Universidade de Évora
Mestrado em Biologia da Conservação

Dissertação

Investigating the Importance of Nocturnal Lepidoptera as Pollinators: a Network Approach

Mestranda
Paula Banza

Abril, 2011

Orientadores

Profª. Drª Anabela Belo, Universidade de Évora

Phd. Darren Evans, Universidade de Hull (Reino Unido)

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Photographs of front cover (left to right)

Phragmatobia fuliginosa; Aspitates ochrearia; Tyta luctuosa.

“Pollen – pollen – everywhere: in the bread you eat, in the air you breathe, in the dust in the street. Small, invisible to the naked eye, but indestructible by ordinary influences, capable of surviving millennia”

Knut Fægri, Professor Emeritus of Botany at the University of Bergen

In “Spores et pollen”, Josette Renault – Miskovsky and Michel Petzold, Editions LA DURAULIE

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ABSTRACT

Pollination can be viewed at the level of an entire ecological community as a network of mutualistic interactions between two trophic levels as most plants utilise multiple pollinators and *vice versa*.

Over the last ten years there has been growing interest in pollination networks and pollination webs have been studied covering a variety of geographical and ecological settings. However, nocturnal pollination as a community-level phenomenon has been overlooked and there are almost no published nocturnal pollination networks.

Moths are probably the most common nocturnal pollinators and they play a significant role in many communities as they are also herbivores and prey.

In this study two types of networks have been described: pollen transfer and flower visitation, nocturnal Lepidoptera pollinators have been identified and the construction of Portugal´s first nocturnal plant-pollinator network has been described. The main properties studied revealed a lower nestedness than expected when compared with other pollination networks, high number of interactions between species reflected on the high values of interaction evenness and interaction diversity; specialization was high for pollen transfer network and low for flower visitation network.

Understanding the ecology of moths is important for the conservation of moth and ecosystem services of pollination.

Keywords: plant-pollinator networks, pollen transport, moths, interactions, community.

A Importância dos Lepidópteros Nocturnos como Polinizadores: uma Abordagem às Redes de Polinização

RESUMO

A polinização pode ser entendida ao nível da comunidade ecológica como uma rede de interacções mutualistas entre dois níveis tróficos, já que a maior das plantas utiliza múltiplos polinizadores e vice-versa.

Nos últimos dez anos houve um crescente interesse nas redes de polinização e muitas têm sido estudadas e descritas cobrindo uma ampla variedade geográfica e ecológica. Contudo o estudo dos polinizadores nocturnos ao nível da comunidade, tem sido descurado e praticamente não existem redes nocturnas de polinização descritas na literatura especializada.

Os Lepidópteros nocturnos são talvez dos mais comuns polinizadores nocturnos e desempenham um papel muito importante nas comunidades biológicas também como presas e herbívoros.

Neste estudo descrevem-se dois tipos de redes de polinização: transferência de pólen e visitação floral; também se identificam alguns lepidópteros polinizadores nocturnos e constrói-se a primeira rede nocturna planta - polinizador para Portugal. As propriedades das redes de polinização estudadas revelaram um valor abaixo do esperado para o aninhamento ponderado e um elevado número de ligações por espécies, o que se reflecte nos valores elevados da diversidade e regularidade das interacções. O grau de especialização é elevado no caso da rede de transferência de polén mas muito baixo no caso da rede de visitação floral.

A compreensão da ecologia das borboletas nocturnas é muito importante para a sua conservação e também para a preservação da polinização enquanto serviço dos ecossistemas.

Palavras – chave: rede de polinização, transporte de polén, borboletas nocturnas interacções, comunidade ecológica.

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1. Introduction

The Lepidoptera is one of the most recent groups of insects; they started to diverge from about 290-220 million years ago (Garcia-Pereira & Garcia-Barros, 2003). Fossil records of Lepidoptera date back to the Jurassic period, yet the evolution of present-day species-rich lineages are probably related to the radiation of angiosperms during the Cretaceous within a relatively short time frame (Withfield & Kjer, 2008). There are about 165,000 described species of Lepidoptera in the world, and perhaps as many more awaiting discovery and description (Waring *et al*., 2003).

Moths are often thought of as the drab, night-flying relatives of butterflies, but a browse through a moth identification guide will tell us the opposite. Many moths are as brightly patterned and colourful as butterflies and, in terms of wing shape, body form and size, moths are much more varied than butterflies. In the adult stage, some moth species do not feed and live only a few days (CEE, 2009) but the majority of adult moths feed on flower nectar and many plants rely on them for pollination. Most larvae feed on plant material using biting-chewing mouthparts but the majority of adults use their proboscis to drink nectar and other liquid substances (Krenn, 2010). The role of Lepidoptera as pollinators has been demonstrated in many cases of mutualistic relationships with flowers and floral specialization (Fenster, *et al*., 2004; Kevan *et al*., 1983; Nilsson, 1988; Proctor *et al*., 1996; Schiestl & Schluter, 2009). Their adaptation to flower morphology has provided many examples of reciprocal adaptations in insect-flower interactions. For example, after Charles Darwin examined the flower of a star orchid possessing an approximately 300 mm-long nectar spur, he predicted the existence of a hawk moth with a proboscis of matching length (Darwin, 1862). This species of moth was actually discovered 40 years later (Nilsson, 1998).

The floral characteristics of plants pollinated by animals are very specific. The colours and scent of the perianth are the main factors for attracting the attention of pollinators.
The development of pollen across many anthers and many flowers frequently results in a characteristic temporary schedule of pollen presentation to pollinators (Thomson & Thomson, 1992).
The reproductive success of a plant depends largely on the amount of pollen it donates to stigmas. In animal-pollinated plants, this amount is influenced by the schedules of pollen presentation, pollen survivorship, and pollinator visits. Although each of these factors acts in a straightforward, comprehensible way when only a single pollinator type is attracted, heterogeneous pollinator fauna produce complicated interactions with the scheduling variables.

Pollination by animals occurs in virtually all terrestrial ecosystems. Modern angiosperms comprise an estimated 250,000 species (Heywood, 1993), and most of these — by some estimates over 90% (Buchmann *et al*., 1996) — are pollinated by animals, especially insects (Kearns,1998). The number of flower-visiting species worldwide may total nearly 300,000 (Nabhan *&* Buchmann 1997). Relatively few plant-pollinator interactions are absolutely obligate. Most are more generalized on the part of both plants and animals, and they also vary through time and space (Feinsinger, 1983; Feinsinger, 1987; Herrera, 1988; Herrera, 1996; Roubik, 1992; Waser *et al*., 1996).

Pollination can be viewed at the level of an entire ecological community as a web, or network, of mutually beneficial (mutualistic) interactions between two trophic levels, as most plants utilise multiple pollinators and vice versa (Waser *et al.* 1996). In this way, pollination systems can be examined in light of the theory of food webs (e.g. Memmott & Waser, 2002; Dicks *et al,* 2002), and other complex networks (e.g. Bascompte *et al*., 2003; Jordano *et al.,* 2003).

Humankind benefits from a multitude of resources and processes that are supplied by natural ecosystems. Collectively, these benefits are known as ecosystem services and include products like clean drinking water and processes such as the decomposition of wastes. The pollination of flowering plants by animals is a crucial ecosystem service of great value to humanity because without it most flowering plants would not reproduce sexually and humans would lose both food and other plant origin products (Buchmann *&* Nabhan, 1996). The economic importance of pollination, as well as its esthetic and ethical values, makes it clear that the conservation of pollination systems should be a high priority to mankind (Kearns, 1998).

Over the last ten years there has been growing interest in pollination networks and pollination webs have been studied covering a variety of geographical and ecological settings (Memmott, 1999; Dicks *et al*., 2002; Bascompte *et al*., 2003; 2007; Memmott *et al*., 2004; Olesen *et al*., 2007; 2008; Ings *et al*., 2008; Bosch *et al*., 2009). However, nocturnal pollination as a community-level phenomenon has been overlooked and there are almost no published nocturnal pollination webs (Devoto *et al*., 2011). Some of the animal reported to pollinate plants at night are moths, some bee families, bats, lizards, rodents, and other small mammals. But moths are probably the most common nocturnal pollinators, both in temperate and tropical areas.

The study of pollination by moths as a community–level phenomenon is the main goal of this work and encompasses the identification of nocturnal Lepidoptera pollinators; identification of the pollination networks established at the study site and the construction of Portugal´s first nocturnal plant-pollinator network.

2. Materials and Methods

2.1 Study site

The study was carried out in an abandoned meadow in the Western Algarve, near Portimão, in a place named “Quinta da Rocha Peninsula”. This area is part of the Ria de Alvor Natura 2000 Site. The Alvor estuary is an important area of wetlands, dunes and farmland protected from the sea by two sand spits, which shape the beaches of Alvor and Meia Praia. The estuary is at the confluence of three tributary streams, forming a lagoon system around two peninsulas – Quinta da Rocha and Abicada. It is the third most important wetland area in the Algarve and the first one in the Western Algarve in terms of size and conservation status. It is characterized by a rich diversity of birds, plants, insects, including butterflies and moths, molluscs, fish, amphibians, reptiles and mammals, with unique geological, ecological and environmental features (Jorge & Kaye, 2001).

Figure 1 - Location of the study site.

The study site was chosen according to the following criteria:

- High floral abundance and diversity
- Common flowering plants that could be found anywhere in Portugal

- Homogenous in terms of flora representation
- Open space surrounded by bushes and trees, therefore ideal for moths to move and find shelter.
- Easy access

Figure 2 - Aerial photograph of the study site annotated with boundaries.

This photograph was taken when there was no leafy herbaceous vegetation present.

Figure 3 - General view of the study site in spring.

2.2 Sampling design

2.2.1. Vegetation sampling

Vegetation was sampled in 20 plots, systematically set in a 4x5 lines grid arrangement separated by 15 m. The Braun-Blanquet method was followed in vegetation sampling but only plants in their floration period were recorded. The name and number of species in flower, their relative cover (%) and height (cm) were recorded, in addition to the total plant cover (%), whether they were in flower or not. The site was sampled on 19th and 24th March, 6th and 21st April, 5th and 18th May and 3rd June 2010 in order to correspond with moth sampling sessions. Every time the vegetation survey was undertaken a flower of each species was collected, placed into a separate plastic bag, identified and labeled for pollen collection. Nomenclature followed Flora Europaea (Tutin *et al*., 1964-1980). Where necessary, the nomenclature of the species was updated according Flora Iberica (Castroviejo *et al*., 1986-2009).

In order to build a pollen reference collection, the pollen was collected from the flower, placed and fixed on to a microscope slide using fuchsin jelly. The slides were kept for later observation in order to compare and identify the pollen transported by moths.

Figure 4 - Sampling the vegetation using the quadrats technique

The number of sampling sessions was determined in order to give a good overview of the plants during the flowering season, so that the greatest number of species in flower could be registered. See Annex 1 for the list of all plant species recorded in the area. It was also necessary to analyze pollen samples from other plant species found in the wider locality of the sample site (within a 500 meter radius) in order to be able to better identify the diversity of pollen carried by moths.

Pollen identification was facilitated by the use of a pollen collection from Évora University and an appropriate bibliography (Abreu & Moreno, 1998; Boi & Llorens, 2007; Smith, 1984). In most cases identification of pollen was to the level of genus e.g., *Urtica spp*, *Pinus spp*, *Cupressus spp*., *Acacia spp*., *Plantago spp.*, *Prunus spp*. with the exception of the Poaceae for which it was only possible to determine the family.

2.2.2. Moth sampling

As moth presence and abundance is very much related to the weather conditions this had to be taken into account when planning moth trapping sessions. The spring of 2010 was rather unusual, with lower minimum temperatures in April and May than in previous years and higher rainfall in April, May and June (unpublished A Rocha Observatory Report, 2011) as presented by the graphs below (figures 5, 6 and 7).

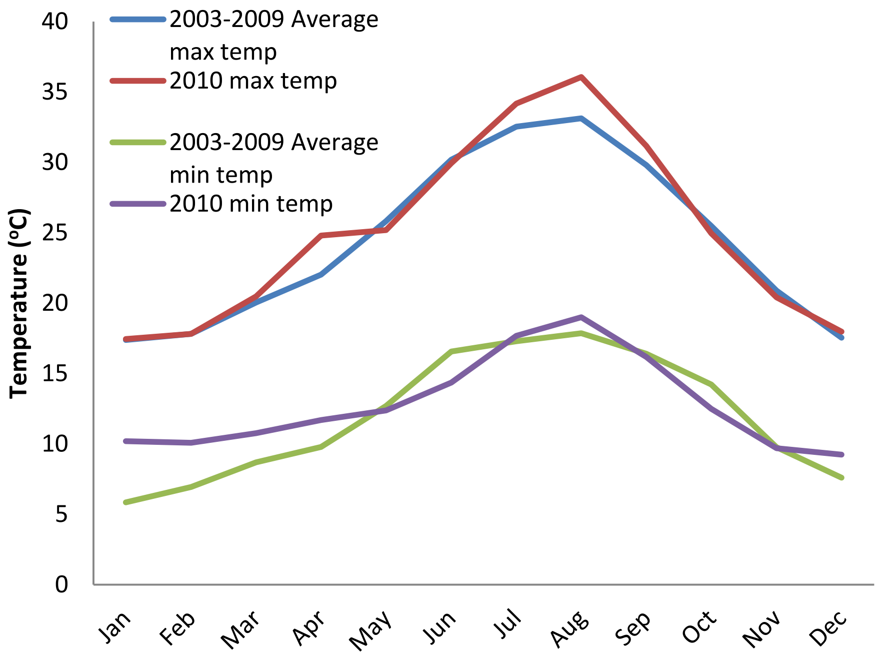

Figure 5 - Average monthly maximum and minimum air temperatures for 2010 and averages for 2003-2009.

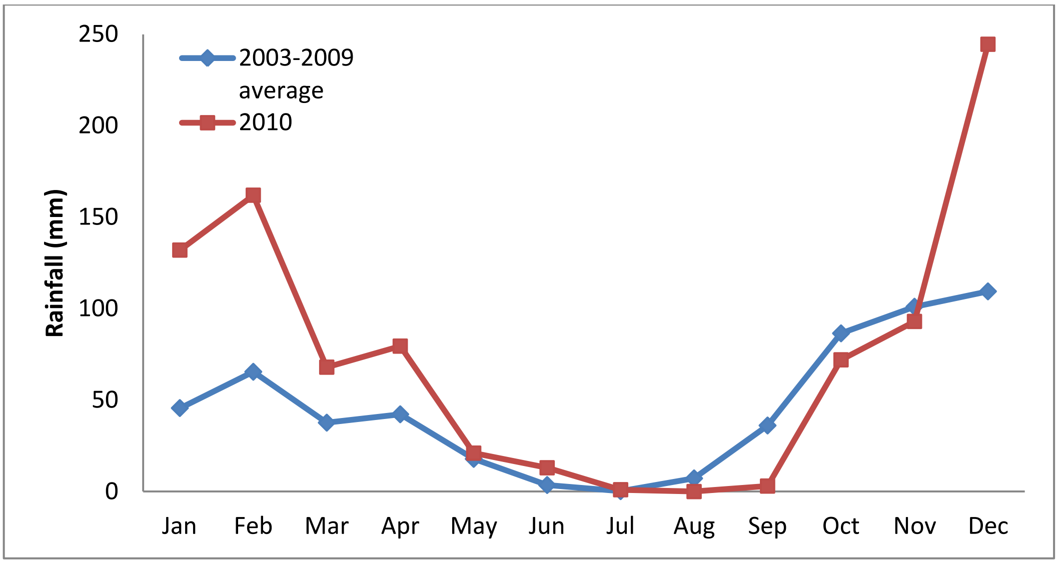

Figure 6 - Monthly rainfall for 2010 and average rainfall for each month from 2003-2009.

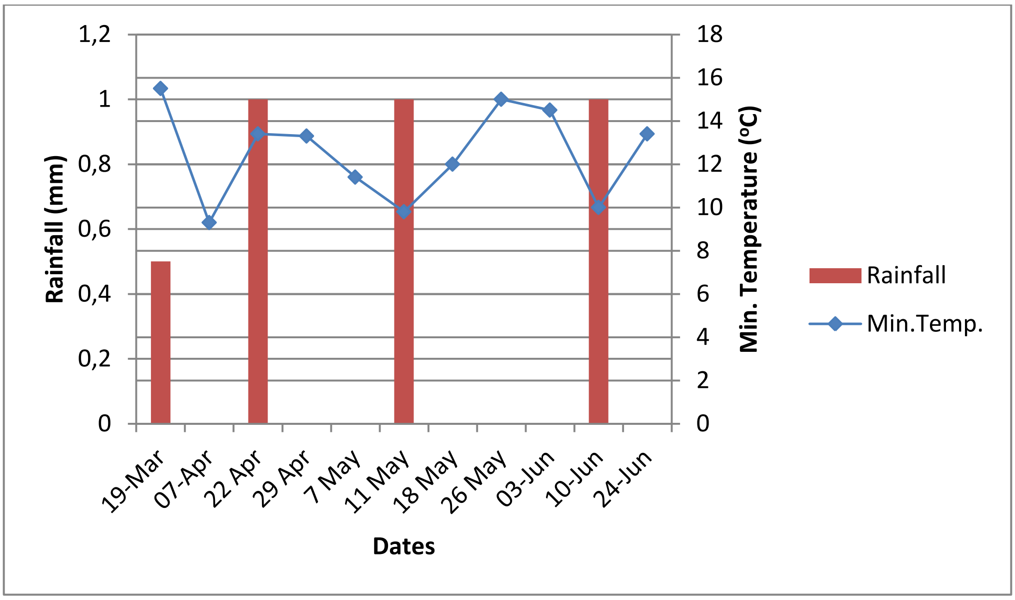

Figure 7 - Rainfall and minimum temperature during the moth sampling sessions.

The dates of the moth sampling sessions were chosen specifically having taken into account the local weather forecast and the brightness of the moon in order to trap as many moths as possible. Rainy and cold nights and/or a bright moon are associated with fewer numbers of moths trapped (pers. obs.).

The pollen loads of moths captured during this work were collected to determine which and how many plant taxa they had visited and these data were used to construct pollen transport networks (Bosch *et al*., 2009; Forup *et al*., 2008; Forup & Memmott, 2005).

A portable 6W UV-light (Philips TL 6W/05) heath trap was used to trap moths. The trap contained a few empty egg cartons to provide a foothold for the moths and was placed on a white sheet in order to aid collection. The trap was set on the ground in the centre of the field at sunset and collected the next day at sunrise. The captured moths were placed in individual tubes and transported to the freezer to kill and store them until processing (Devoto *et al.*, 2011). Moth trapping sessions were conducted on 19^{th} March, 7^{th}, 21^{st}, 29^{th} April, 7^{th}, 18^{th}, 26^{th} May, and the 3^{rd}, 10^{th} 24^{th} June.

Figure 8 - Moth trap (A) **and collecting the moths** (B).

2.2.3. **Moth-carried pollen sampling**

In order to sample the pollen carried by the moths, the moths were firstly placed in a re-hydration box for more than 12 hours before their heads were swabbed with a circle of fuchsin jelly. The area of the head between the base of the antennae, the labium and the eyes was swabbed, as this is the area of the body most likely to touch plant reproductive structures while feeding. Moths land on the flowers and have a very long proboscis to access the nectar from the plants so that most pollen grains are found on the head (Devoto *et al.*, 2011). Whenever possible, the proboscis was uncoiled and swabbed as well.

Figure 9 - Collecting the pollen from the moths.

The fuchsin jelly was melted onto a microscope slide and kept for later pollen identification and counting. To avoid fungal contamination a fine layer of colourless nail varnish was used to seal the slide content. After pollen had been removed, the moths were identified using a reference collection from “Associação A Rocha” and appropriate bibliography (Waring *et al*., 2003; Manley, 2008).

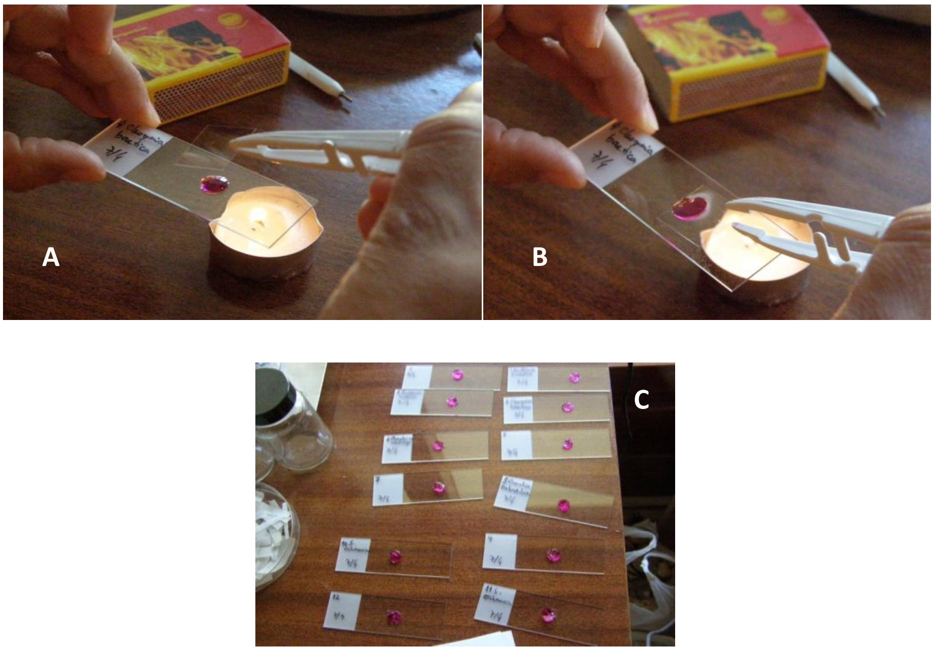

Figure 10 - Prepararation of the slides (A **and** B**). Some of the slides (**C**).**

2.3. Constructing the nocturnal plant-pollinator networks

An “interaction” between plant and moth was only included in the analysis when at least five pollen grains from the same plant species were counted in the pollen load of a single moth. This was taken as evidence that the moth really visited that plant species and reduced the potentially biasing effect of pollen contamination, which may have occurred due to heterospecific pollen transfer by visitors between co-flowering plant taxa or in the light trap or subsequent handling of the moths (Devoto *et al*., 2011). Pollen identification from wind-pollinated species (e.g. *Pinus spp*., *Olea europea*, *Ceratonia siliqua*, *Cupressus spp*., *Eucalyptus globulus*, *Acacia spp*., *Casuarina spp*.) was not considered for the network analysis because the pollen grains from those plants carried by the moths do not represent a pollen transfer - they are anemophilus and not entomophilus. The pollen grains from undetermined plant taxa were not considered for the network analysis.

Information on the quantity and identity of the pollen carried by each moth species from the study site was pooled to build a quantitative pollen-transport web representative of the habitat. When an individual slide contained more than 100 pollen grains the total number of grains was estimated.

For a better understanding of the global network it was decided to build a pollen transfer network and a visitation network. The first shows total pollen transfer but doesn't take into account pollinator abundance. The second ignores the amount of pollen but instead focuses on the number of interactions between plants and pollinators showing which plants are being visited most and by which moths. The interaction was determined by whether or not the moth was carrying pollen (presence/absence).

The field work and laboratory work provided data from which it was possible to examine patterns of moth biodiversity such as species richness, degree of specialization of the whole network (measured as H2´; Bluthgen *et al*., 2006), interaction evenness (based on Shannon diversity of interactions; Bersier *et al*., 2002), and weighted nestedness (Galeano *et al*., 2008; Devoto *et al*., 2011). All the network properties were calculated using the function "network level" from the R-package "bipartite", version 1.15 (Dormann *et al*., 2009; Dormann *et al*., 2011).

The microscope slides of identified plant pollen, prepared earlier in this study, were used to identify the pollen grains taken from the moths trapped at the study site. Taking this information together, it was possible to assess the importance of moth species, as pollinators, according to the methodology followed by Devoto (2011) by ranking the species by:

- the number of individuals of each of the moth species that carried pollen grains;
- the total number of plant taxa recorded in the pollen load of each moth species;
- the total number of pollen grains making up the pollen load of each moth species.

3. Results

3.1. Moths and Plants

During the sampling period, 50 plant taxon were identified. Around 13 plant taxon within a 500 meters circle from the field site were identified as well. The moths carried pollen from 36 plant taxon. Pollen grains of seven sampled plant taxon were never found on the captured moths within this study. The results are shown on Table 1. The bars represent the flowering period of each plant taxon during the sampling period. The thickness of the bars represents the relative abundance of the plant taxon.

Simpson's Diversity Index was used to analyse the vegetation results and averaged 0.94 ± 0.107 between sampled plots and 0.94 ± 0.091 between sampling dates.

The species more abundant were *Pallenis spinosa*, *Daucus carota*, *Scorpiurus muricatus*, *Euphorbia spp*. (mainly *E. exigua* and *E. helioscopia*) and *Sheradia arvensis*.

The plant species flowering during the entire period of field work were *Centaurea pullata*, *Stachys arvensis* and *Euphorbia exigua*. The species present for a short period of time were *Bellardia trixago*, *Melilotus indica, Leontodon taraxacoides*, *Ornithogalum narbonense*, *Trifolium spp*. and *Linum tenue*.

Table 1 - Distribution and abundance of plant taxa.

Dates Plant Species	19 Mar	24 Mar	6 Apr	21 Apr	5 May	18 May	3 Jun
Anagallis arvensis		—	—	—	—	—	
Anchusa italica			—		—	—	—
*Amni visnaga**				—			
Bellardia trixago				—	—	—	
Calendula arvensis	—			—			
Centaurea pullata	—	—	—	—	—	—	—
*Chicorium intybus**					—		
Convolvulus althaeoides spp. Althaeoides				—	—	—	
Cynoglossum clandestinum	—						
Cynoglossum creticum		—	—	—		—	—
Chrysanthemum coronarium		—	—	—	—		
Daucus carota						━	━
Erodium malacoides	—	—		—		—	
Euphorbia exigua	━	━	━	━	━	━	━
Euphorbia helioscopia	━	━	━	━			
Euphorbia peplus	—	—	—				
Fedia cornucopiae	—	—	—	—			
Galactites tomentosa				—			
Gallium verrucosum	—	—					
Geranium dissectum	—						
Geranium molle		—	—				
Gladiolus italicus		—	—	—	—		
Hedypnois cretica		—	—	—			
*Helychrysum stoechas**					—		
Lathyrus aphaca		—	—	—			
Leontodon taraxacoides						—	—
Linum tenue						—	—
Medicago polymorpha	—	—	—	—	—	—	
*Melilotus indica**				—			
Muscari comosum		—	—	—	—		
Nigella damascena			—	—	—	—	
Ophrys lutea		—	—				
*Ornithogalum narbonense**				—			
Orobanche sanguinea				—	—	—	
Oxalis pes-caprae	—	—	—				
Pallenis spinosa				━	━	━	━
Raphanus raphanistrum		—	—	—	—		
Rapistrum rugosum		—	—				
Scolymus hispanicus				—	—	—	—
Scorpiurus muricatus		━	━	━	━	━	━
Senecio vulgaris	—						
Sherardia arvensis	━	━	━	━		—	
Silene vulgaris				—		—	
Stachys arvensis	—	—	—	—	—	—	—
Trifolium campestre				—		—	
Trifolium stellatum				—			
Vicia sativa		—	—	—	—		
Valerianella discoidea		—	—				
*Verbascum sinuatum**					—		

3.2. The nocturnal plant-pollinator networks

Overall, 262 moths from 100 different species were captured in 10 trap-nights during the sampling period between 19th of March and 3rd of June. From those, 102 moths carried a significant amount of pollen (i.e. five or more pollen grains of a plant taxon) which represents around 39% of the total. The total number of pollen grains counted and identified was 9119 from 201 individual moths (total number of individuals carrying pollen). The average pollen load *per* individual carrying pollen was 45.4 grains. Of the 102 moths, 58 species carried five or more pollen grains from 27 plant taxa (not including wind-pollinated plant species). The most important moth species in terms of pollen transfer are shown in Table 2. (See Annex 2 for the complete moth dataset).

Table 2 - The main moth species recorded as pollen vectors.

Names	Family	Total	Nº ind w/pollen	Nº plant taxa	Nº interaction w/plants	Total Nº pollen grains
Aleucis distinctata	Geometridae	**1**	1	11	6	78
Aspitates ochrearia	Geometridae	**2**	2	10	7	154
Catharoe basochesiata	Geometridae	**2**	1	12	6	86
Scopula marginepunctata	Geometridae	**2**	2	13	8	80
Cleonymia baetica	Noctuidae	**1**	1	15	12	169
Cucullia calendulae	Noctuidae	**1**	1	12	6	69
Proxenus hospes	Noctuidae	**5**	4	14	10	196
Tyta luctuosa	Noctuidae	**9**	6	21	17	207
Ethmia bipunctella	Ethmiidae	**10**	6	17	5	96
Pterolonche traugottolseniella	Pterolonchidae	**8**	2	14	10	104
Cnephasia sp.	Tortricidae	**10**	8	21	5	109
Cnephasia longana	Tortricidae	**4**	4	18	5	53
Cochylimorpha decolorella	Tortricidae	**1**	1	8	6	52
Endothenia gentianaena	Tortricidae	**12**	12	17	5	108
Endothenia marginana	Tortricidae	**10**	9	25	5	136
Epinotia thapsiana	Tortricidae	**3**	3	17	5	63
Ephestia parasitella	Pyralidae	**1**	1	7	2	2648
Eudonia lineola	Pyralidae	**35**	30	36	19	2641
Phycitodes saxicola	Pyralidae	**1**	1	16	7	149
Mecyna asinalis	Pyralidae	**2**	2	16	2	47
Phycitodes saxicola	Pyralidae	**1**	1	16	6	149

It was not possible to identify three of the moths which were important for the network, and although they were positively identified as micro-moths, nothing could be said about the families. For some of the moths (i.e. *Cnephasia sp.*, *Agdistis sp.*) the identification was possible only at the genus level.

Table 3 and Figures 11 and 12 show the results of the main properties of the pollen transfer network and visitation network.

Table 3 - Main properties of nocturnal quantitative pollen transfer network and flower visitation network

	Pollen transfer	Flower visitation
Number of moth species	58	58
Number of plant taxa	27	27
Links per species	1,64	1,65
Interaction diversity	0,93	1,08
Interaction evenness	0,44	0,97
Weighted nestedness	0,44	0,47
Specialization (H_2')	0,78	0,13

The species composition, abundance and relative importance of plant taxa as pollen sources and vectors were reflected in the topology of the networks (table 3).

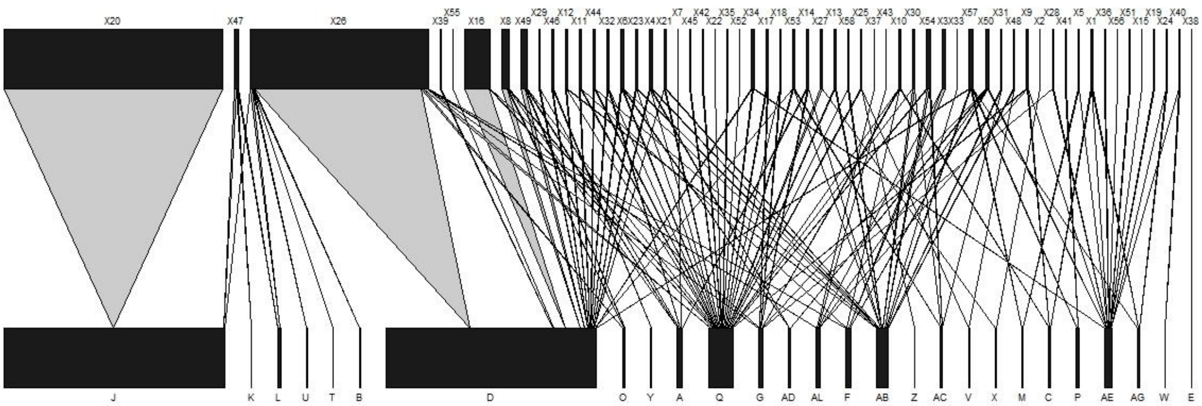

Figure 11 - Nocturnal plant-pollinator pollen transfer network.

The width of links between rectangles represents the number of individual moth of a given species that had a significant number of pollen grains of a given plant taxon on their bodies.

The codes for the plant species are as follow: *Urtica spp.* (**A**), *Lathyrus aphaca* (**B**), *Erodium malacoides* (**C**), *Anagallis arvensis* (**D**), *Allium cepa* (**E**), *Plantago spp.* (**F**), *Ornitogalum narbonense* (**G**), *Muscari comosum* (**H**), *Vicia sativa* (**I**), *Cynoglossum creticum* (**J**), *Valerianella discoidea* (**K**), *Nigella damascena* (**L**), *Leontodon taraxacoides* (**M**), *Ophrys spp.* (**N**), *Rapistrum rugosum* (**O**), *Crysanthemum coronarium* (**P**), *Melilotus indica* (**Q**), *Anchusa italica* (**R**), *Silene vulgaris* (**S**), *Convolvolus althaeoides* (**T**), *Scorpiurus muricatus* (**U**), *Senecio vulgaris* (**V**), *Galactites tomentosa* (**W**), *Trifolium sp.* (**X**), *Beta maritima* (**Y**), *Fedia cornucopiae* (**Z**), *Euphorbia spp.* (**AA**), *Bellardia trixago* (**AB**), *Sherardia arvensis* (**AC**), *Linum tenue* (**AD**), *Daucus carota* (**AE**), *Medicago polymorpha* (**AF**), *Prunus spp.* (**AG**), *Stachys arvensis* (**AH**), *Amni visnaga* (**AI**), *Chicorium intybus* (**AJ**), *Scolymus hispanicus* (**AK**), *Raphanus raphanistrum* (**AL**).

The codes for the moths species are as follow: *Acontia lucida* (**X1**), *Agdistis sp.* (**X2**), *Agonopteryx rutana* (**X3**), *Aleucis distinctata* (**X4**), *Aplasta ononaria* (**X5**), *Catharoe basochesiata* (**X6**), *Chloroclysta siterata* (**X7**), *Cleonymia baetica* (**X8**), *Cnephasia longana* (**X9**), *Cnephasia sp.* (**X10**), *Cnephasia stephensiana* (**X11**), *Cochylimorpha decolorella* (**X12**), *Coscinia cribaria* (**X13**), *Cuculia calendulae* (**X14**), *Unknown* (**X15**), *Eilema caniola* (**X16**), *Endothenia gentianaena* (**X17**), *Endothenia marginana* (**X18**), *Endotrichia flammealis* (**X19**), *Ephestia parasitella* (**X20**), *Epinotia thapsiana* (**X21**), *Eteobalea intermediella* (**X22**), *Ethmia bipunctella* (**X23**), *Euchromius gozmanyi* (**X24**), *Eudonia angustea* (**X25**), *Eudonia lineola* (**X26**), *Eupithecia centaureata* (**X27**), *Exaeretia lutosella* (**X28**), *Gymnoscelis rufifasciata* (**X29**), *Hecatera corsica* (**X30**), *Homaloxestis briantella* (**X31**), *Hypena obsitalis* (**X32**), *Idaea dimidiata* (**X33**), *Idaea lutulentaria* (**X34**), *Isophrictis kefetrsteiniella* (**X35**), *Mecyna asinalis* (**X36**), *Mendesia echiella* (**X37**), *Metzeneria torosulella* (**X38**), *Mnyotipe spinosa* (**X39**), *Mythimna vitellina* (**X40**), *Unknown 1* (**X41**), *Unknown 2* (**X42**), *Ocneria rubea* (**X43**), *Paradrina noctivaga* (**X44**), *Peribatodes ilicaria* (**X45**), *Phragmatobia fuliginosa* (**X46**), *Phycitodes saxicola* (**X47**), *Platyedra subcinerea* (**X48**), *Proxenus hospes* (**X49**), *Pterolonche traugottolseniella* (**X50**), *Pyralis obsoletalis* (**X51**), *Pyroderces argyrogrammes* (**X52**), *Scopula marginepunctata* (**X53**), *Aspitates ochrearia* (**X54**), *Symmoca signatella* (**X55**), *Tephronia codetaria* (**X56**), *Tyta luctuosa* (**X57**), *Udea ferrugalis* (**X58**).

In this network, most pollen from *Annagalis arvensis* (**D**) and *Cynoglossum creticum* (**J**) was transported by *Ephestia parasitella* (**X20**) and *Eudonia lineola* (**X26**) (See photograph below, Figure 13). Six moth species, *Eudonia lineola* (**X26**), *Tyta luctuosa* (**X57**) *Cleonymia baetica* (**X8**), *Proxenus hospes (***X49**), *Pterolonche traugottolseniella* (**X50**), *Scopula marginepunctata* (**X53**) and carried pollen from above eight different plant species.

In a flower visitation network we can find which species were key pollinators. In this case it showed that four plant species – *Anagallis arvensis* (**D**), *Bellardia trixago* (**AB**), *Melilotus indica* (**Q**)*, Daucus carota* (**AE**) – were frequently visited by four moth species – *Eudonia lineola* (**X26**), *Proxenus hospes (***X49**), *Tyta luctuosa* (**X57**) and *Aspitates ochrearia* (**X53**). See also table 4 which shows some of the moths which were important as pollen vectors for this community.

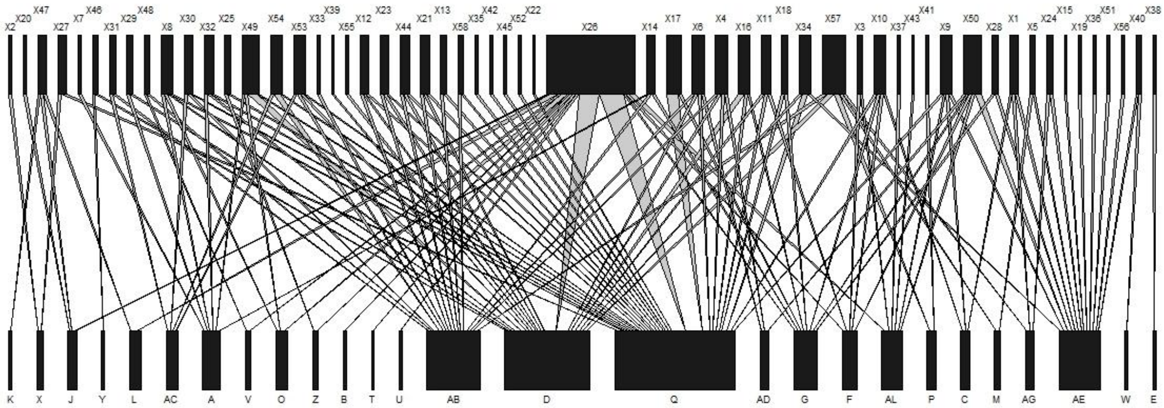

Figure 12 - Nocturnal flower visitation network.

The width of links between rectangles represents the number of a given plant taxon that are being visited by a number of individual moths of a given species.

The codes in this Figure are the same as the ones on Figure 11.

Table 4 - Examples of important moths that carried pollen grains.

Moths carrying the highest number of pollen grains	Moths carrying the highest number of pollen grains from different plant taxa	Moths with the highest number of plant interactions (5 or more pollen grains of the same taxon)
Aspitates ochrearia	*Cnephasia longana*	*Aleucis distinctata*
Cleonymia baetica	*Cnephasia sp.*	*Aspitates ochrearia*
Cnephasia sp.	*Endothenia gentianaena*	*Catharoe basochesiata*
Eilema caniola	*Endothenia marginana*	*Cochylimorpha decolorella*
Endothenia marginana	***Eudonia lineola***	*Cleonymia baetica*
Ephestia parasitella	*Ethmia bipunctella*	*Cucullia calendulae*
Eudonia lineola	*Epinotia thapsiana*	***Eudonia lineola***
Phycitodes saxicola	*Mecyna asinalis*	*Proxenus hospes*
Proxenus hospes	*Phycitodes saxicola*	*Pterelonche traugottolseniella*
Tyta luctuosa	*Platyedra subcinerea*	*Scopula marginepunctata*
	Tyta luctuosa	***Tyta luctuosa***

Note: Species in **bold** are represented in all columns.

Figure 13 - Photograph of *Eudonia lineola*.

This is a very common moth species for the area, and can usually be found flying in the spring (March – June).

4. Discussion

Pollination is amongst the more important ecosystem services for humankind. The ecosystem services are critical to the functioning of the Earth´s life-support system and the fact that they are often neglected in policy decisions may compromise the sustainability of humans in biosphere (Constanza *et al*., 1997).

Ecosystem changes affect the distribution, abundance, and effectiveness of pollinators According to the Millennium Ecosystem Assessment, there is "*established but incomplete* evidence of a global decline in the abundance of pollinators and pollinator declines have been reported in at least one region or country on every continent except Antarctica, which has no pollinators". In a study comparing the declines of pollinators in Britain and The Netherlands there are evidences showing that "pollinator declines were most frequent in habitat and flower specialists, in univoltine species, and/or in nonmigrants species. In conjunction with this evidence, outcrossing plant species that are reliant on the declining pollinators have themselves declined, relatively to other plant species. Taken together, these findings strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species" (Biesmeijer *et al*., 2006).

In the last decade there´s been an increasing amount of research on diurnal pollinators (Memmott, 1999; Dicks *et al*., 2002; Bascompte *et al*., 2003; 2007; Memmott *et al*., 2004; Olesen *et al*., 2007; 2008; Ings *et al*., 2008; Bosch *et al*., 2009) but recent works (Devoto *et al*., 2011) suggest that nocturnal pollinators are also very important for the functioning of a community. Having this in mind, the present study assumes a particular meaning because it was conducted in one of the "Hot Spots" for Biodiversity in the world and the only one in Europe, the Mediterranean Region, with huge importance for conservation (Médail and Quézel, 1999; Blondel and Aronson, 1999).

Out of the 262 moths caught over 10 sampling sessions, between March and June 2010, 39% carried a significant amount of pollen. The pollen transfer network was dominated by two moth species, *Eudonia lineola* and *Ephestia parasitella*, which carried most of the pollen load from the network. Around 31% of the moths interacted with five or more different plant species (i.e. carried pollen from five or more plant species). Out of those six species carried pollen from a wide number of plant species: *Eudonia lineola, Tyta luctuosa, Cleonymia baetica, Aspitates ochrearia, Proxenus hospes* and *Scopula marginepunctata*.

In the majority of cases, however, only one individual of each moth species was caught. It is therefore difficult to draw any conclusions about their relative importance as pollen vectors. With the exception of *Eudonia lineola,* which was the most common species (35 individuals were caught in total), only 5 other species (*Endothenia gentianaena*, *E. marginana*, *Cnephasia spp*., *Ethmia bipunctella*, and *Aplasta ononaria*) were caught in significant numbers (12, 10, 10, 10 and 9, respectively). The low numbers of individuals caught of each moth species could perhaps be attributed to the unfavourable weather conditions (see the Material and Methods section). It is not possible to conclude whether network structures would be different if more individuals from each species were caught. More research and increased sampling effort is needed.

Vegetation species richness of the study site was high and species abundance distribution was fairly homogeneous, both across space and time, as can be inferred by Simpson's Diversity Index averaged values close to 1 and small standard deviations, 0.94 ± 0.107 and 0.94 ± 0.091, respectively.

The abundance of a particular plant species is not necessarily a good predictor of the level of pollen transfer from that species within the community. One plant species, for example, with a highest abundance was *Pallenis spinosa* yet no moths were discovered carrying pollen grains from this plant.

Similarly, the abundance of a moth species was not a good predictor of its importance as a pollen vector in the community, e.g. the only individual from the species *Ephestia parasitella* caught carried the biggest pollen grains load, but only from one plant (*Cynoglossum creticum*).

4.1. Properties of the nocturnal pollen-transport networks

Mutualistic networks such as plant-pollinator networks share some common properties such as the presence of many specialist but few generalists (Waser *et al*., 1996, Jordano *et al*., 2003, Devoto *et al*, 2011) and a nested pattern of interactions (Memmott, 1999, Bascompte *et al*., 2003). See Annex 5 for more information on results of these works.

When we analyse the pollen transfer network from this study we can see that it appears to be highly specialised (Specialisation H2´ was 0.78) because only a few individual moths (*Eudonia lineola* and *Ephestia parasitella*) carried most of the pollen. However, when we compare these results with the results from the visitation network, it appears that the visitation network is not highly specialised (Specialisation H2´ was 0.13). The visitation network was constructed with the same data used for the pollen transfer network, only this time using the presence/absence of pollen to create interactions. This is something new in network ecology: a visitation network based on the presence/absence of pollen. Most diurnal pollination networks are, in fact, flower visitor networks but tell us nothing about whether the insect is carrying pollen or is indeed a true pollinator. Creating a visitation network using pollen data is arguably a better and more informative method in pollination ecology (even if it doesn't tell us whether or not an insect is actually pollinating a plant).

The work of Bascompte (2003) showed that mutualistic networks are generally highly nested, that is, the more specialist species interact only with proper subsets of those species interacting with the more generalists. Also, nestedness increases with the complexity (number of interactions) of the network: for a given number of species, communities with more interactions are significantly more nested. Interestingly, this study differs from the previously shown patterns of nestedness in diurnal networks, as both moth networks, pollen transfer (weighted nestedness: 0.44) and flower visitation (weighted nestedness: 0.47), do not appear to be nested.

The higher values for Interaction diversity and Interaction evenness for flower visitation (0.93 and 1.08, respectively) reflects the number of moth-plant interactions in this network. For the pollen transfer the lower Interaction evenness (0.44) may be explained by the fact that most pollen was carried by only two species of moth.

There are two compartments for both pollen transfer and flower visitation networks. One is the interaction of *Ephestia parasitella* with *Cynoglossum creticum* and the other compartment represents the rest of the moth and plant species in the network.

In general, the sample size of moths, when grouped by species, was too small to reach statistically sound conclusions, i.e., in most cases only one individual of each moth species was caught and therefore analysed for pollen. The experimental analysis was found to be very time consuming and therefore it was not possible to undertake a more intensive trapping regime in this study. Furthermore moth trapping was accomplished under unusual unfavourable weather conditions.

Despite the fact that the sampling effort can affect network structures, the results of this study are unique and highlight the fact that moths may be providing an important and overlooked ecological function. Given these results and the current concerns regarding the decline of Europe´s bees, can we hope pollination processes to be more robust to bee decline than previously thought?

4.2. Implications for the conservation of moths and for the ecosystem service of pollination

The study of the properties of the network is very relevant to understand better the organization of plant-animal mutualisms and their interactions in the community. This information can be used to understand more about moths and their importance as pollinators in the community. To date, there are very few studies in Portugal regarding moths and the available information is mainly at the species level.

There is very little information regarding abundance and population trends of moths in Portugal because, as a whole, this group of insects has not been investigated enough to even provide basic presence/abundance and distribution data. The community level approach can provide useful information about the presence and abundance of moth species and can also be used to help understand potential causes of decline and design scientifically sound restoration conservation.

As no plants recorded at the study site were moth pollination specialists and all plant taxa present were also likely to have been visited by diurnal pollinators, we do not know how important moths are as pollen vectors in this particular community.
Regarding further work, it would be very interesting to study the same community but with diurnal pollinators in order to build diurnal pollination networks. Simultaneously, it would also be very interesting to repeat this same work with moths, what would allow comparing the results from those networks and their relative importance in pollination process and, by enlarging the sample, to clarify how important the different moth species are regarding pollen transfer and flower visitation.

5. Conclusion

The main goal of this study was to characterise pollination by moths as a community-level phenomenon The study achieved its main objectives of the construction of Portugal´s first nocturnal plant-pollinator network, identification of nocturnal Lepidoptera pollinators and of the pollination networks established at the study site.

It is clearly demonstrated the importance of moths as vectors for pollen and the importance of adding the nocturnal information for understanding the “whole picture” of what´s happening in a community. It opens a unique area of research in Mediterranean pollination ecology by suggesting further research on the role moths may be playing as pollinators for this particular setting.

Another positive implication of this study was the gathering of more information on Portuguese moths. This should contribute to raise awareness for the importance of conservation measures regarding the moths and their importance for pollination as one of the most important ecosystem services.

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ANNEXES

ANNEX 1 – Plant Species Complete List

Botanical Family	Plant Species	Pollen found in the moths
Apiaceae	***Ammi visnaga****	
	Daucus carota	***X***
Asteraceae	***Calendula arvensis***	
	Centaurea pullata	
	Chicorium intybus*	***X***
	Chrysanthemum coronarium	***X***
	Cynara humilis	***Cynara spp.*****
	Cynara cardunculus	***Cynara spp.*****
	Galactites tomentosa	***X***
	Leontodon taraxacoides	***X***
	Senecio vulgaris	***X***
	Hedypnois cretica	
	Helicrysum stoechas*	
	Scolymus hispanicus	
Boraginaceae	***Anchusa italica***	
	Cynoglossum clandestinum	
	Cynoglossum creticum	***X***
Brassicaceae	***Raphanus raphanistrum***	***X***
	Rapistrum rugosum	***X***
Caryophyllaceae	***Silene vulgaris***	
Casuarinaceae	***Casuarina spp.+***	***X***
Chenopodiaceae	***Beta maritima+***	***X***
Convolvulaceae	***Convolvulus althaeoides spp. althaeoides***	***X***
Cupressaceae	***Cupressus spp.+***	***X***
Euphorbiaceae	***Euphorbia exigua***	
	Euphorbia helioscopia	
	Euphorbia peplus	
Fabaceae	***Acacia spp.+***	***X***
	Ceratonia siliqua+	***X***
	Lathyrus aphaca	***X***
	Medicago polymorpha	
	Melilotus indica*	***X***
	Scorpiurus muricatus	***X***
	Trifolium campestre**	***Trifolium spp.*****
	Trifolium stellatum**	***Trifolium spp.*****
	Vicia sativa	
Geraniaceae	***Erodium malacoides***	***X***
	Geranium dissectum	
	Geranium molle	
Iridaceae	***Gladiolus italicus***	
Lamiaceae	***Stachys arvensis***	
Liliaceae	***Muscari comosum***	***X***
	Ornithogalum narbonense*	***X***
	Allium cepa+	***X***
Linaceae	***Linum tenue***	***X***
Myrtaceae	***Eucalyptus globulus+***	***X***
Oleaceae	***Olea europea+***	***X***
Orchidaceae	***Ophrys lutea***	
Orobanchaceae	***Orobanche sanguinea***	
Oxalidaceae	***Oxalis pes-caprae***	
Pinaceae	***Pinus spp.+***	***X***
Plantaginaceae	***Plantago spp.+***	***X***
Poaceae	***Undetermined species+***	***X***
Primulaceae	***Anagallis arvensis***	***X***
Ranunculaceae	***Nigella damascena***	***X***
Rosaceae	***Prunus spp.+***	***X***
Rubiaceae	***Galium verrucosum***	
	Sherardia arvensis	***X***
Valerianaceae	***Fedia cornucopiae***	***X***
	Valerianella discoidea	
Scrophulariaceae	***Bellardia trixago***	***X***
	Verbascum sinuatum*	
Urticaceae	***Urtica spp.+***	***X***

The plant species with * were found in the study site but not in the quadrats.

Trifolium spp.** **represents a pool of species of** ***Trifolium stelattum*** **and** ***Trifolium campestre*** **because it was not possible to distinguished among their pollen grains.**

Cynara **spp.*** represents a pool of of** ***Cynara humilis*** **and** ***Cynara cardunculus*** **because it was not possible to distinguished among their pollen grains.**

The plant species with + represent plant taxon not present at the field site but in a 500 metres circle from it. For most of them it was only possible to identify the genus (*Casuarina spp., Cupressus spp., Acacia spp., Pinus spp., Plantago spp., Prunus spp., Urtica spp.)* or the family *(*Poaceae).

ANNEX 2 – The Moth Species Recorded as Pollen Vectors

Names	Family	Total	Nº ind w/pollen	Nº plant taxa	Nº interaction w/plants	Total Nº pollen grains
Aleucis distinctata		**1**	1	11	6	78
Aplasta ononaria	Geometridae	**9**	4	12	2	21
Aspilates ochrearia		**2**	2	10	7	154
Catharoe basochesiata		**2**	1	12	6	86
Chloroclysta siterata		**1**	1	5	1	12
Eupithecia centaureata		**3**	3	11	2	46
Gymnoscelis rufifasciata		**5**	2	10	3	32
Idaea dimidiata		**1**	1	5	2	23
Idaea lutulentaria		**4**	4	13	4	66
Idaea subsericeata		**1**	1	2	2	4
Idaea degeneraria		**1**	0	0	0	0
Menophra abruptaria		**1**	0	0	0	0
Menophra japygiaria		**1**	1	4	0	7
Orthonama obstipata		**1**	0	0	0	0
Peribatodes ilicaria		**1**	1	2	1	11
Rhodometra sacraria		**1**	0	0	0	0
Scopula marginepunctata		**2**	2	13	8	80
Tephronia sepiaria		**1**	0	0	0	0
Tephronia codetaria		**2**	2	11	1	25
Ocneria rubea	Lymantridae	**1**	1	6	1	16
Apaidia mesogona	Arctiidae	**1**	1	6	0	11
Coscinia cribaria		**3**	2	12	5	84
Eilema caniola		**4**	4	13	4	334
Eilema pygmaeola		**4**	2	9	0	18
Phragmatobia fuliginosa		**5**	2	12	3	36
Acontia lucida	Noctuidae	**1**	1	7	3	31
Cleonymia baetica		**1**	1	15	12	169
Coccidiphaga scitula		**1**	1	4	0	5
Conisania andalusica		**1**	1	5	0	8
Cucullia calendulae		**1**	1	12	6	69
Dicestra sodae		**1**	0	0	0	0
Eublemma ostrina		**1**	1	5	0	11
Hecatera corsica		**1**	1	8	4	49
Hecatera weissi		**1**	1	5	0	11
Hoplodrina ambigua		**1**	1	7	0	11
Hypena obsitalis		**3**	3	13	3	70
Mniotype spinosa		**2**	2	6	1	33
Mythimna vitellina		**1**	1	3	2	15
Mythimna scirpi		**1**	1	5	0	9
Mythimna unipuncta		**2**	2	11	0	22

Names	Family	Total	Nº ind w/pollen	Nº plant taxa	Nº interaction w/plants	Total Nº pollen grains
Paradrina noctivaga		**1**	1	12	4	52
Platiperygea proxima		**1**	0	0	0	0
Proxenus hospes		**5**	4	14	10	196
Tyta luctuosa		**9**	6	21	17	207
Aglaope infausta	Zygaenidae	**1**	1	8	0	17
Crassicornella agenjoi	Tineidae	**2**	0	0	0	0
Reisserita chrysopterella		**1**	0	0	0	0
Plutella xylostella	Plutellidae	**1**	1	2	0	2
Agonopteryx rutana	Depressariidae	**1**	1	10	2	52
Exaeretia lutosella		**1**	1	3	2	17
Coleophora solidaginella	Coleophoridae	**1**	1	4	0	8
Elachista nuraghella	Elachistidae	**1**	1	2	0	2
Mendesia echiella		**2**	1	9	1	18
Ethmia terminella	Ethmiidae	**1**	1	3	0	4
Ethmia bipunctella		**10**	6	17	5	96
Eteobalea intermediella	Cosmopterigidae	**1**	1	5	1	13
Pyroderces argyrogrammos		**1**	1	6	1	11
Anarsia lineatella	Gelechidae	**1**	0	0	0	0
Isophrictis kefersteiniella		**1**	1	6	1	16
Mesophleps corsicellus		**1**	0	0	0	0
Metzneria torosulella		**8**	6	13	1	49
Platyedra subcinerea		**3**	3	15	3	58
Stibaromacha ratella	Symmocidae	**1**	1	5	0	11
Symmoca signatella		**1**	1	11	2	28
Symmocoides oxybiellus		**2**	1	3	0	5
Homaloxestis briantiella	Lecithoceridae	**1**	1	14	4	55
Pterolonche traugottolseniella	Pterolonchidae	**8**	2	14	10	104
Enolmis acanthella	Scythrididae	**2**	2	8	1	19
Episcythris triangulella		**2**	1	5	0	11
Bactra lancealana	Tortricidae	**1**	1	2	0	2
Cnephasia conspersana		**1**	1	0	0	0
Cnephasia longana		**4**	4	18	5	53
Cnephasia stephensiana		**1**	1	15	3	77
Cnephasia sp.		**10**	8	21	5	109
Cochylimorpha decolorella		**1**	1	8	6	52
Crocidosema plebejana		**1**	0	0	0	0
Endothenia gentianaena		**12**	12	17	5	108
Endothenia marginana		**10**	9	25	5	136
Endothenia sp.		**1**	0	6	0	12
Epinotia thapsiana		**3**	3	17	5	63
Agdistis sp.	Pterophoridae	**1**	1	5	2	20
Apomyelois ceratoniae	Pyralidae	**1**	0	0	0	0

Names	**Family**	**Total**	**Nº ind w/pollen**	**Nº plant taxa**	**Nº interaction w/plants**	**Total Nº pollen grains**
Dolicharthria punctalis		**2**	2	7	0	10
Endotricha flammealis		**1**	1	5	1	16
Ephestia parasitella		**1**	1	7	2	2648
Epischnia bankesiella peroni		**1**	0	0	0	0
Epischnia illotella		**1**	1	6	0	7
Euchromius gozmanyi		**2**	2	5	2	21
Eudonia angustea		**3**	3	11	6	90
Eudonia lineola		**35**	30	36	19	2641
Homoeosoma sinuellum		**1**	1	2	0	2
Lamoria anella		**1**	1	7	0	15
Mecyna asinalis		**2**	2	16	2	47
Phycitodes saxicola		**1**	1	16	6	149
Pyralis obsoletalis		**6**	4	10	1	28
Udea ferrugalis		**1**	1	7	5	31
Udea numeralis		**6**	5	13	0	22
Unknown		**1**	1	3	1	**9**
Unknown 1		**1**	1	6	1	**25**
Unknown 2		**1**	1	7	1	18
Total		**262**	**201**		**262**	**9119**

ANNEX 3 - List of Fungi spores carried by the moths

Fungi Spores carried by the moths
Stemphylium sp.
Tetraploa
Venturia
Alternaria
Curvularia
Drachsiera/Helminthosporium
Nigrospora
Dictyosporium
Asperisporium
Pithomyces
Alatospora
"Corneta"
Coprinus

Identification based on SMITH, 1984 .

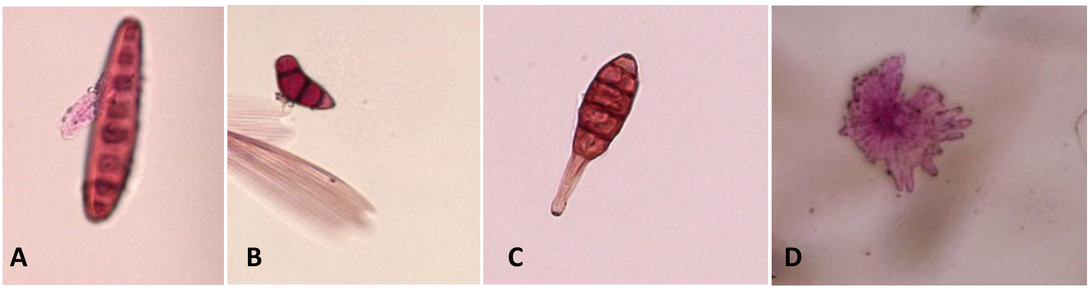
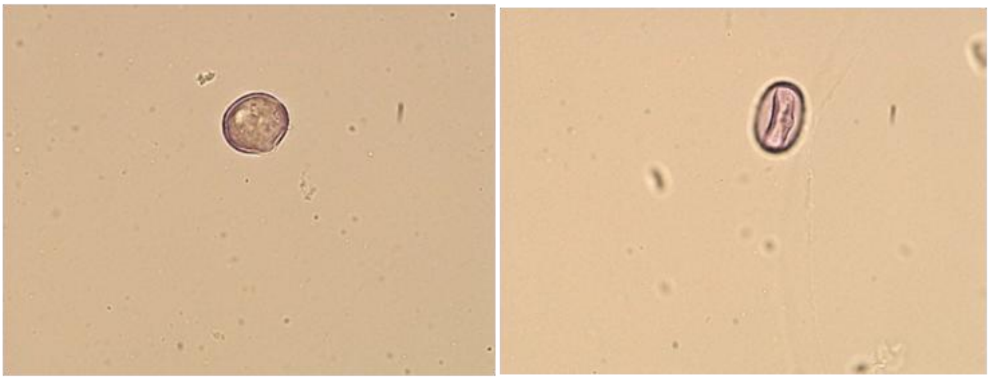


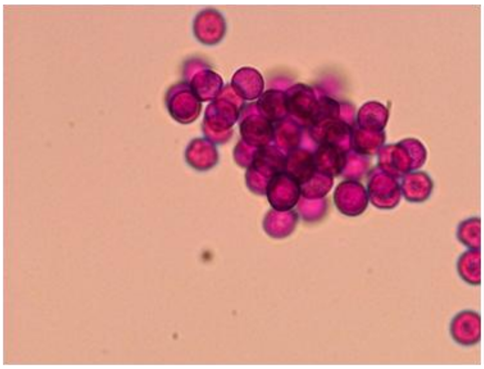
Figure 1 – Photos of some fungi spores carried by the moths.

A - *Drachsiera/Helminthosporium*; B – *Curvularia*; C – *Alternaria*; D – Unknown.

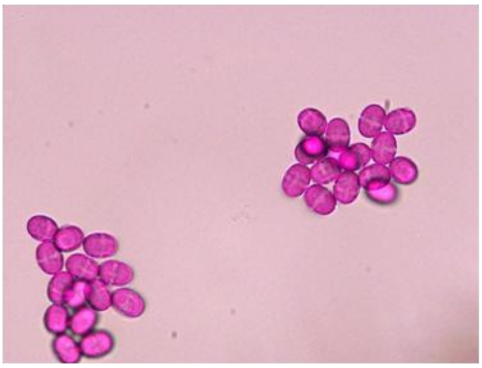
ANNEX 4 – Photos of some pollen grains



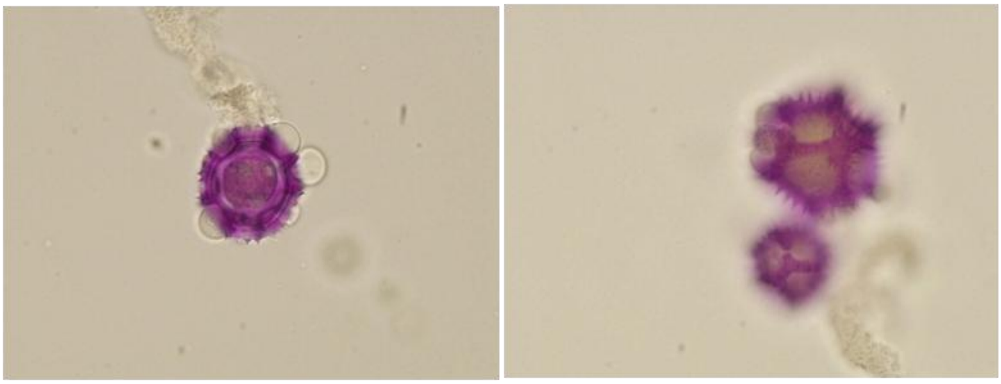
Melilotus indica



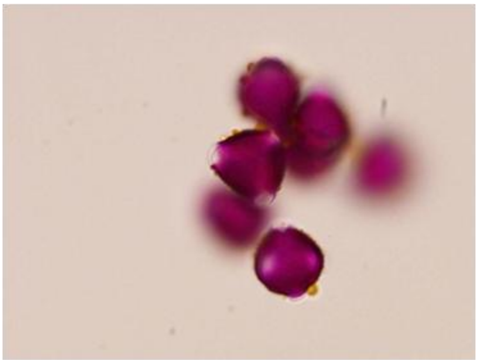
Urtica spp.



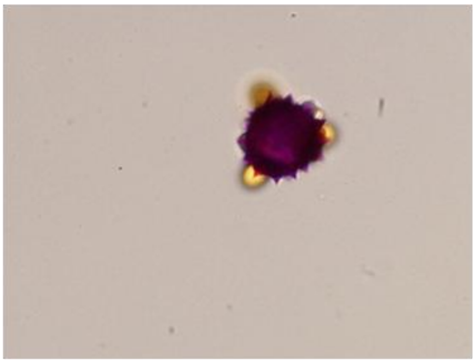
Cynoglossum creticum



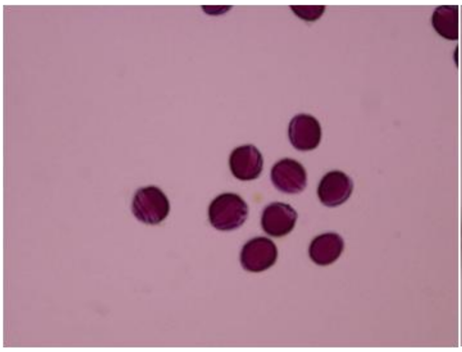
Chicorium intybus



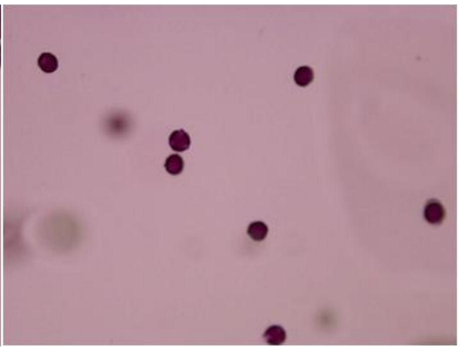
Anagallis arvensis



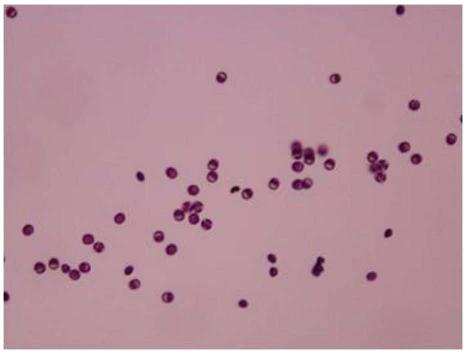
Crysanthemum coronarium



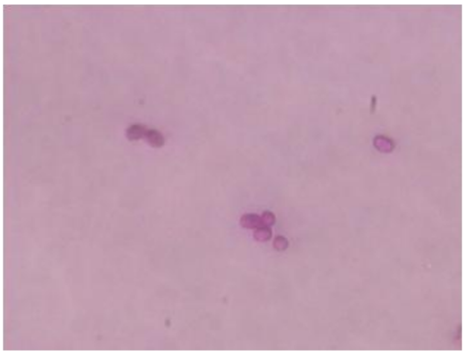
Gladiolus italicus



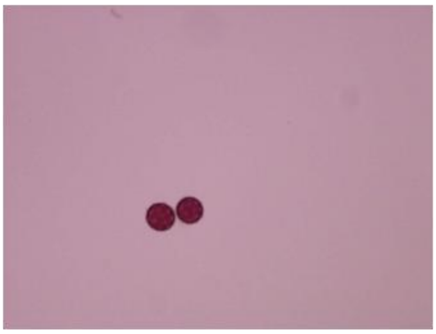
Nigella damascena



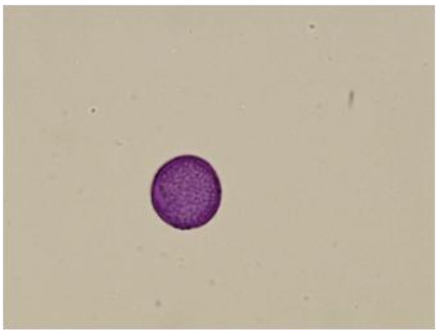
Raphanus raphanistrum



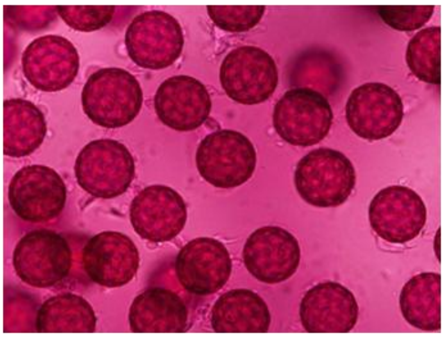
Scorpiurus muricatus



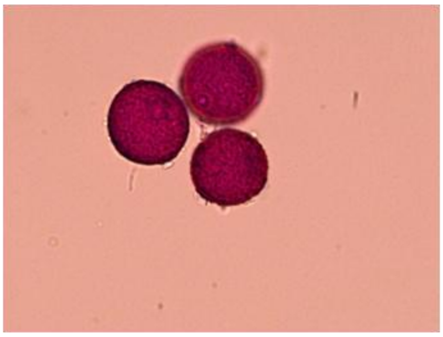
Silene vulgaris



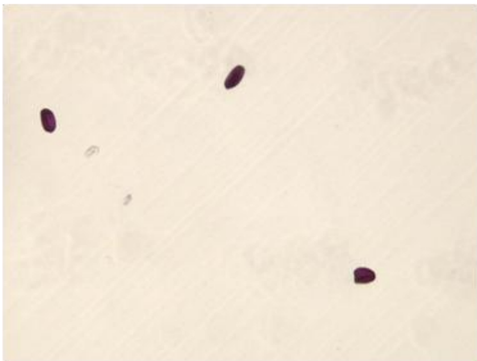
Trifolium spp.



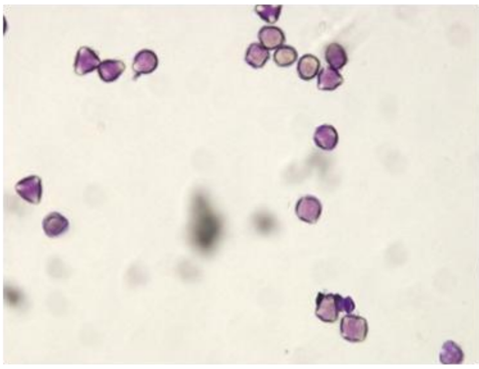
Beta maritima



Plantago spp.

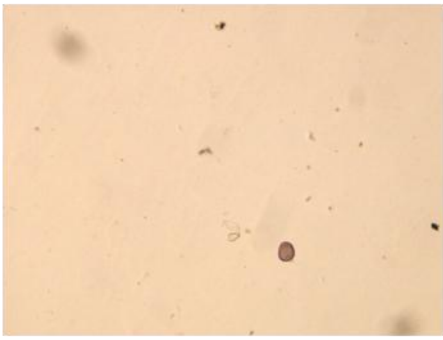


Ornitogalum narbonense



Prunus spp.

Bellardia trixago



Daucus carota

ANNEX 5 – Other examples of pollination networks

Example 1 – Taken from: " The 'night shift': nocturnal pollen-transport networks in a boreal pine forest" (Devoto *et al*., 2011).

Table 1 and Figure 1 show the main results of the study conducted in a boreal pine forest and the construction of the first nocturnal plant-pollinator network. The study was conducted in two consecutive years (2007 and 2008) and the results show that "the nocturnal network exhibited the same properties as diurnal networks: presence of many generalists but few extreme generalists, a nested pattern of interactions and the prevalence of asymmetric interactions" (Devoto *et al*., 2011)

Table 1 - Main properties of nocturnal quantitative pollen-transport networks in Scottish pine woods in 2007 and 2008.

	2007	**2008**
Number of moth species	17	15
Number of plant taxa	5	9
Links per species	1	1.16
Interaction diversity	2.91	1.66
Interaction evenness	0.80	0.42
Weighted nestedness	0.45	0.71
Specialization (H_2')	0.55	0.23

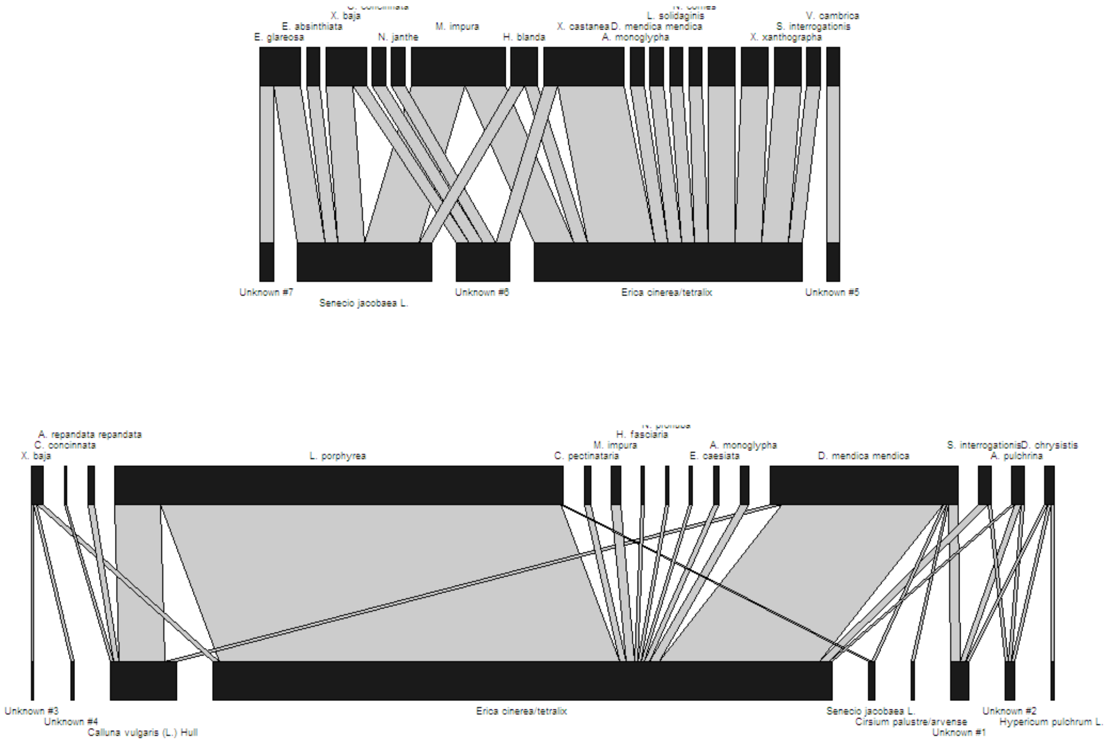


Figure 1 - Nocturnal plant-pollinator webs in Caledonian pine forest in 2007 (top) and 2008 (bottom). The width of links between rectangles represents number of individual moths of a given species that had a significant number of pollen grains of a given plant taxon on their bodies. The web from 2007 is represented four times larger than it would be if both webs were drawn to the same scale.

Example 2 – Taken from. "The structure of a plant-pollinator food web" (Memmot, 1999).

Table 2 and Figure 2 show the main results of the study conducted in a meadow plot in England during July 1997. The study focused on diurnal flower visiting insects from four Insect Orders: Diptera, Hymenoptera, Lepidoptera and Coleoptera. "Generalization appears to be the norm for both plants and insects in this community" (Memmott, 1999). The results were used to construct a plant visitation web (Figure 2).

Table 2 - Main properties of nocturnal quantitative pollen-transport networks in July 2007.

Number of higher trophic species	79
Number of lower trophic species	25
Links per species	2.88
Interaction diversity	1.70
Interaction evenness	0.79
Weighted nestedness	0.73
Specialization (H_2')	0.27

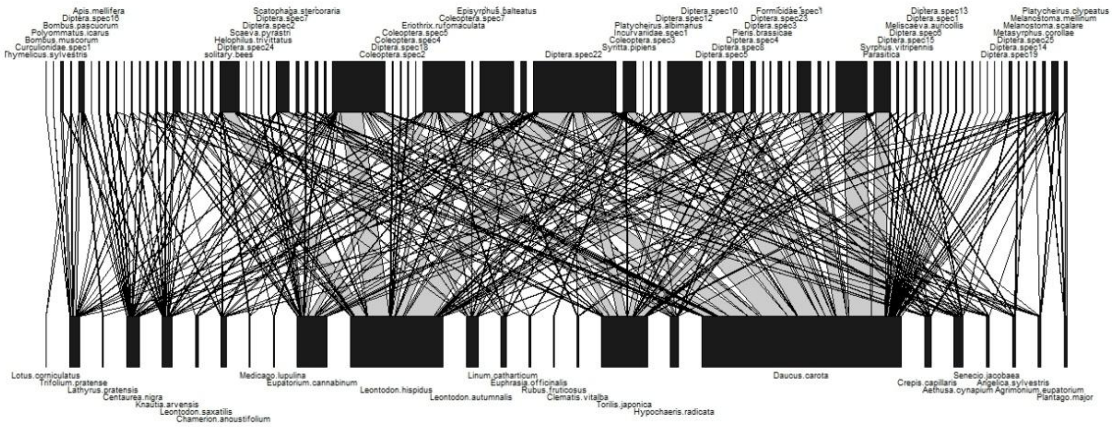


Figure 2 – The results of the quantitative sampling for a plant-pollinator community showing the trophic links (pollen and/or nectar feeding) during July 1997. Each species of plant and insect represented by a rectangle: the lower lines represents flower abundance, the upper lines represents insect abundance (Col, Coleoptera; Dipt, Diptera; Hym, Hymenoptera; Lep, Lepidoptera). The width of the rectangle and the size of the interaction between them is proportional to their abundance at the field site. Plants shown as a dotted line were present at the field site, but not recorded by the sampling. Interactions shown as a dotted line were observed less than 10 times during the sampling period.