulus (L. 1758)		1			
		Merodon equestris (F. 1794)		1			
		Platycheirus albimanus (F. 1781)	1		1		
		Platycheirus peltatus (Meigen 1822)					1
Order	Family	Taxon	A	B	C	D	E
		Platycheirus scutatus (Meigen 1822)			1		
		R. campestris (Meigen 1822)			4	3	7
		Sphaerophoria scripta (L. 1758)			1		1
		Syritta pipiens (L. 1758)	1	1	1		
		Volucella bombylans (L. 1758)	1				
Hymenoptera	Andrenidae	*Andrena carantonica* (Perez 1902)					1(♀)
		Andrena flavipes (Panzer 1799)			1(♀)		
		Andrena nigroaenaea (Kirby 1802)					1(♀)
	Apidae	*A. mellifera* (L. 1758)	4(☿)	3(☿)			1(☿)
		Bombus hypnorum (L. 1758)		1(☿)			1(☿)
		B. lapidarius (L. 1758)	2(☿)		1(☿)	2(♀, ☿)	1(☿)
		Bombus lucorum (L. 1761)		1(♂)			
		B. pascuorum (Scopoli 1763)		1(☿)	1(☿)		
		Bombus pratorum (L. 1761)					4(3♂, 1 ☿)
		Bombus terrestris (L. 1758)	1(♀)				
	Colletidae	*Hylaeus confusus* (Nylander 1852)			1(♂)		
	Halictidae	*Lasioglossum fulvicorne* (Kirby 1802)					1(♀)
		Lasioglossum malachurum (Kirby 1802)	1(♀)				
		Lasioglossum parvulum (Schenck 1853)					1(♀)
		Sphecodes sp.	1(♀)				
	Megachilidae	*O. bicolor* (Schrank 1781)				1(♀)	
Lepidoptera	Crambidae	*Pyrausta* sp.			1		
	Geometridae	*Siona lineata* (Scopoli 1763)		1			
	Hesperiidae	*Erynnis tages* (L. 1758)					1
		Carterocephalus palaemon (Pallas 1771)					2
	Nymphalidae	*Aglais io* (L. 1758)		1			
		Lasiommata megera (L. 1767)			1		
		Melitaea cinxia (L. 1758)		1			
	Noctuidae	*Euclidia glyphica* (L. 1758)			1	1	
	Pieridae	*Pieris napi* (L. 1758)			2		

Formicidae were present in all the populations but weren’t caught. ♀ = female; ♂ = male; ☿ = worker.

Table 3. Non-exhaustive list of insects observed on *O. militaris inflorescences* in different countries

Order	Family	Taxon	Country (ISO code)	References
Coleoptera	Cantharidae	*Cantharis lateralis* (L. 1758)	RU	12
		Cantharis rustica (Fallen 1807)	GB	9
	Cerambycidae	*Pachyta quadrimaculata* (L. 1758)	RU	10
		Rutpela maculata (Poda 1761)	GB	9
	Cetoniidae	*T. hirta* (Poda 1761)	RU, RU, AT	10*, 12*, 15*
	Chrysomelidae	*Cassida viridis* (L. 1758)	GB	9
		Smaragdina salicina (Scopoli 1763)	RU	12
	Malachiidae	*Malachius bipustulatus* (L. 1758)	RU	12
		Malachius coccineus (Waltl 1838)	RU	12
	Oedemeridae	*Oedemera nobilis* (Scopoli 1763)	RU	10, 12
Diptera	Bibionidae	*Bibio marci* (L. 1758)	GB	9
	Bombyliidae	*B. major* (L. 1758)	AT	13
		B. venosus (Mikan 1796)	AT	13
	Calliphoridae	*Onesia* sp.	GB	7
	Empididae	*Empis tessellata* (F. 1794)	FR, GB	3, 9
	Muscidae	*Musca* sp.	AT	13
		Thricops semicinereus (Wiedemann 1817)	GB	7
	Opomyzidae	*Opomyza germinationis* (L. 1758)	GB	7
	Scathophagidae	*S. stercoraria* (L. 1758)	GB	7
	Syrphidae	*Baccha elongata* (F. 1775)	GB	7
		C. cautum (Harris 1776)	GB	9
		Chrysotoxum octomaculatum (Curtis 1837)	AT	13
		E. balteatus (De Geer 1776)	GB, AT	9, 13
		Eristalis pertinax (Scopoli 1763)	GB	9
		Leucozona lucorum (L. 1758)	GB	7
		Melanostoma mellinum (L. 1758)	GB	7
		Melanostoma scalare (F. 1794)	GB	9
		Platycheirus ambiguus (Fallen 1817)	AT	13
		P. scutatus (Meigen 1822)	GB	7
		R. campestris (Meigen 1822)	GB	7
Hemiptera	Cercopidae	*Cercopis vulnerata* (Rossi 1807)	GB	9
	Scutelleridae	*Eurygaster* spp.	GB	9
Hymenoptera	Andrenidae	*A. carantonica* (Perez 1902)	AT	13
		Andrena cineraria (L. 1758)	RU	12*
		Andrena curvungula (Thomson 1870)	FR	8*
		A. enslinella (Stoeckhert 1924)	AT	13*, 14*
Order	Family	Taxon	Country (ISO code)	Reference
		A. flavipes (Panzer 1799)	AT	13
		Andrena hattorfiana (F. 1775)	AT	13*, 14*
		Andrena helvola (L. 1758)	AT	13
		Andrena minutula (Kirby 1802)	AT	13
		Andrena subopaca (Nylander 1848)	AT	13
		Andrena taraxaci (Giraud 1861)	AT	13
		Andrena tarsata (Nylander 1848)	AT	13
	Apidae	*Anthophora aestivalis* (Panzer 1801)	AT	13*
		Anthophora borealis (Morawitz 1864)	RU	10*, 12*
		A. mellifera (L. 1758)	FR, FR, NL, GB, AT, AT	2*, 3*, 5*, 9*, 13*, 14*
		B. lapidarius (L. 1758)	FR, GB, FR, GB, AT	3, 7, 8, 9, 13
		B. lucorum (L. 1761)	GB	7, 9
		B. pascuorum (Scopoli 1763)	FR, GB	3*, 9
		B. pratorum (L. 1761)	GB	1*, 9
		B. terrestris (L. 1758)	GB, AT	9*, 13
		Bombus vestalis (Geoffroy 1785)	GB	1*
		Ceratina callosa (F. 1794)	AT	13
		Ceratina cyanea (Kirby 1802)	AT	13
		Eucera sp.	FR	3, 4
		N. fabriciana (L. 1767)	BE	11*
		N. ruficornis (L. 1758)	BE	11*
		N. succincta (Panzer 1798)	BE	11*
		Tetralonia sp.	FR	3
	Formicidae	*Myrmica ruginodis* (Nylander 1846)	GB	7
	Halictidae	*H. eurygnathus* (Bluethgen 1931)	AT	13*, 14*
		Halictus simplex (Bluethgen 1923)	AT	13*, 14*

(continued)

Table 3. continued

Order	Family	Taxon	Country (ISO code)	References
		Lasioglossum albipes (F. 1781)	AT	13
		Lasioglossum calceatum (Scopoli 1763)	AT	13
		Lasioglossum morio (F. 1793)	AT	13
		Lasioglossum nigripes (Lepeletier 1841)	AT	13
		Lasioglossum pauxillum (Schenck 1853)	AT	13
		Lasioglossum xanthopum (Kirby 1802)	AT	13
		Sphecodes ferruginatus (von Hagens 1882)	AT	14*
	Megachilidae	*H. adunca* (Panzer 1798)	BE	11*
		Osmia aurulenta (Panzer 1799)	AT	13*, 14*
		O. bicolor (Schrank 1781)	AT	13*
		Osmia rufa (L. 1758)	BE	11*
	Sphecidae	*Ammophila sabulosa* (L. 1758)	GB	9
		Sphex funerarius (Gussakovskij 1934)	GB	9
Order	Family	Taxon	Country (ISO code)	Reference
	Vespidae	*Vespula vulgaris* (L. 1758)	GB	9
Lepidoptera	Geometridae	*Pseudopanthera macularia* (L. 1758)	AT	13
	Hesperiidae	*C. palaemon* (Pallas 1771)	DE, AT	6, 13
	Lycaenidae	*P. icarus* (Rottemburg 1775)	DE	6
	Nymphalidae	*A. io* (L. 1758)	GB	9
		Boloria euphrosyne (L. 1758)	DE	6
	Papilionidae	*Parnassius mnemosyne* (L. 1758)	RU	12
	Pieridae	*A. cardamines* (L. 1758)	DE	6
		Colias hyale (L. 1758)	DE	6
		Gonepteryx rhamni (L. 1758)	DE	6
		Leptidea sinapis (L. 1758)	DE	6
		Pieris brassicae (L. 1758)	DE, GB, GB	6, 7, 9
		P. napi (L. 1758)	DE, GB, GB, AT	6, 7, 9, 13
		Pieris rapae (L. 1758)	GB	9

1, Bateman and Rudall (2014); 2, Berger (2003); 3, Berger (2004); 4, Berger (2010); 5, Claessens and Kleynen (2011); 6, Ebert and Rennwald (1993); 7, Farrell (1985); 8, Godfery (1933); 9, Harding (1996); 10, Krivosheev et al. (2009); 11, Petit (1998); 12, Shamigulova (2012); 13, Vöth (1987); 14, Vöth (1999); 15, Vöth (2003). *potential pollinator *sensu* Schatz et al. (2005).

O. militaris, which exploit the food-seeking behavior of insects and lure visitors—Jersáková et al. 2006), substances secreted by the stigma, pollen or occasionally to find a shelter; lepidopterans seek nectar essentially in species with a long spur (Berger 2003), like *Gymnadenia* spp. or *Platanthera* spp. Caught and observed coleopterans were "simple visitors" (*sensu* Berger 2003); they apparently landed randomly on inflorescences and never actively visited flowers. *A. mellifera* was already known as a potential pollinator (i.e. insects collected in an inflorescence while carrying one or more pollinia—after Schatz et al. 2005) of *O. militaris* but it was apparently not the case of *B. lapidarius*, although this species was previously observed on *O. militaris* in different countries (Table 3). The high number of pollinia found on two of the four caught honeybees (13 and 12, respectively) indicates repeated visits to *O. militaris*.

Several factors can explain why an insect is only a visitor and not a pollinator of a plant species (Pellmyr 2002; Shivanna and Tandon 2014). In the case of *O. militaris*, the behavior and or the morphology of visitors on the orchid flowers normally prevents these insects from picking up pollinia. To do so, an insect first has to press the spur opening. Then the bursicle's membrane breaks and the viscidia are released and glued to the insect's head (Claessens and Kleynen 2011). As long as individuals from a given insect species are not documented as having collected at least one pollinium, they should only be considered as ineffective visitors. This categorization could be temporary or permanent; for example, some insect groups, like orthopterans (but see Micheneau et al. 2010), usually are never considered as pollinators. Caught insects bearing pollinia are potential pollinators and could be either incidental (transporting pollinia by chance) or confirmed ones. A potential pollinator becomes confirmed when the observer has seen the removal of one or more pollinia from a flower and the deposit of pollen on the stigma of another flower from the same species (Schatz et al. 2005; but see Berger (2006) who details the limits of this definition).

More than 90 insect species (half are hymenopterans) are known to visit the flowers of *O. militaris* (Table 3). Not all these flower visitors are effective at pollen delivery. One-fourth (almost exclusively hymenopterans, with the exception of the coleopteran *Tropinota hirta* Poda) have to be considered as potential pollinators. Among them, some species were caught in this study but bore no pollinia (Apidae: *Bombus pascuorum* Scopoli, *B. pratorum* (L.), and *B. terrestris* (L.); Megachilidae: *Osmia bicolor* Schrank). The low sample size per species (in average, two specimens) could explain why we did not observe pollinia on these species. Moreover, a few potential pollinators from Table 3 are not present or extremely rare in Belgium: *Andrena enslinella* Stoeckhert (Andrenidae: Rasmont et al. 2013), *Anthophora aestivalis* Panzer, *A. borealis* Morawitz (Apidae: Rasmont 2014), and *Halictus eurygnathus* Bluethgen (Halictidae: Pauly et al. 2016); their role in *O. militaris* pollination is probably very low in this country. In addition, in the study site "Oseraie de Lanaye" (Table 1), Petit (1998) found specimens from three species of *Nomada* Scopoli (Apidae: *Nomada fabriciana* (L.), *Nomada ruficornis* (L.), and *Nomada succincta* Panzer) and *Hoplitis adunca* Panzer (Megachilidae) with *O. militaris* pollinia. However, none of these species was observed in our study. Finally, as mentioned earlier, *A. mellifera* was the only species bearing pollinia in common in this study (Table 2) and in previous ones (Table 3).

According to Vöth (1987), confirmed pollinators (at least in Lower Austria) are short-tongued hymenopterans from the genera

Andrena (F.) (Andrenidae: *A. enslinella* and *A. hattorfiana* F.) and *Halictus* Latreille (Halictidae: *H. eurygnathus* and *H. simplex* Bluethgen). *A. hattorfiana* (Rasmont et al. 2013), *H. eurygnathus*, and *H. simplex* (Pauly et al. 2016) are found in Belgium, but these species were not observed on *O. militaris* during the censuses. As mentioned in the previous paragraph, *A. enslinella* is absent from the Belgian territory (Rasmont et al. 2013). Vöth's conclusion is based upon the presence of pollinia on insects and the similarity between spur length (and lip length) and proboscis length (and body length). According to this interpretation, pollinia adhesion and subsequent transport would be unadapted to the morphology of long-tongued bees (in Vöth's study, Apidae: *A. mellifera*, *B. lapidarius*, and *B. terrestris*) because they reach the end of the spur before the viscidium adheres to the insect (Vöth 1987, van der Cingel 1995). However, real pollination events are not described by Vöth (1987) and his conclusion is thus mostly putative. Bateman and Rudall (2014) hypothesized that bumblebees (*B. pratorum* and *B. vestalis* Geoffroy) could be confirmed pollinators in a British population. In Vöth's study, the two mentioned bumblebee species (*B. lapidarius* and *B. terrestris*) weren't pollinators because no specimen bore pollinia (but see Harding 1996) and their proboscis was longer than the spur. *B. lapidarius* and *B. terrestris* are long-tongued bees but this is not the case of the short-tongued *B. pratorum* and *B. vestalis* (Goulson 2010, Bateman and Rudall 2014). The results of Vöth (1987) and those of Bateman and Rudall (2014) suggest the importance of short-tongued (non-)Apidae bees as confirmed pollinators of *O. militaris*. In general, long-tongued bees, like *A. mellifera* and *B. lapidarius* in this study, probably remove pollinia accidentally during their visits. In certain cases, the number of removing events can be high but specimens bearing numerous pollinia should be rare, although we mostly observed honeybees bearing many pollinia. The function of short-tongued bees should be examined in more details through direct observation of pollination events and study of pollination efficiency. The status of incidental pollinators attributed to long-tongued bees could also be confirmed through experiments involving *A. mellifera* or *B. terrestris* (e.g. hives provided by Biobest, Westerlo, Belgium). Direct field observations and captures also remain of the greatest importance to determine the role of insect visitors and study the visitor spectrum of a plant species.

Acknowledgments

We warmly thank Thomas Merckx, Marc Migon, Jonas Mortelmans, Alain Pauly, Jean-Luc Renneson, and Nicolas Vereecken for their help in the identification of several specimens of dipterans, hymenopterans, and lepidopterans. We thank Philippe Blerot (Service public de Wallonie, Département de la Nature et des Forêts) for providing us insect collecting permit for several Walloon *O. militaris* populations. We would also like to thank Benoît Bailleux, Jean-Louis Gathoye, and Philippe Toussaint for giving us information about populations and their location. We are grateful to Laurent Berger and Jean Claessens for providing us bibliographic documents and photographs respectively. We thank Ardenne et Gaume asbl and local administrations for access to sites. We finally thank two anonymous reviewers for their constructive comments, which helped us to improve the article. This is BRC347 contribution from the Biodiversity Research Centre (Université catholique de Louvain).

References Cited

Alexandersson, R., and J. Ågren. 1996. Population size, pollinator visitation and fruit production in the deceptive orchid *Calypso bulbosa*. Oecologia. 107: 533–540.

Bateman, R. M., and P. J. Rudall. 2014. Bumblebee-mediated pollination of English populations of the Military Orchid (*Orchis militaris*): its possible relevance to functional morphology, life history and climate change. New J. Bot. 4: 122–133.

Berger, L. 2003. Observations sur le comportement de quelques pollinisateurs d'Orchidées (1ère partie). L'Orchidophile. 158: 201–216.

Berger, L. 2004. Observations sur le comportement de quelques pollinisateurs d'Orchidées (3e partie). L'Orchidophile. 160: 21–37.

Berger, L. 2006. Quelques notions de base sur la pollinisation des orchidées. L'Orchidophile. 170: 183–202.

Berger, L. 2010. De la similitude du comportement des mâles d'abeilles *Eucerini* sur différents genres d'orchidées, ainsi que celui d'autres abeilles qui ont des mœurs comparables. Bulletin De La Société Française D'Orchidophilie Rhône-Alpes. 22: 16–41.

Claessens, J., and J. Kleynen. 2011. The flower of the European orchid: form and function. Jean Claessens & Jacques Kleynen, Voerendaal, Netherlands.

Cozzolino, S., F. P. Schiestl, A. Müller, O. De Castro, A. M. Nardella, and A. Widmer. 2005. Evidence for pollinator sharing in Mediterranean nectar-mimic orchids: absence of premating barriers?. Proc. R. Soc. B. 272: 1271–1278.

Delforge, P. 2012. Guide des orchidées de France, de Suisse et du Benelux, 2nd ed. Delachaux et Niestlé, Paris, France.

Ebert, G., and E. Rennwald. 1993. Die Schmetterlinge Baden-Württembergs, band 1: Tagfalter I. Eugen Ulmer GmbH&Co, Stuttgart, Germany.

Farrell, L. 1985. Biological flora of the British Isles: *Orchis militaris* L. J. Ecol. 73: 1041–1053.

Godfery, M. J. 1933. Monograph & Iconograph of native British Orchidaceae. Cambridge University Press, Cambridge, United Kingdom.

Gómez, J. M., and R. Zamora. 2006. Ecological factors that promote the evolution of generalization in pollination systems, pp. 145–66. *In* N.M. Waser and J. Ollerton (eds.), Plant–pollinator interactions: from specialization to generalization. The University of Chicago Press, Chicago, IL.

González, A. V., M. Murúa, and P. A. Ramírez. 2014. Temporal and spatial variation of the pollinator assemblages in *Alstroemeria ligtu* (Alstroemeriaceae). Rev. Chil. Hist. Nat. 87:5

Goulson, D. 2010. Bumblebees: behaviour, ecology, and conservation. Oxford University Press, New York, NY.

Gross, K., M. Sun, and F. P. Schiestl. 2016. Why do floral perfumes become different? region-specific selection on floral scent in a terrestrial orchid. PLoS One. 11: e0147975.

Harding, M. 1996. The ecology of the military orchid, pp. 44–57. *In* P. Ratcliffe and J. Claridge (eds.), Thetford forest park: the ecology of a pine forest. Forestry Commission, Technical Paper 13.

Jersáková, J., S. D. Johnson, A. Jürgens. 2009. Deceptive behavior in plants. II. Food deception by plants: from generalized systems to specialized floral mimicry, pp. 223–46. *In* F. Baluška (ed.), Plant-environment interactions: from sensory plant biology to active plant behavior. Springer-Verlag, Berlin Heidelberg, Germany.

Jersáková, J., S. D. Johnson, P. Kindlmann. 2006. Mechanisms and evolution of deceptive pollination in orchids. Biol. Rev. 81: 219–235.

Kretzschmar, H., W. Eccarius, and H. Dietrich. 2007. The Orchid Genera Anacamptis, Orchis, Neotinea: phylogeny, taxonomy, morphology, biology, distribution, ecology, hybridisation. EchinoMedia Verlag Dr. Kerstin Ramm, Bürgel, Germany.

Krivosheev, M. M., I. V. Suyundukov, and A. S. Shamigulova. 2009. Некоторые особенности репродуктивной биологии *Orchis militaris* L. на Южном Урале. Вестник Оренбургского государственного университета. 6: 168–171.

Lortie, C. J., A. E. Budden, and A. M. Reid. 2012. From birds to bees: applying video observation techniques to invertebrate pollinators. J. Poll. Ecol. 6: 125–128.

Metsare, M., A. Ilves, M. Haldna, T. Kull, and K. Tali. 2015. Four seed-quality measures in orchids with different pollination systems. Acta Botanica Gallica. 162: 263–269.

Micheneau, C., J. Fournel, B. H. Warren, S. Hugel, A. Gauvin-Bialecki, T. Pailler, D. Strasberg, and M. W. Chase. 2010. Orthoptera, a new order of pollinator. Ann. Bot. 105: 355–364.

Micheneau, C., S. D. Johnson, and M. F. Fay. 2009. Orchid pollination: from Darwin to the present day. Bot. J. Linn. Soc. 161: 1–19.

Nakase, Y., and K. Suetsugu. 2016. Technique to detect flower-visiting insects in video monitoring and time-lapse photography data. Plant Species Biol. 31: 148–152.

Neiland, M. R., and C. C. Wilcock. 1998. Fruit set, nectar reward, and rarity in the Orchidaceae. Am. J. Bot. 85: 1657–1671.

Nilsson, L. A. 1992. Orchid Pollination Biology. Trends Ecol. E. 7: 255–259.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals?. Oikos. 120: 321–326.

Pauly, A., Y. Pesenko, and V. Radchenko. 2016. Les *Halictus* Latreille, 1804 d'Europe et du Bassin Méditerranéen. Atlas Hymenoptera. http://www.atlashymenoptera.net/page.asp?id=70

Pellmyr, O. 2002. Pollination by animals, pp. 157–84. *In* C.M. Herrera and O. Pellmyr (eds.), Plant-animal interactions: an evolutionary approach. Blackwell Science, Oxford, United Kingdom.

Peter, C. I., and S. D. Johnson. 2013. Generalized food-deception: colour signals and efficient pollen transfer in bee-pollinated species of *Eulophia* (Orchidaceae). Bot. J. Linn. Soc. 171: 713–729.

Petit, J. 1998. Sur quelques Hyménoptères aculéates nouveaux ou intéressants pour la Montagne Saint-Pierre et la région voisine (Province de Liège, Belgique). Lambillionea. 98: 255–266.

Rasmont, P. 2014. Atlas of the European Bees: genus Anthophora, 1st edn. STEP Project, Atlas Hymenoptera, Mons, Gembloux. http://www.zoologie.umh.ac.be//hymenoptera/page.asp?ID=260

Rasmont, P., S. P. M. Roberts, D. Michez, O. Schweiger, M. Franzen, T. De Meulemeester, B. Tomozein, and V. Radchenko. 2013. Atlas of the European Bees: genus Andrena, 1st ed. STEP Project, Atlas Hymenoptera, Mons, Gembloux. http://www.zoologie.umh.ac.be//hymenoptera/page.asp?ID=243

Renner, S. S. 2006. Rewardless Flowers in the Angiosperms and the Role of Insect Cognition in Their Evolution, pp. 123–44. *In* N.M. Waser and J. Ollerton (eds.), Plant-Pollinator Interactions: from Specialization to Generalization. The University of Chicago Press, Chicago, IL.

Schatz, B., M. Démares, B. Lorella, F. Séité, and D. Prat. 2005. Définitions autour de la relation insectes-orchidées. L'Orchidophile. 165: 129–136.

Schiestl, F. P., and S. D. Johnson. 2013. Pollinator-mediated evolution of floral signals. Trends Ecol. E. 28: 307–315.

Scopece, G., S. Cozzolino, and A. Dafni. 2014. Darwin on the pollination of *Orchis* - what he taught us and what we can tell him today, pp. 23–46. *In* R. Edens-Meier and P. Bernhardt (eds.), Darwin's Orchids: then and Now. The University of Chicago Press, Chicago, IL.

Shamigulova, A. S. 2012. Особенности биологии и экологии, динамика ценопопуляций *Orchis militaris* L. (Orchidaceae) в степной зоне Башкирского Зауралья. Candidate of Biological Sciences, Bashkir State University, Ufa, Russia.

Shivanna, K. R., and R. Tandon. 2014. Reproductive Ecology of Flowering Plants: a Manual. Springer, New Delhi, India.

Singer, R. B., B. Gravendeel, H. Cross, and S. R. Ramirez. 2008. The Use of Orchid Pollinia or Pollinaria for Taxonomic Identification. Selbyana. 29: 6–19.

Sun, M., K. Gross, and F. P. Schiestl. 2014. Floral adaptation to local pollinator guilds in a terrestrial orchid. Ann. Bot. 113: 289–300.

The Plant List. 2013. Orchidaceae. http://www.theplantlist.org/1.1/browse/A/Orchidaceae/

Tremblay, R. L., J. D. Ackerman, J. K. Zimmerman, and R. N. Calvo. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. Biol. J. Linn. Soc. 84: 1–54.

van der Cingel, N. A. 1995. An Atlas of Orchid Pollination: European Orchids. A.A. Balkema Publishers, Rotterdam, Netherlands.

Vöth, W. 1987. Bestäubungsbiologische Beobachtungen an *Orchis militaris* L. Die Orchidee. 38: 77–84.

Vöth, W. 1999. Lebensgeschichte und Bestäuber der Orchideen am Beispiel von Niederösterreich. Stapfia. 65: 1–257.

Vöth, W. 2003. Orchideen und ihre Käfer. Journal Europäischer Orchideen. 35: 381–405.

Widmer, A., S. Cozzolino, G. Pellegrino, M. Soliva, and A. Dafni. 2000. Molecular analysis of orchid pollinaria and pollinaria-remains found on insects. Mol. Ecol. 9: 1911–1914.