Entomofauna

ZEITSCHRIFT FÜR ENTOMOLOGIE

Band 34, Heft 27: 357-368 ISSN 0250-4413 Ansfelden, 2. Januar 2013

Pollination activity of *Zygaena filipendulae* (LINNAEUS, 1758) (Lepidoptera: Zygaenidae) in *Anacamptis pyramidalis* orchid on the North Bull Island (Ireland)

Elisa VALLIUS, Ulf BUCHSBAUM & Vladimir NAZAROV

Summary

The pollination biology of the *Anacamptis pyramidalis* (L.) RICH. was studied at five localities on North Bull Island in Ireland. During numerous observation hours different insect visitors on the Pyramidal orchid and other pink-purple flowered plants were captured. But only three species of Lepidoptera - *Zygaena filipendulae* (LINNAEUS, 1758), *Heliothis peltigera* (DENIS & SCHIFFERMÜLLER, 1775) and *Maniola jurtina* (LINNAEUS, 1758) – were found to carry pollinaria of *A. pyramidalis* on their proboscis. In overall 47 individual pollen vectors for this orchid were registered. *Z. filipendulae* dominated as visitors and pollinators from Pyramidal orchid in all studied localities. In most cases, *Z. filipendulae* individual carried from 3 to 5 pollinaria. The male and female *Z. filipendulae* demonstrated different pollination activity. In total, males were found to carry more pollinaria than females (86.7% vs. 13.3%). However the female pollen vectors had more pollinaria attached to them than male moths (5.5 vs. 2.9).

Zusammenfassung

Die Bestäubungsbiologie von *Anacamptis pyramidalis* (L.) RICH. von fünf Lokalitäten auf North Bull Island in Irland wurde untersucht. Während zahlreicher Beobachtungsstunden konnten verschiedene Insektenarten als Besucher von Pyramiden-Orchideen-Blüten registriert werden. Nur drei Arten davon - *Zygaena filipendulae* (LINNAEUS, 1758), *Heliothis peltigera* (DENIS & SCHIFFERMÜLLER, 1775) und *Maniola jurtina* (LINNAEUS, 1758) - tragen die Pollinarien von der Pyramiden-Orchidee auf ihrem Saugrüssel. Insgesamt wurden 47 solche Pollinarienträger nachgewiesen. *Z. filipendulae* dominierte in allen untersuchten Lokalitäten. Sie trägt in der Regel 3-5 Pollinaria der Pyramiden-Orchidee auf dem Saugrüssel. Männliche und weibliche Exemplare von Widderchen zeigen eine unterschiedliche Bestäubungsaktivität. Die Männchen tragen die Pollinaria viel häufiger als die Weibchen (86.7% vs. 13.3%). Die Weibchen haben durchschnittlich mehr Pollinaria pro Exemplar als die Männchen (5.5 vs. 2.9).

key words: orchids, pollination, pollen vectors, visitors, floral mimicry, *Anacamptis pyramidalis*, *Zygaena filipendulae*, Zygaenidae, Lepidoptera

Introduction

It is well known that insects are the most important pollinators for plants and of course also for orchids. Orchids have especially wide variety of adaptations related to pollinator attraction (e. g. DARWIN 1862, GOTTSBERGER 1999). These adaptations differ due to pollination strategy (rewarding vs. deceptive) and special qualities of pollen vectors. Some orchids produce a special scent (KAISER 1993) to attract pollinators as other species are pollinated via floral mimicry or special morphology without any pollinator reward (NILSSON 1983, DAFNI 1984, DARWIN 1862, SCHIESTL & COZZOLINO 2008, SWEZEY 1945).

The Pyramidal orchid (*Anacamptis pyramidalis* (L.) RICH.) occurs almost throughout whole Europe. It ranges from the southern Scandinavia to northern Africa in south, and from the British Isles to Caucasus in east (FOLEY & CLARKE 2005). *A. pyramidalis* occurs on base-rich soils, and the habitats include rather dry, open, calcareous grassland where competition is not too excessive (FOLEY & CLARKE 2005). In British Isles the species is frequently found in coastal grasslands, especially on stabilised dune systems (FOLEY & CLARKE 2005).

Flowers of the Pyramidal orchid has sweet scent (dominated by benzenoids, ANDERSSON et al. 2002) and they are frequently visited by day- and night-flying Lepidoptera (e.g. FOLEY & CLARKE 2005). Flowers do not produce nectar (BELL et al. 2009), but FOLEY & CLARKE (2005) mention that butterflies are “attracted to the liquid in the spur”. Presence of this liquid and its possible role in the pollination of A. pyramidalis has not been discussed widely in the literature. Pollen vectors of *A. pyramidalis* are quite well studied on the northern and southern parts of range are . IBoth field studies (LIND & LINDEBORG 1989, NAZAROV & EFETOV 1993, KULL & TUULIK 2002, LIND et al. 2007) and examination of insect collections (NAZAROV et al. 2005, NAZAROV & BUCHSBAUM 2004, 2006) have shown that Zygaenidae species play very important role as pollinators of the Pyramidal orchid in the areas studied.

However, we could not find specific information about the pollinators of Pyramidal orchid from western Europe. Therefore in this new investigation on North Bull Island (Ireland) the authors examined the importance of burnet moths for pollination of Pyramidal orchid on the west-north parts of its area.

Material and Methods

The North Bull Island is located in the Dublin Bay north from Dublin Harbour. This dune island started to develop in 1700´s as a result of changes in tidal currents following the construction of embankment protecting the harbour in south side, and this development was accelerated after construction of the North Bull Wall in 1825 (FLOOD 1977). Today the North Bull Island is about 5 km long consisting of salt marsh, dunes and sand beach.

In 2006, diurnal lepidopterans were investigated in five micropopulations of the Pyramidal orchid (*Anacamptis pyramidalis* (L.) RICH.) (A - E in Fig. 1). Additionally, lepidopterans were monitored in the populations of *Dactylorhiza incarnata* ssp. *coccinea*, *D. fuchsii* and their putative hybrids (VALLIUS 2010) and occasionally also outside the orchid area. All study sites were located in the southern dune area of the island (F and G in Fig. 1, respectively Fig.1. A, B).

These micropopulations were observed about 5 hours in total during several days All visitors on the flowers of Pyramidal orchid were captured for identification and detecting for orchid pollinaria on its proboscis. Additionally this group of insects was checked outside of the orchid micropopulation for orchid pollinaria during additional 5 hours. Insect individuals that did not have any attached pollinaria on them, were released after checking. All specimens carrying at least on pollinium were collected and stored in a freezer for laboratory analyses.

The orchid pollinaria on the captured insects were investigated in the laboratory for identification. Each pollinaria was measured using LOMO binocular microscope MBS-10. A special Pollinia Database at RBO web-portal was used for the determination of orchid species on the basis of the morphological measures of pollinaria (http://www.r-b-o.eu).

Results

Although some species of Lepidoptera (*Maniola jurtina* (LINNAEUS, 1758) (Satyridae) and *Heliothis peltigera* (DENIS & SCHIFFERMÜLLER, 1775) (Noctuidae)) and bumblebee workers (Bombus, Apidae) were found to visit the flowers of Pyramidal orchid, most insects collected from the inflorescences were males and females of *Zygaena filipendulae* (LINNAEUS, 1758). A total of 16 specimens of this species were captured carrying pollinaria of the Pyramidal orchid (Tab. 1).

Additionally, a total of 31 specimens of Lepidoptera with orchid pollinaria were collected from the inflorescences of other plants as well inside as outside the micropopulation of *A. pyramidalis*. These plants, like Pyramidal orchid, have red, purple

or pink flowers (Table 2). The morphological analysis gave evidence that all orchid pollinaria on the proboscis of all Lepidoptera specimens studied, originated from *A. pyramidalis*.

Z. filipendulae was found to dominate as a visitor and pollinator of the Pyramidal orchid. 15 specimens of these insects carrying *A. pyramidalis* pollinaria were captured on the Pyramidal orchid flowers and 28 specimens on the flowers from other plants (Tab. 1 & 2). The males of *Z. filipendulae* were detected on the flowers of Pyramidal orchids more frequently than females (86.7% vs. 13.3%). Higher proportion of males than females were also found to carry *A. pyramidalis* pollinaria (88.0% vs. 12.0%). The pollinia load of male *Zygaena* was, however, smaller than that of females. In *A. pyramidalis* micropopulations, on average 2.9 pollinaria were detected on the proboscises of males, whereas the females carried on average 5.5 pollinaria. The same trend was observed outside the micropopulations of *A. pyramidalis* (3.7 vs. 4.3).

Discussion

The male and female individuals of *Z. filipendulae* were found to demonstrate a strong pollination activity for *Anacamptis pyramidalis* on the North Bull Island. In most cases, *Z. filipendulae* individual carried from 3 to 5 pollinaria. Some were detected even with 10 pollinaria from the Pyramidal orchid on its proboscises that indicates to very strong pollination activity on orchid flowers.

The role of *Z. filipendulae* for pollination of Pyramidal orchid from other parts its range is well-known. In general is it obviously less important as a pollinator species than other *Zygaena*. For example, the proportion of *Z. filipendulae* in *Zygaena* pollinators of Pyramidal orchid is only 8.8% in the central and south Europe (NAZAROV et al. 2005) and even 3.7% on the Crimea (NAZAROV & EFETOV 1993). On the island of Öland in Sweden, *Z. filipendulae* were found to have no role in pollination of *A. pyramidalis* population studied (LIND et al. 2007). In the previously studied areas, *Z. purpuralis* (BRÜNNICH, 1763), *Z. viciae* ([DENIS & SCHIFFERMÜLLER], 1775), *Z. lavandulae* (ESPER, 1783), *Z. loti* ([DENIS & SCHIFFERMÜLLER], 1775) and *Z. minos* ([DENIS & SCHIFFERMÜLLER], 1775). LIND et al. (2007) have been shown to dominate as pollinators of *A. pyramidalis*. However, these *Zygaena* species are not recorded from Ireland (DE FREINA & WITT 2001, NAUMANN et al. 1999). Therefore Z. *filipendulae* takes over the pollination role for Pyramidal orchid almost completely on the North Bull Island.

The other difference in the pollination activity of Z. *filipendulae* between the North Bull Island and all other studied *A. pyramidalis* is the number of pollinaria carried by male and female individuals. The female specimens of *Z. filipendulae* carried markedly more pollinaria than males (5.5 vs. 2.9). In other studies on pollination of Pyramidal orchid both sexes have carried equal pollinia load on their proboscises (on average 2.3 vs. 2.2). Additionally the number of Pyramidal orchid pollinia on the proboscises of *Z. filipendulae* females was two times higher on the North Bull Island than in other populations studied.

An interesting fact about the pollination in North Bull Island population is, that *A. pyramidalis* occurred in community together with other red and pink flowering orchid

species (*Dactylorhiza incarnata, D. fuchsii* and their hybrids). Other Lepidoptera species and bumblebee workers (*Bombus* sp.) were also observed to visit these nectarless orchids. Because these insects can not get either nectar or pollen from these orchids, they rely on other pink coloured but rewarding plants like e. g. *Trifolium pratense* in they search for food. The Insects with the orchid pollinaria more often could get catched at nectarferous plants. This is probably a result of a generalized form of Batesian mimicry, in which similarity to rewarding plants determines reproductive success of nectarless species (GUMBERT & KUNZE 2001). This can be explained by 'magnet-species hypothes' (LAVERTY 1992), in which the pollination of nectarless species is facilitated by the presence of food plants with a flower coloration similar to that of the deceptive species.

Even though some inter-specific visits between *Dactylorhiza* and *Anacamptis* by Lepidoptera and *Bombus* -workers was witnessed,the Lepidopteran species were never found to carry any pollinaria of *Dactylorhiza*, and bumble bee workers carried no *A. pyramidalis* pollinaria. The reason for this are probably the morphological differences both among orchid and insect taxa.

Some *Z. filipendulae* were collected on the nectarferous pink flowers of Red Clover *(T. pratense)*. It also plays a very important role in the pollination of *A. pyramidalis* on the North Bull Island. Firstly, because these flowers are model plants in the mimicry complex, in which burnet moths foraging in clover inflorescences are frequently visiting the flowers of non-rewarding *A. pyramidalis*. Secondly, intact *Z. filipendulae* populations are important for the preservation of *A. pyramidalis*. The food plants clovers (*Trifolium*) and other legumes (SPEIGHT 1977) exist in the habitat and support of a stable population level of this species.

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Author's adresses:
Elisa VALLIUS
Department of Biological and Environmental Science
P. O, Box 35
FIN-40014 University of Jyväskylä
Finland
E-Mail: elisa.vallius@springhouse.fi

Ulf BUCHSBAUM
Zoologische Staatssammlung München
Münchhausenstraße 21
D – 81247 München
Germany
E-Mail: UlfBuchsbaum.Lepidoptera@zsm.mwn.de

Vladimir Nazarov
Haydnstraße 35
D – 85622 Feldkirchen
Germany
E-Mail: nazarov@gmx.net

Fig. 1. The location of investigated *Anacamptis pyramidalis* micropop

Table 1. The insect species are captured on the flowers from Pyramidal orchid

Insects	Sex	Number of pollinaria	Site
Zygaena filipendulae	M	1	A
Zygaena filipendulae	M	2	A
Zygaena filipendulae	M	3	A
Zygaena filipendulae	F	2	A
Zygaena filipendulae	M	2	B
Zygaena filipendulae	M	1	*C*
Zygaena filipendulae	M	6	C
Zygaena filipendulae	M	7	C
Zygaena filipendulae	M	1	D
Zygaena filipendulae	M	5	D
Zygaena filipendulae	M	3	D
Zygaena filipendulae	M	2	D
Zygaena filipendulae	M	3	D
Zygaena filipendulae	M	6	D
Zygaena filipendulae	F	9	D
Zygaena filipendulae	M	4	E

M – male; F – female

Table 2. The insect species with *Anacamptis pyramidalis* pollinaria are collected outside the Pyramidal orchid micropopulations.

Insects	Sex	Number of pollinaria	Place of colleting	Site
Maniola jurtina	M	0	*Dactylorhiza fuchsii*	A
Zygaena filipendulae	M	4	*Dactylorhiza fuchsii*	A
Zygaena filipendulae	M	3	*Dactylorhiza fuchsii*	A
Zygaena filipendulae	M	4	*Dactylorhiza hybrid*	A
Zygaena filipendulae	M	4	*Dactylorhiza hybrid*	S
Maniola jurtina	M	2		B
Zygaena filipendulae	F	7	*Dactylorhiza fuchsii*	

Insects	Sex	Number of pollinaria	Place of colleting	Site
Zygaena filipendulae	M	2	*Dactylorhiza maculata*	F
Zygaena filipendulae	M	3	*Dactylorhiza maculata*	F
Zygaena filipendulae	M	3	*Dactylorhiza incarnata*	F
Zygaena filipendulae	M	2	*Dactylorhiza* hybrid	F
Zygaena filipendulae	M	1	*Dactylorhiza* hybrid	F
Heliothis peltigera	F	1	*Trifolium repens*	F
Zygaena filipendulae	M	3	Hay	C
Zygaena filipendulae	M	3	*Dactylorhiza* hybrid	C
Zygaena filipendulae	M	6		G
Zygaena filipendulae	M	5	*Dactylorhiza* hybrid	G
Zygaena filipendulae	M	4	*Trifolium pratense*	D
Zygaena filipendulae	M	2	*Trifolium pratense*	D
Zygaena filipendulae	M	3	*Trifolium pratense*	D
Zygaena filipendulae	M	1	*Dactylorhiza maculata*	D
Zygaena filipendulae	M	2	*Dactylorhiza incarnata*	D
Zygaena filipendulae	M	8	*Dactylorhiza fuchsii*	D
Zygaena filipendulae	M	5	*Dactylorhiza* hybrid	D
Zygaena filipendulae	M	1	*Dactylorhiza* hybrid	D
Zygaena filipendulae	M	10	*Dactylorhiza* hybrid	D
Zygaena filipendulae	M	4	*Dactylorhiza* hybrid	D
Maniola jurtina	M	4	Hay	
Zygaena filipendulae	F	1	*Dactylorhiza* hybrid	G
Zygaena filipendulae	M	3	*Dactylorhiza* hybrid	E
Zygaena filipendulae	M	2	*Dactylorhiza* hybrid	E
Zygaena filipendulae	M	5	*Dactylorhiza* hybrid	E
Zygaena filipendulae	F	5	*Dactylorhiza* hybrid	E

M – male; F – female

Druck, Eigentümer, Herausgeber, Verleger und für den Inhalt verantwortlich:
Maximilian SCHWARZ, Konsulent f. Wissenschaft der Oberösterreichischen Landesregierung, Eibenweg 6, A-4052 Ansfelden, E-Mail: maximilian.schwarz@liwest.at.

Redaktion: Erich DILLER, ZSM, Münchhausenstraße 21, D-81247 München;
Roland GERSTMEIER, Lehrstuhl f. Tierökologie, H.-C.-v.-Carlowitz-Pl. 2, D-85350 Freising;
Fritz GUSENLEITNER, Lungitzerstr. 51, A-4222 St. Georgen/Gusen;
Wolfgang SPEIDEL, MWM, Tengstraße 33, D-80796 München;
Thomas WITT, Tengstraße 33, D-80796 München.

Adresse: Entomofauna, Redaktion und Schriftentausch c/o Museum Witt, Tengstr. 33, 80796 München, Deutschland, E-Mail: thomas@witt-thomas.com; Entomofauna, Redaktion c/o Fritz Gusenleitner, Lungitzerstr. 51, 4222 St. Georgen/Gusen, Austria, E-Mail: f.gusenleitner@landesmuseum.at