Title: Nocturnal pollinators strongly contribute to pollen transport of wild flowers in an agricultural landscape

Running Head: Moths as important pollen transporters

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Abstract

Dramatic declines in diurnal pollinators have created great scientific interest in plant-pollinator relationships and associated pollination services. However, existing literature is generally focused on diurnal pollinating insect taxa, especially on Apidae (Hymenoptera) and Syrphidae (Diptera) pollinators, while nocturnal macro-moths that comprise extremely species-rich flower-visiting families have been largely neglected. Here, we report that in agricultural landscapes macro-moths can provide unique, highly complex pollen transport links, making them vital components of overall wild plant-pollinator networks in agro-ecosystems. Pollen transport occurred more frequently on the moths’ ventral thorax rather than on their mouthparts that have been traditionally targeted for pollen swabbing. Pollen transport loads suggest that nocturnal moths contribute key pollination services for several wild plant families in agricultural landscapes, in addition to providing functional resilience to diurnal networks. Severe declines in richness and abundance of settling moth populations highlight the urgent need to include them in future management and conservation strategies within agricultural landscapes.
Introduction

In recent decades, bee and other insect pollinator populations have undergone dramatic global declines, with strong negative implications for insect-pollinated crop yields (1,2). These declines are increasingly entering political debates (3) and the wider public consciousness. Nevertheless, the focus of scientific and public attention remains largely limited to a few pollinator groups, mainly diurnal solitary and social bees representing approximately 20,000 species (4). Hoverflies (Diptera: Syrphidae) (5), butterflies (Lepidoptera: Rhopalocera) (6), hawkmoths (Lepidoptera: Sphingidae) (6) and nocturnal tropical bees (7,8) have also received some attention.

In contrast, most nocturnal and crepuscular insects representing much more species-rich taxa have been strongly neglected by pollination research (Table S1). They include mega-diverse settling macro-moth families like geometrid and noctuid moths, that alone comprise a quarter of the 160,000 known moth species (6). Limited existing studies into pollen-transport capabilities of settling macro-moth species have provided some indications of their flower visitations (9–14). Nonetheless, detailed plant-pollinator network studies revealing the extent of nocturnal moth pollen transport in comparison to diurnal pollinators are currently lacking. This partly relates to *Apis* spp. (honey bees) and *Bombus* spp. (bumblebees) being regarded as superior pollinators (15,16) by routinely harvesting and transporting large amounts of pollen. They are, however, also known to preferentially target the most prolific nectar and pollen sources (17–19). Settling macro-moths could be less-effective pollinators by comparison, but their high diversity and abundance may render them important and complementary components of diurnal pollination networks.
Here, we present novel insights into the complexity of pollen transport networks linked to nocturnal settling moths in intensively cultivated agricultural landscapes, while also providing comparisons with diurnal flower-visititation networks in the same landscape. We test the hypothesis that nocturnal macro-moths, due to their great abundance, are linked to plant-pollinator networks that have a similar or even greater complexity to networks linked to diurnal pollinators. We furthermore hypothesise that some plant species in the investigated landscape will chiefly depend on moths as pollen transporters.

Methods
This study was conducted during the growing seasons (March-October) of 2016 and 2017 at the margins of nine ponds (see Table S2) whose steep field-to-pond moisture gradients promoted highly diverse vegetation. Ponds were located at the edge, or directly within, agriculturally-intensive arable fields cropped with cereal grains or sugar beet, with oilseed rape and beans also commonly encountered, in Norfolk, eastern England, near the villages of Bodham (52.9177° N, 1.1574° E, elevation 72.4 m) and Briston (52.8540° N, 1.0618° E, elevation 54.3 m). Six of the small study ponds (surface area ~ 150 m², average depth ~ 1 m) had herb-rich, open-canopy margins, while three ponds had margins filled with dense woody vegetation. The width of the pond margins varied between 5 and 10 m.
Flowering plant survey and pollen library

All insect-pollinated plant species flowering at the time of the pollinator surveys were recorded and identified to species level (20) during once-monthly surveys throughout the study interval. Pollen was extracted from insect-pollinated flowers using an insect pin dipped in a mixture of fuchsin jelly and glycerol (21) to create a pollen reference collection. The pollen was checked against an identification atlas (22) to ensure accurate identification.

Diurnal and nocturnal pollinator surveys

Pollinating invertebrates were sampled using three methods to obtain a standardised, comprehensive record of plant interaction networks with the target groups. Nocturnal moth communities were surveyed once a month using a 6W-actinic rigid portable light trap (Watkins & Doncaster, Leominster, UK) connected to an external 12V 22Ah sealed battery (Lucas Electrical, Coleshill, UK). Moths were individually captured and euthanised to avoid pollen contamination between specimens. Pollen was then extracted from 50% (838) of captured moth specimens. Diurnal pollinator sampling was undertaken using time-lapse photography for one day each month using two Timelapse Cam 8.0 camera systems (© EBSCO Industries, Inc., Birmingham, AL) installed within the pond margin and aimed at flower patches. Photographs were taken at 30-second intervals and analysed for flower visitation. Furthermore, visual surveys were conducted monthly within each pond margin for 30 minutes, recording all pollinating invertebrates accessing the front of a flower. Only nocturnal and diurnal specimens clearly identifiable to genus or species level using morphological features (23–26) were included in network analysis.
**Pollen extraction and analysis**

Pollen from all sampled moth species (representatives from Noctuidae, Sphingidae, Geometridae, Erebidae, Lasiocampidae, Hepialidae, Crambidae and Notodontidae) was removed using an insect pin dipped in a mixture of glycerol and fuchsin jelly (21), unrolling the proboscis with the pin, swabbing the labial palps, and then rubbing the resultant residue on a prepared slide (27). Swabbing was then performed on the under-thorax of moth specimens using a separate insect pin. All detected grains from insect-pollinated plants were matched against the pollen library and other existing records (22), and all grains identifiable to genus or species level were included in network construction.

**Statistical analysis**

With different survey methods employed, accumulation curves were created for each method and group (Non-Apidae, Apidae, and combined diurnal insect observations, and nocturnal moth observations) to analyse for potential undersampling of diurnal and nocturnal insect populations. Curves were constructed using the number of individuals gathered over twelve sampling periods using the vegan package (28), while plant-pollinator interaction networks were constructed using the bipartite package (29) in R (Version 3.5.1 GUI El Capitan build, © 2016). Diurnal pollinator flower-visitation networks were separated into Apidae and Non-Apidae networks, given that social bees within Apidae have largely been the focus of pollinator research to date (15,16) and their colonies are regularly managed and promoted by humans, creating interaction differences with other, largely solitary pollinators. This allowed for direct comparison of pollen transport by moths with the flower-visiting efforts of social bees as well as with the less understood efforts of solitary diurnal species. Resulting network differences were analysed using network level metrics produced during network construction, including linkage density (reflecting network complexity), interaction diversity expressed as
Fisher’s alpha and Shannon’s diversity (measuring interaction richness), specialisation ($H_2'$, indicating the importance of specialist versus generalist interactions), and the species-level Paired Difference Index (PDI) (reflecting specialisation of individual species) for commonly visited flowers.

**Results**

Accumulation curves exhibited considerable decreases in curve gradation for visual observations of the Non-Apidae and combined diurnal pollinator pools, as well as for the combined diurnal pollinator pool for total output of visual observation and time-lapse based surveys, while not showing a distinct plateau (Figure S1). In contrast, the accumulation curves for Apidae-only species in both visual observation and time-lapse methods noticeably plateaued towards the end of the sampling. The accumulation curve for Non-Apidae populations obtained during time-lapse photography showed a distinct increase in the species pool along the entire curve, whereas the accumulation curve for moths also continued to rise, but with a discernible reduction in gradation towards the end.

The nocturnal pollen-transport network (Figure 1a) contained 103 moth species, dominated by Noctuidae (owlet moths), Erebidae (underwing and tiger moths) and Geometridae (looper moths) (Figure 2). Moths carried pollen from 47 insect-pollinated plant species on their bodies, with pollen from Rosaceae, Fabaceae, Apiaceae, and Lamiaceae most commonly found. Transported pollen included 7 plant species where no visitation from diurnal pollinators was observed (see Table S3) and 38 species also included in observed diurnal pollinator networks. *Rubus fruticosus* agg. L. (blackberry, Rosaceae) and two clovers, *Trifolium repens* L., and *T. dubium* Sibth. (Fabaceae), were very prominent in the nocturnal pollinator network (Figure 1a), with a more generalist PDI score (Table 1) indicating that their pollination may be strongly promoted by a wide range of moth species. While the
recorded network is conservative, as it only represents individuals carrying pollen, several network-level metrics (Table 1) including linkage density (moths: 12.9, non-Apidae: 7.8, Apidae: 8.0) and interaction diversity (Fisher’s Alpha - moths: 368.4, non-Apidae: 140.9, Apidae: 45.2; Shannon’s Diversity - moths: 5.5, non-Apidae: 4.7, Apidae: 4.3) indicate them to contain greater levels of complexity than diurnal networks. The moth-based network showed similar nestedness to the non-Apidae network (moths: 5.2, non-Apidae: 4.7, Apidae: 10.6) and occupied an intermediate position with regards to specialisation (H2’ - moths: 0.3, non-Apidae: 0.4, Apidae: 0.2).

Of the 838 individuals swabbed, 381 moth specimens (45.5%) were found to transport pollen. For pollen-transporting moths, 81% carried more than one pollen grain, 30% carried between 2-5 grains, 51% ≥ 5 grains, and 19% ≥ 10 grains. Furthermore, 20% of moths carried pollen originating from multiple plant species. Of the detected pollen grains, 57% were encountered on the under-thorax of the moths (Figure S2).
Figure 1. Pollen transport and flower visiting networks in a lowland agricultural landscape. Networks represent (a) nocturnal moths based on individuals carrying pollen of given plant species, and flower visitation networks consisting of (b) non-social bee and wasp species, hoverflies, and butterflies and (c) social bee species. Species contributing to >10% of interactions are named, and the number of contributing species, as well as linkage density and connectance, are indicated above the networks. Width of boxes in each column is proportional to the number of individuals found with pollen from a given species or from the number of visitation records. Width of connecting links corresponds with the recorded number instances of pollen or flower visitation by the pollinator with a specific flower species.
Figure 2. Richness and abundance of settling moths with pollen. Number of individuals and species within four most prominent moth families found transporting pollen in agricultural landscape. Secondary y-axis corresponds with number of species from each family (as there are fewer species than number of individuals). Lighter shades represent total individuals/species swabbed. Darker shades represent those total individuals/species found with pollen.
Table 1. Interaction network metrics comparison between nocturnal and diurnal plant-pollinator networks around agricultural ponds. Table showing (a) network-level parameters and (b) paired difference index (PDI) for some plants common to all networks. For nestedness, the scale is 1-100 with 1 being perfectly nested and 100 being random. For the PDI, the scale is 0-1, with 0 indicating total generalism and 1 indicating total specialism in the plants’ relationship with pollinators.

<table>
<thead>
<tr>
<th></th>
<th>Nocturnal Network</th>
<th>Non-Apidae Diurnal Network</th>
<th>Apidae Only Diurnal Network</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Linkage Density</strong></td>
<td>12.922</td>
<td>7.759</td>
<td>8.028</td>
</tr>
<tr>
<td><strong>Fisher’s Alpha</strong></td>
<td>368.370</td>
<td>140.933</td>
<td>45.192</td>
</tr>
<tr>
<td><strong>Shannon’s Diversity</strong></td>
<td>5.461</td>
<td>4.677</td>
<td>4.301</td>
</tr>
<tr>
<td><strong>Specialisation (H')</strong></td>
<td>0.286</td>
<td>0.399</td>
<td>0.197</td>
</tr>
<tr>
<td><strong>Nestedness</strong></td>
<td>5.205</td>
<td>4.712</td>
<td>10.603</td>
</tr>
<tr>
<td><strong>Connectance</strong></td>
<td>0.063</td>
<td>0.099</td>
<td>0.318</td>
</tr>
<tr>
<td><strong>Links Per Species</strong></td>
<td>2.040</td>
<td>2.802</td>
<td>2.825</td>
</tr>
<tr>
<td><strong>Interaction Strength Asymmetry</strong></td>
<td>0.075</td>
<td>0.060</td>
<td>-0.135</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Nocturnal Network</th>
<th>Non-Apidae Diurnal Network</th>
<th>Apidae Only Diurnal Network</th>
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</thead>
<tbody>
<tr>
<td><strong>Centaura nigra</strong></td>
<td>0.942</td>
<td>0.973</td>
<td>0.948</td>
</tr>
<tr>
<td><strong>Cirsium arvense</strong></td>
<td>0.971</td>
<td>0.883</td>
<td>0.790</td>
</tr>
<tr>
<td><strong>Epilobium hirsutum</strong></td>
<td>0.976</td>
<td>0.937</td>
<td>0.800</td>
</tr>
<tr>
<td><strong>Heracleum sphondylium</strong></td>
<td>0.971</td>
<td>0.993</td>
<td>0.880</td>
</tr>
<tr>
<td><strong>Hypericum perforatum</strong></td>
<td>0.956</td>
<td>0.992</td>
<td>0.937</td>
</tr>
<tr>
<td><strong>Mentha aquatica</strong></td>
<td>0.961</td>
<td>0.918</td>
<td>0.830</td>
</tr>
<tr>
<td><strong>Ranunculus repens</strong></td>
<td>0.879</td>
<td>0.993</td>
<td>0.729</td>
</tr>
<tr>
<td><strong>Rubus fruticosus agg.</strong></td>
<td>0.910</td>
<td>0.996</td>
<td>0.633</td>
</tr>
<tr>
<td><strong>Salix cinerea agg.</strong></td>
<td>0.991</td>
<td>—</td>
<td>0.900</td>
</tr>
<tr>
<td><strong>Trifolium dubium</strong></td>
<td>0.973</td>
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<td>0.950</td>
</tr>
<tr>
<td><strong>Trifolium repens</strong></td>
<td>0.937</td>
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<td>0.847</td>
</tr>
<tr>
<td><strong>Vicia cracca</strong></td>
<td>0.971</td>
<td>0.984</td>
<td>0.909</td>
</tr>
</tbody>
</table>
Solitary bees, wasps, syrphids, and butterflies formed a network of 76 pollinators, with 45 plant species (Figure 1b) visited by 632 individuals, while 11 species of social Apidae bees (*A. mellifera* and *Bombus* spp.) comprising 1,548 individuals visited 46 plant species (Figure 1c). Plant species preferentially visited by diurnal visitors chiefly belong to the Lamiaceae, Rosaceae, Apiaceae, and Asteraceae (see Table S4). The nocturnal network had an overall higher linkage density and interaction diversity than either diurnal network, with a higher level of specialisation than social bees, but a lower level of specialisation than the Non-Apidae diurnal network (Table 1).

**Discussion**

With direct comparability of the three interaction networks created in our study arguably impeded by the different survey methods employed (30), the observed high network complexity in the nocturnal moth pollen-transport network (being the most conservatively sampled network) reflects the value of such comparisons. Acknowledging that neither flower visitation nor pollen presence on pollinators’ bodies represent direct proof of pollination (31), our observations strongly suggest that nocturnal moths are influential components of wild plant-pollinator networks in agro-ecosystems. Therefore, moths may provide additional important resilience to pollination networks, potentially counter-balancing ongoing diurnal pollinator declines (3).

Our results are in line with hypothesis 1, as evidenced by the greater size and complexity of the moth network in comparison with previous studies (9,10,12), and by moths transporting pollen from a high number of plants also visited by diurnal pollinators in the agricultural landscape. For hypothesis 2, however, results are inconclusive, as all 7 plants exclusively present in the nocturnal network are known to be pollinated by diurnal insects (32–36). Our
observation likely reflects the different methods employed to sample each community that could have led to undersampling. Accumulation curves indicate that the Apidae-only networks in the studied habitat likely represent a near-complete picture of the existing network, while Non-Apidae diurnal populations were sampled well using the visual observation method, with some further additions in sampling strength contributed by the time-lapse method, with a small degree of undersampling persisting for this group. Moth populations similarly appear to have been undersampled, meaning that the networks presented here remain partial, although distinct gradation reductions in most curve termini are indicative that the main network links are represented in our analysis.

In line with our results, recent moth pollen-transport research in an agro-ecosystem (13) has found strong evidence of Rosaceae and Fabaceae pollen being transported by moths, further establishing moths as important pollen transporters at least for wild members of these two families. Spill-over effects from enhanced diurnal pollinator visits to wild flower-dominated habitats leading to increased crop pollination services have been previously documented (37–39). For moths, however, there are currently considerable knowledge gaps concerning spill-over effects and the role these taxa play in crop pollination. Although crop-based pollen has been previously found on settling moths (11), measurable research on the provision of pollination services that could benefit specific crops, especially from spill-over, is currently lacking and requires urgent further investigation.

Previous research of pollen transport in settling moths commonly focused on their proboscis. However, settling moths sit on the flower while feeding (40,41), with their often distinctly hairy bodies easily accidentally touching the flower’s reproductive organs (41). Here, we show that most pollen is indeed transported on the ventral thorax of moths, from where it
might easily be deposited during subsequent flower visits (42,43). Emerging research already indicates that some plants may be specifically pollinated by moths transporting the pollen on their ventral thorax (43), however, the effectiveness of this mechanism, particularly when compared to bees’ pollen harvest from their target plants (17–19,44), requires further investigation.

This pivotal study comes as moth populations experience extreme declines across the globe (45–49), with worrying implications that we may be losing critical pollination services at a time when we are barely beginning to understand them. Reductions in flowering plant populations throughout the landscape are likely contributing to moth declines (50,51). Activities aimed at counter-acting moth losses by providing enhanced food resources and improved habitat structures (52,53) may be helped by pollen-transport network analysis that provides fundamental insights into plant species dependent on visits of adult moths, and key nectar sources for dwindling moth populations in agricultural landscapes, thus greatly informing conservation efforts.

Our investigations demonstrate that nocturnal settling macro-moths in agro-ecosystems have highly complex, formerly unknown plant interactions, with moths regularly transporting pollen of plants found in highly managed landscapes. The massive decline of moths from these landscapes (45,47) may thus represent a significant loss to pollination services for these wild plants. We echo calls for future crop pollination research to include nocturnal pollinators to understand their specific role in pollination services (7,10,11). Beyond this, additional research involving settling moths is directly needed to quantify their effectiveness in pollen deposition, and specifically their potential for pollinating crops, especially arising from spill-over from semi-natural habitats, as this potential remains largely unknown. Our observations
that nocturnal moth networks in agro-ecosystems are contributing substantially towards pollen-transport after the sun sets raises questions on the capability of nocturnal moths to partly counterbalance pollination gaps of non-cultivated plants caused by decreases amongst diurnal pollinator populations. Moth pollen transport networks should therefore become an integral part of studies investigating pollination services, while moth conservation needs to be crucially integrated into modern agricultural management. We ignore them at our peril.

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