



Research

Cite this article: Lefebvre V, Fontaine C, Villemant C, Daugeron C. 2014 Are empidine dance flies major flower visitors in alpine environments? A case study in the Alps, France. *Biol. Lett.* **10**: 20140742.
<http://dx.doi.org/10.1098/rsbl.2014.0742>

Received: 15 September 2014

Accepted: 16 October 2014

Subject Areas:

ecology, behaviour, plant science

Keywords:

mountain ecology, foraging behaviour, flower visitors, Empidinae, Mercantour National Park

Author for correspondence:

Christophe Daugeron

e-mail: daugeron@mnhn.fr

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2014.0742> or via <http://rsbl.royalsocietypublishing.org>.

Animal behaviour

Are empidine dance flies major flower visitors in alpine environments? A case study in the Alps, France

Vincent Lefebvre¹, Colin Fontaine², Claire Villemant¹
and Christophe Daugeron¹

¹Muséum national d'Histoire naturelle, ISYEB, UMR 7205 MNHN, CNRS, UPMC, EPHE, 45 rue Buffon, CP 50, Paris 75005, France

²Muséum national d'Histoire naturelle, CESCO, UMR 7204 MNHN, CNRS, UPMC, 55 rue Buffon, CP 53, Paris 75005, France

Pollination is one of the most important ecosystem services and bees the most important pollinators. As a population decline of bees has been documented in numerous regions of the world, it is crucial to develop understanding on other possible pollinators. Here, we study the potential pollination impact of Diptera, and among them Empidinae, in an alpine environment, where the abundance of bees is naturally lower. Interactions between 19 entomophilous plants and their flower visitors were recorded in a subalpine meadow in the French Alps during six weeks. Visitation frequencies were used to build the flower–visitor network. Our results show that interactions between flies and plants are dominant; flies represent more than 60% of all visitors, with 54% of them being Empidinae. We especially found that flies, Empidinae and bees are the main visitors of 11, three and one plants, respectively. When considering both bees and Syrphidae together, six plants were more visited by Empidinae; when considering bees and Syrphidae separately, 10 plants were more visited by Empidinae than by bees or Syrphidae. The results support the idea that flies widely replace bees as main flower visitors at altitude, and among them the Empidinae might play a key role in pollination.

1. Introduction

Pollination by insects is one of the most important ecosystem services, but is threatened by pollinator decline [1]. Bees, which are known to be the main pollinators of flowering plants, are particularly affected [2]. In this context, it is highly relevant to study the pollinator impact of other insects, especially in areas where bees are naturally scarcer, such as cool temperate to cold habitats. Diptera, of which many families are well known to be flower visitors, are often more abundant and diverse than bees in such habitats [3]. It was hypothesized that high elevation areas offer favourable ecological conditions (wet soils, stream edges) for fly larvae and, unlike bees, flies take better advantage of microhabitats for thermoregulation [4] as they do not spend their time building and provisioning nests. Therefore, at higher elevations, Diptera could take over from Hymenoptera and be more efficient pollinators than other well-known pollinators that thrive in low-altitude conditions, such as solitary bees. Bumblebees, which can also thermoregulate efficiently, seem to be the exception among bees.

The subfamily Empidinae (Diptera, Empididae), or empidine dance flies, has never been formally studied as a group of pollinators, although most observations suggest that it could be a key group of insects for the pollination of angiosperms in various mountains and cold habitats of the world (e.g. in the Snowy Mountains of Australia, the Arctic regions or in Patagonia, see [5–7]). The anthophilous empidine species belong to the tribe Empidini, which includes about 1200 known species.

Many of them visit flowers frequently, due to their exclusive dependence on nectar as a food source on which they feed using their elongated proboscis (electronic supplementary material, figure S1). While foraging, they often carry high loads of pollen due to increased hair density on some body parts, further suggesting their potential role as pollinators.

As a prerequisite for assessing the pollinator impact of flies and more particularly Empidinae in mountain environments, our study was to build the flower–visitor network of a subalpine meadow, with the aim to test the following assumptions: (i) the dominance of flies as flower visitors relative to other orders of insects foraging for pollen or nectar and (ii) the dominance of Empidinae as flower visitors over two groups known as the most efficient pollinators in temperate areas, bees and syrphids (Diptera, Syrphidae).

2. Material and methods

(a) Study site

The study was conducted from 29 May to 10 July 2012 in the north of Mercantour National Park, France (electronic supplementary material, figure S2). The site, called Plateau des Meyries (1800 m, 6°46'36.7" E, 44°23'53.4" N), is a semi open subalpine area of about 6 ha, largely flat and with no particular slope, including a large meadow dotted with larches (*Larix decidua*). Mowing usually occurs in late summer.

(b) Plant visitation

Pollination depends on many factors, some of which are used as more or less relevant proxies. For example, flower visitation is considered either as a poor pollination proxy [8] or an acceptable one [9]. However, an insect has a potential pollinator impact only if it visits a flower and deposits conspecific pollen grains on its stigma. Therefore, building the flower–visitor network of an ecosystem is a prerequisite to detect potential pollinators, provided that the quality of the foraging behaviour is taken into account. In this study, the network is based on visits for which the foraging insect came into contact with either the stamen or stigma of the flower.

(c) Monitoring of plants

Monitoring involved observation sessions for each selected plant species (electronic supplementary material, table S1) on non-rainy days with a temperature above 12°C. Each session was focused on a single plant species: insect visits on every flower of this plant species were counted at sight by walking in the survey area. Sessions lasted 10 min at most depending on the plant density and were distributed throughout the day between 9.30 and 17.00. As many sessions as possible were done for each plant species during its flowering time.

The monitored plants were chosen for being visited by foraging insects. Indeed, many plants known as entomophilous were actually not visited by insects during the study (e.g. some species of Orchidaceae). Finally, 19 species for which at least one session every 2 days was available were retained for analysis.

Owing to their different flowering time (electronic supplementary material, figure S3), the number of sessions varied for each plant species. This did not impact on the results as we did not compare plants but the number of visits on a given plant by various pollinators.

(d) Identification of insects

Flower visitors were identified on-site as Empidinae, Syrphidae, other Diptera, bees, other Hymenoptera, Coleoptera, Lepidoptera, Heteroptera and Neuroptera. Empidinae were also

collected on each plant species for accurate identification to species or morphospecies level in the laboratory.

(e) Data analyses

All statistical analyses were performed using R [10]. The flower–visitor network was generated with the bipartite package [11] using visitation frequencies: the thickness of a link between a plant species and a group of visitors is equal to the visitation frequency calculated as N_v/T , where N_v is the total number of visits by this group of insects on this plant species and T the total time of monitoring for this plant species (electronic supplementary material, table S1).

The correspondence analysis to visualize associations of flowers and visitors was performed with ade4 package for R [12] using the relative proportions of each group of visitors on each flowering plant species.

The significance of the differences in the number of visits of each taxon on a given plant species was tested with the non-parametrical Wilcoxon test, as data from counting insects on flowers is not normally distributed (electronic supplementary material, table S2).

3. Results

The flower–visitor network is dominated by two groups of insects: Diptera and Hymenoptera with 62% and 23% of all visits, respectively (figure 1). Diptera composed more than 50% of all visits for 14 plants, and the proportion of flies to other insects was significantly higher for 11 of them (Wilcoxon test, $p < 0.05$).

Fifty-four per cent of the flower-visiting Diptera were empidines. They represent at least 50% of all visits for seven plant species, this proportion being significantly higher for three of them (Wilcoxon test, $p < 0.05$). The number of visits of empidines was significantly higher than visits by syrphids and bees considered together for six plant species (Wilcoxon test, $p < 0.05$). When considering syrphids and bees separately, four additional species were significantly more visited by empidines than by syrphids (*Campanula rotundifolia*, *Hieracium cymosum*, *Onobrychis viciifolia* and *Ornithogalum umbellatum*; Wilcoxon test, $p < 0.05$) and another four species were significantly more visited by empidines than by bees (*Myosotis decumbens*, *Ranunculus bulbosus*, *Persicaria bistorta* and *H. cymosum*, Wilcoxon test, $p < 0.05$).

Most of the flower-visiting Hymenoptera observed during the study were bees (approx. 88%), but bees were the main visitors only for *Rubus idaeus* (Wilcoxon test, $p < 0.001$), with at least 85% of the visits by *Apis mellifera* and *Bombus* spp. In addition, three plant species were significantly more visited by bees than by empidines (*Knautia arvensis*, *Centaurea uniflora* and *O. viciifolia*; Wilcoxon test, $p < 0.05$).

None of the flowering plants had syrphids as main visitor. However, *Meum athamanticum* (Apiaceae) was significantly more visited by syrphids than by empidines (Wilcoxon test, $p < 0.01$).

The data are particularly well structured: the correspondence analysis (figure 2) segregates two distinct clusters of plants, each one overlapping with empidines and bees, respectively; a third cluster reveals that flowers visited by syrphids mostly attract insects other than empidines and bees (various flies, sawflies and beetles).

Twenty-two species of Empidinae were collected foraging on flowers. Thirteen of them were collected on one plant species only, but some were found on up to five different plant species.

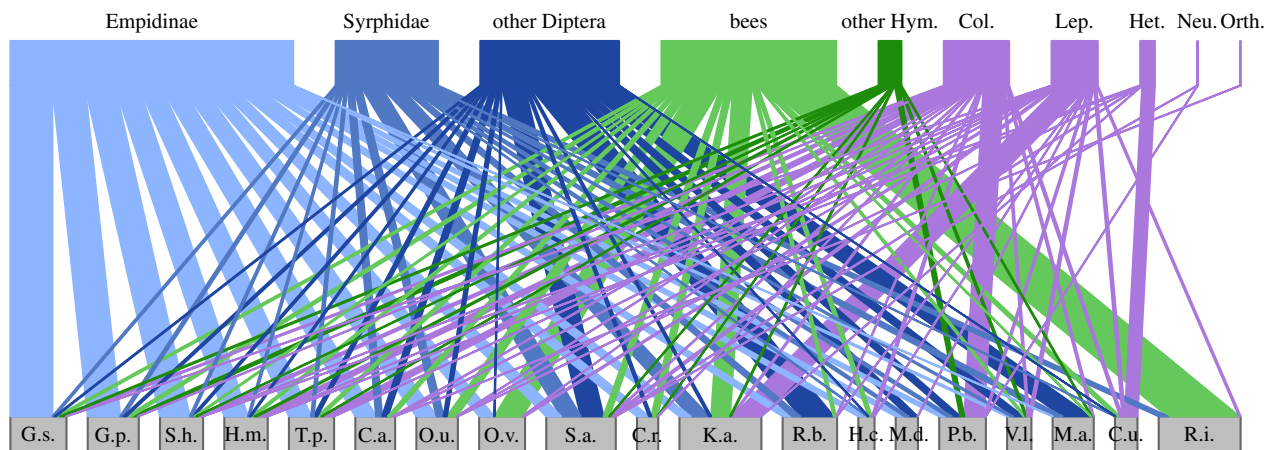


Figure 1. Flower–visitor network obtained in a subalpine meadow at 1800 m in the Mercantour National Park. Insects: Hym., Hymenoptera; Col., Coleoptera; Lep., Lepidoptera; Het., Heteroptera; Neu., Neuroptera; Orth., Orthoptera. Plants: G.s., *Geranium sylvaticum*; G.p., *Geranium pyrenaicum*; S.h., *Scorzonera hispanica*; H.m., *Hypochaeris maculata*; T.p., *Tragopogon pratensis*; C.a., *Cerastium arvense*; O.u., *Ornithogalum umbellatum*; O.v., *Onobrychis vicifolia*; S.a., *Sisymbrium austriacum*; C.r., *Campanula rotundifolia*; K.a., *Knautia arvensis*; R.b., *Ranunculus bulbosus*; H.c., *Hieracium cymosum*; M.d., *Myosotis decumbens*; P.b., *Persicaria bistorta*; V.l., *Viburnum lantana*; M.a., *Meum athamanticum*; C.u., *Centaurea uniflora*; R.i., *Rubus idaeus*.

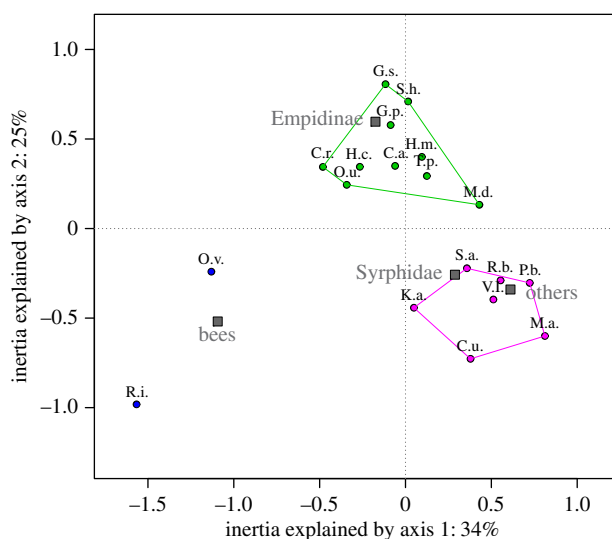


Figure 2. Two-dimensional graphical representation of the correspondence analysis of the flowers/visitors data table. Main contribution to axis 1 (34% of inertia) comes from bees visiting *O. vicifolia* and *R. idaeus*, and main contribution to axis 2 (25% of inertia) comes from Empidinae. For abbreviations, see legend of figure 1.

4. Discussion

For the set of plant species monitored in the study site, our results do confirm the conspicuousness of anthophilous Diptera compared with bees. Although flower visitation does not equal pollination, the prevalence of flies as flower visitors in subalpine areas suggests that they could be major pollinator agents at this elevation. Among them, Empidinae seem to play a key role compared with bees or syrphids. This can be explained by their abundance as well as their diversity: 22 species were collected on flowers during the study, but more than 70 species were identified for the purpose of the recent ‘All Taxa Biodiversity Inventory’ recently conducted in the Mercantour National Park (2009–2011) (Daugeron and Lefebvre 2012, unpublished data).

Owing to their foraging behaviour and abundant hairiness, they are more likely to fertilize the visited plants than most other Diptera: as they only feed on nectar, they

commute between flowers continuously, while many opportunist flies also feeding on other resources (e.g. Muscoidea, Tachinoidea) seem to have a low frequency of visit and a reduced activity on flowers.

Although Diptera are usually considered as generalist foragers, this might not apply to some empidine species. For instance, *Empis* sp. 2 was observed 52 times on *C. rotundifolia*, but never seen or captured on another flower, nor was *C. rotundifolia* visited by other empidine species. This suggests a strong ecological link between the two taxa. Certain empidine species could thus be specialized for foraging and pollination, which could be explained by co-evolution events. However, the factors making a given flowering plant species preferred by an empidine are still unknown.

Empidinae, bees and syrphids have to visit flowers because of their life cycle, which entirely or in part depends on nectar and/or pollen. At this elevation, solitary bees showed, as expected, a relative scarcity, but surprisingly syrphids did not appear as major visitors of the monitored plant species.

Our study shows that empidines are a key component of the flower–visitation networks in temperate altitude meadows and could have a higher fertilization impact than bees and other well-known pollinators. In this respect, they could be an excellent model for understanding the role played by both ecological and historical factors in the evolution of plant–pollinating insect relationships at altitude, and why bees are widely replaced by flies. For that it could be particularly relevant to determine the altitude at which the pollinator shift from Hymenoptera to Diptera occurs, and how the mutualistic relationships between the flora and the available pollinators change with elevation. If strong specific mutualisms could be revealed, it would be interesting to assess if the diversity of angiosperms in alpine areas is partly related to the diversity of empidine flies.

Acknowledgements. We thank François Breton, Marie-France Leccia (Mercantour National Park), Jean-Philippe Sibley (MNHN), Adrian Plant (National Museum of Wales), Gabrielle Names (École Normale Supérieure, Paris), Jean-Luc and Charline Printemps (Centre Seolane, Barcelonnette) and three anonymous reviewers.

Funding statement. This study was partially funded by the Mercantour National Park and the Service du Patrimoine Naturel (MNHN).

References

1. Steffan-Dewenter I, Potts SG, Packer L. 2005 Pollinator diversity and crop pollination services are at risk. *Trends Ecol. Evol.* **20**, 651–652. (doi:10.1016/j.tree.2005.09.004)
2. Fitzpatrick Ú, Murray TE, Paxton RJ, Breen J, Cotton D, Santorum V, Brown MJF. 2007 Rarity and decline in bumblebees—a test of causes and correlates in the Irish fauna. *Biol. Conserv.* **136**, 185–194. (doi:10.1016/j.biocon.2006.11.012)
3. Mani MS. 1968 *Ecology and biogeography of high altitude insects*. The Hague, The Netherlands: Dr W. Junk.
4. Kearns CA. 1992 Anthophilous fly distribution across an elevation gradient. *Am. Midl. Nat.* **127**, 172–182. (doi:10.2307/2426332)
5. Inouye DW, Pyke GH. 1988 Pollination biology in the Snowy Mountains of Australia: comparisons with Montane Colorado, USA. *Aust. J. Ecol.* **13**, 191–210. (doi:10.1111/j.1442-9993.1988.tb00968.x)
6. Hocking B. 1968 Insect–flower associations in the high arctic with special reference to nectar. *Oikos* **19**, 359–388. (doi:10.2307/3565022)
7. Daugeron C, D’Haese C, Plant A. 2009 Phylogenetic systematics of the Gondwanan *Empis macrorrhyncha* group (Diptera, Empididae, Empidinae). *Syst. Entomol.* **34**, 635–648. (doi:10.1111/j.1365-3113.2009.00490.x)
8. King C, Ballantyne G, Willmer PG. 2013 Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods Ecol. Evol.* **4**, 811–818. (doi:10.1111/2041-210X.12074)
9. Vazquez DP, Morris WF, Jordano P. 2005 Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol. Lett.* **8**, 1088–1094. (doi:10.1111/j.1461-0248.2005.00810.x)
10. R Core Team. 2011 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. (<http://www.R-project.org>)
11. Dormann CF, Fruend J, Bluethgen N, Gruber B. 2009 Indices, graphs and null models: analyzing bipartite ecological networks. *Open Ecol. J.* **2**, 7–24. (doi:10.2174/1874213000902010007)
12. Dray S, Dufour AB. 2007 The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.* **22**, 1–20.