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Evidence of butterfly wing pollination in the martagon lily *Lilium martagon* L. Evidència sobre la pol·linització del marcòlic *Lilium martagon* L. a través de les ales de papallones

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It has been estimated that about 90 % of all flowering plants on Earth are pollinated by animals, above all by insects (Ollerton *et al.*, 2011). Insect pollination is therefore a highly important ecosystem service (Kremen *et al.*, 2007) but one that is at risk due to recent generalized declines in insect populations (Steffan-Dewenter *et al.*, 2005; Biesmeijer *et al.*, 2006). Although bees (Hymenoptera: Anthophila) are considered to be the most effective insect pollinators, many studies indicate that the contribution of other groups is not negligible (e.g. Proctor *et al.*, 1996; Rader *et al.*, 2016; Stefanescu *et al.*, 2018). Lepidoptera have been shown to play an important role in the pollination of some plants and the close relationship between certain Lepidoptera and the plants they pollinate has led to the description of a number of pollination syndromes (i.e. psychophily, phalaenophily and sphingophily; see definitions in Willmer, 2011), that is, the evolutionary convergence between certain morphologies and reward pat-

terns in flowers that enable them to exploit more efficiently the ability and preferences of moths and butterflies. Butterflies, for example, tend to visit psychophilous flowers with flat platforms that assist landing in inflorescences that have a mild and pleasant aroma, offer small-to-medium volumes of dilute nectar, and have brightly coloured petals. In some cases, it has been demonstrated that butterflies are indeed the main pollinators of such flowers (e.g. Johnson & Bond, 1992; Bloch *et al.*, 2006).

On 4 June 2017, CA observed along the river Ridaura (Santa Cristina d'Aro, Baix Empordà, 31T DG9430) a female small white (*Pieris rapae* Linnaeus, 1758) with extensive brownish patches on the underside of both hind-wings (Table 1, Fig. 1a). This abnormal colouration was first thought to be a rare aberration until other butterfly species with similar patches of colour were recorded again in the same area the following year (Table 1, Figs. 1b-c). Subsequently, JC and

Table 1. Records of butterflies with pollen from *Lilium martagon* on their hind-wings. *No pollen was found on this butterfly due to its small size (see text).

Butterfly species	Site	Date	Nectaring
<i>Gonepteryx cleopatra</i> (Pieridae)	Sta. Cristina d'Aro (Girona)	15-18.VI.18	on <i>Echium vulgare</i> and <i>Lilium martagon</i>
<i>Maniola jurtina</i> (Nymphalidae)	Riera de Canyamars (Barcelona)	22.VI.18	on <i>Lilium martagon</i>
<i>Pieris brassicae</i> (Pieridae)	Sta. Cristina d'Aro (Girona)	18.VI.18	on <i>Lilium martagon</i>
	Riera de Canyamars (Barcelona)	20.VI.18	on <i>Lilium martagon</i>
<i>Pieris napi</i> (Pieridae)	Sta. Cristina d'Aro (Girona)	11.VI.18	on <i>Rubus</i> sp.
		18.VI.18	
<i>Pieris rapae</i> (Pieridae)	Sta. Cristina d'Aro (Girona)	4.VI.17	on <i>Galactites tomentosa</i>
		18.VI.18	
<i>Pyronia bathseba</i> (Nymphalidae)	Riera de Canyamars (Barcelona)	20.VI.18	on <i>Lilium martagon</i>
<i>Satyrrium esculi</i> * (Lycaenidae)	Sta. Cristina d'Aro (Girona)	18.VI.18	on <i>Lilium martagon</i>



Figure 1. Butterflies with *Lilium martagon* pollen on hind wings: *Pieris rapae* (a), *Pieris napi* (b), *Gonepteryx cleopatra* (c, d), *Pieris brassicae* (e). *Satyrium esculi* nectaring on *Lilium martagon* (f). Detail of the tepal with the basal groove leading to the nectary (g). Detail of pollen grains carried by *G. cleopatra* (h), and of the pollen kitt appearing as yellow drops among grains after being washed in ethanol 70° (i). Photos by Carlos Alvarez-Cros (a-c), and Jordi Corbera (d-i).

CA (the first and the second authors) visited the area to try to obtain samples and detected this curious phenomenon again on several other Pieridae. For the first time they were able to determine that the origin of these brownish patches was pollen from martagon lilies (*Lilium martagon* L.) deposited on the hind-wings as butterflies imbibed the nectar concentrated at the base of the tepals of the flowers (Figs. 1d-e). This was confirmed by comparing under a microscope pollen samples from the anthers of *L. martagon* with the dust-like material covering the butterflies' hind-wings, brushed off and mounted on a slide for inspection (Fig. 1h). Some days later, additional observations were made at another site (Riera de Canyamars, Dosrius, Maresme, 31T DG5506) and, in total,

evidence was found of the wing transportation of pollen in six butterfly species, four Pieridae and two Nymphalidae/Satyrinae (Table 1).

The behaviour of butterflies when visiting *L. martagon*, together with the morphology of the flower, explains why pollen grains appear on butterfly hind-wings but not on the hairs of the body. The flower's nectar is located in nectaries hidden in grooves at the base of the tepals (Fig. 1g), which is only accessible to insects that can probe with long tongues (i.e. the proboscis of Lepidoptera). When the butterfly lands on the tepal and searches for nectar, the hind-wings touch the anthers of the stamens, which in this flower protrude at length from the corolla (Fig. 2). However, only medium-to-



Figure 2. Aging of the flower of *Lilium martagon*, showing the different position of the stigma that eventually allows wing pollination by nectaring butterflies. Flowers of *Lilium martagon* open downwards (a); during the protandrous period the style is straight and reaches beyond the anthers (b, c); when the stamens wither, the style rotates about 90° upwards (d) leaving the stigma in an ideal position for touching butterflies' wings; after fertilization, the whole flower rotates 180° upwards (e) and the fruit begins to grow (f). Photos by Jordi Corbera.

large-sized butterflies are effective in collecting pollen grains while nectaring: when a false ilex hairstreak (*Satyrrium esculi* Hübner, 1804) was recorded on *L. martagon*, it was obvious that its wings were too small to touch the anthers and, hence, virtually no pollen was collected (Fig. 1f). Moreover, as the flower ages, the style – which during protandrous phase hangs vertically in the centre of the six stamens (Figs. 2b-c) – curves up almost 90° (Fig. 2d). This means that when a medium-to-large-sized butterfly visits the flower, its hind-wings come in close contact with the stigma, thereby making the transfer of pollen grains from a previous flower very likely. In fact, we found butterfly scales on two out of the four stigma analysed in the laboratory.

When pollen from the flowers or from the wings of the collected butterflies was transferred to a slide, it was obviously very sticky. Pollen grains adhered to each other and formed large pollen packages. In contrast to anemophilous flowers, this clumped distribution of pollen grains is typical in insect-pollinated flowers (Timerman *et al.*, 2014). In the case of the genus *Lilium*, this viscosity results from the presence of the so-called pollenkitt, a thin oily material that forms a superficial layer around the pollen grains (Clément *et al.*, 1998) and serves, among other things, to increase pollen adherence on

insect pollinators (Pacini & Hesse, 2005). When washed with ethanol 70°, the lipidic nature of the pollenkitt became apparent in the form of yellow drops among the pollen grains (Fig. 1i).

Our observations clearly show that butterfly wings act as the main –if not only– pollinating vector in *L. martagon*. Despite being unusual in Lepidoptera pollination, the same phenomenon has also been noted in a few other systems (e.g. Cruden & Hermann-Parker, 1979; Byragi Reddy & Subba Reddi, 1995; among others), including a close relative of the martagon lily, the wood lily *Lilium philadelphicum* L. (Edwards & Jordan, 1992). In two more recent studies, Kiepiel & Johnson (2014) described how the South African *Clivia miniata* (Lindl.) Regel (Amaryllidaceae) is mainly pollinated by swallowtail butterflies that transfer pollen via their wings. In turn, Epps *et al.* (2015) experimentally showed that two large-sized butterflies, *Speyeria cybele* (Fabricius, 1775) and, especially, *Papilio glaucus* Linnaeus, 1758, were the two pollinators of the flame azalea *Rhododendron calendulaceum* (Michx.) Torr. (Ericaceae) in North America and that they transfer pollen almost exclusively via their wings. However, in the latter case, pollen adhered in large quantities to the butterflies' wings due to viscin threads rather than the pollenkitt as in *L. martagon*.

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The system described here is very similar to that described by Edwards & Jordan (1992) for the North American wood lily. Based on comprehensive field observations, these authors concluded that flowers are primarily visited by large butterflies such as the swallowtail *Papilio glaucus* and the monarch *Danaus plexippus* (Linnaeus, 1758), which are the main pollinators of this plant. Edwards & Jordan (1992) have also described how pollen adheres to the underside of the wings of these large-sized butterflies when they walk around the flower platform and probe the base of the tepals for nectar, exactly as we have observed in *L. martagon*. They also suggest that the small butterflies that may occasionally nectar on the wood lily are most probably inefficient pollinators because their wings do not always touch the anthers or the stigmatic surface during their visits.

In any case, these different examples show that large-to-medium-sized butterflies, whose wings act as the pollinating vector, may be the main pollinators of a number of flowering plants in temperate ecosystems. In all described examples, flowers are characterized by their large size and generally bright colours (e.g. red, pink, orange or yellow), as well as highly protruding stamens and pistils. We believe that this phenomenon may be more common than previously thought and that similarly shaped and coloured large flowers to those reported here are candidates for butterfly wing pollination.

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