Pollination of the Lady’s slipper orchid (*Cypripedium calceolus*) in Scandinavia – taxonomic and conservational aspects

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Since Darwin (1862), orchid pollination has attracted the attention of many biologists for its intricacy, variety and ecological and evolutionary importance (Nilsson 1992, Tremblay 1992, Christensen 1994, Voth 1999, 2001, Roberts 2003, Tremblay et al. 2005, Blinova 2008). A particular case of pollination involves deceptive flowers, which are able to attract pollinators without actually offering any reward. This syndrome has been extensively studied and may characterize some 40% of all orchid species (Proctor et al. 1996, Schiestl et al. 1999, 2003, Schiestl and Ayasse 2002, Johnson et al. 2003, Schiestl and Roberts 2003, Tremblay et al. 2005, Blinova 2008). Nine species were recognized as effective pollen vectors (*Andrena cineraria*, *A. carantonica*, *A. haemorhous*, *A. helvola*, *A. nigroaenea*, *A. praecox*, *Colletes cunicularius*, *LasioGLOSSum fretellum* and *L. fulvicorne*), four of them for the first time in Scandinavia. This is the first time that a species of *Colletes* is reported to carry pollen of *Cypripedium* in this region. All but one specimens were females. Our results suggest a taxonomically heterogeneous pollinator fauna for *Cypripedium calceolus* and are discussed in light of the management of this species.

The Lady’s slipper orchid *Cypripedium calceolus* L. is considered one of the most beautiful orchids of Europe. Consequently, the species has suffered from over-collecting and is now critically endangered in many countries. Although pollination success is suspected to influence the long-term survival of *Cypripedium calceolus*, relatively little is known about the identity of its pollinators in mainland Sweden – a region that comprises the largest European populations. In order to identify which species pollinate eight representative populations in mainland Sweden, we observed and sampled visitors to flowers using a standardized protocol. Specimens were identified and any pollen smear found on their body was examined for the presence of *Cypripedium* pollen. Nine species were recognized as effective pollen vectors (*Andrena cineraria*, *A. carantonica*, *A. haemorhous*, *A. helvola*, *A. nigroaenea*, *A. praecox*, *Colletes cunicularius*, *LasioGLOSSum fretellum* and *L. fulvicorne*), four of them for the first time in Scandinavia. This is the first time that a species of *Colletes* is reported to carry pollen of *Cypripedium* in this region. All but one specimens were females. Our results suggest a taxonomically heterogeneous pollinator fauna for *Cypripedium calceolus* and are discussed in light of the management of this species.

The pollination of *Cypripedium calceolus* was originally described by Darwin (1862) and further investigated by Nilsson (1979). Pollinators are attracted to the labellum of *Cypripedium* flowers by a sweet scent (Bergström et al. 1999). They enter the flower through the labellum’s central opening (Fig. 1B) but find no reward, because the flower does not produce any nectar. To leave the flower, insects eventually seek the transparent sections in the dorsal part of the labellum, climbing towards the posterior opening. This is facilitated by a ladder of hairs on the inner side of the labellum. Insects of the right size manage to force their way through the narrow posterior openings, thereby touching the orchid’s column. Pollen grains are deposited on the insect’s body and delivered the next time the procedure is repeated.

Despite detailed studies on the pollination mechanism of *Cypripedium calceolus*, several questions remain concerning
which insects act as pollinators. Nilsson (1979) concluded that the most common pollinator of this orchid on the Baltic island of Öland was the solitary bee _Andrena haemorhoidia_, but that some other species in the genera _Lasioglossum_, _Andrena_ and _Halictus_ acted occasionally as pollen vectors. A similar study was performed by Erneberg and Holm (1999), who investigated the pollination of the Danish populations. Surprisingly, their results indicated that bees from the genus _Andrena_ were not regular visitors, being too large to function as efficient pollinators. Instead, medium-sized females of _Halictus tumulorum_ and _Lasioglossum calceatum_ were the most regular and frequent pollen vectors. Other studies outside Scandinavia have revealed yet other pollinator species, such as _Andrena cineraria_, _Colletes cunicularius_ and the flies _Chrysotoxum festivum_, _Syphus ribesii_ and _Musca corniha_ for Southern Ural (Ishmuratova et al. 2006) and _Syphus_ spp. and _Eristalis_ spp. for the Murmansk region (Blino 2002).

Nilsson (1979) and Erneberg and Holm (1999) provided information on the pollination of two Scandinavian populations, but to date no study has investigated the pollinators of the largest populations of _Cypripedium calceolus_ in Europe, namely those in mainland Sweden (Aronsson 2007). The species occurs throughout the main limestone areas of Sweden (with the exception of the southernmost parts), but the largest populations are notably concentrated in three regions (Fig. 2): the eastern province of Uppland, the mid-northern provinces of Jämtland and Dalarna, and the region between lakes Vänern and Vättern in the province of Västergötland. In Västergötland, limestone soils derive mainly from weathering of local limestone table mountains, whereas in Uppland such soils have been transported from limestone bedrocks in the Baltic Sea by the ice sheets during glacial times (Bernes 1994). In all these regions, the edaphic conditions have had a conspicuous effect on the local flora, accommodating a variety of species that are uncommon in the rest of the country.

Considering that the results from earlier pollination studies were not fully consistent with each other, the question of which insects act as pollinators in mainland Sweden remains unanswered. The problem is further complicated by the fact that the phenology of the Scandinavian populations of _Cypripedium calceolus_ differs: plants from Öland and Denmark generally flower in May and the beginning of June, whereas the populations in mainland Sweden flower later (Hultén and Fries 1986). Because the flying period for solitary bees greatly varies among species (Torp 1994, Schmid-Egger and Scheuchl 1997), this phenological difference is expected to affect the taxonomic composition of the pollinators. To address these issues, this study aims to identify the pollen vectors of _Cypripedium calceolus_ in different wild populations of mainland Sweden and discuss the results in light of previous studies and management strategies.

**Material and methods**

**Choice of sites**

The selection of study sites was based on four main criteria: 1) geographic distribution, as we wanted our conclusions to be as general as possible for mainland Sweden, rather than only applicable to a small area; 2) representativeness in terms of vegetation type, as populations growing in different forest types were expected to have different insect faunas (e.g. coniferous vs deciduous forests); 3) estimated number of shoots, because we considered large populations to exhibit less stochastic variation in pollination than smaller ones; and 4) accessibility.

In 2003 we studied three populations in the province of Västergötland: Hjälmsäter on Mt Kinnekulle (estimated to contain approximately 1750 shoots), Rosenlund’s Garden on the east side of Mt Billingen (estimated to contain over 500 shoots), and Blomberg also on Mt Kinnekulle, estimated to contain some 120–200 shoots (Johansson 1998, Furustam 2007, pers. obs.). The ground in these localities is sloping, and the tree layer is mainly composed of _Fraxinus excelsior_ and _Ulmus glabra_, whereas the shrub layer is dominated by _Corylus avellana_ and young trees.

In 2007 we studied five populations in the northeastern parts of the province of Uppland: Mäsjön (~3000 shoots), Holmskaten by the Baltic Sea (~6000 shoots), Göknsäre (~3000 shoots), a locality on the island of Raggön with ~100 shoots, and finally a locality called Rönnfjärden containing ~100 shoots (numbers reported between 2004 and 2006 by E. Zachrisson and L.-T. Nordin to the observation database maintained by ‘The County Administrative Board of Uppsala’). In contrast to the study sites in Västergötland, the tree layer at these five localities in Uppland is dominated
by *Picea abies*, growing on calcareous soils with a rich herbaceous flora. Apart from *Picea abies*, other species form the tree layer locally, most notably *Pinus sylvestris* and deciduous trees such as *Betula pendula* and *Populus tremula*. In Raggarön *Picea abies* is mixed with *Acer platanoides*, *Populus tremula* and *Quercus robur*. This locality, as well as Holmskaten, is abundant in *Corylus avellana* in the bush layer, while *Frangula alnus* is common at Måsjön. The localities studied are summarized in Table 1 and outlined in Fig. 2.

**Insect capture**

We intended to compare our results with earlier works, and therefore used the same capture methods as those adopted by Erneberg and Holm (1999). The sites were visited during sunny days, when pollinators have been shown to be most active (Nilsson 1979, Erneberg and Holm 1999). When insects entered the labellum of a particular flower, we held a transparent plastic bag covering it, and the behaviour of the insect was noted until it flew out again. The insect was then put down with ethyl acetate and stored individually for later identification. To minimize negative effects on the seed set of these populations and standardize the collection efforts, we opted to limit our collecting within the same population.

**Identification of pollinators**

Three operational groups of insects were defined a priori: 1) non-pollinators: insects leaving the labellum through the central opening or those leaving through the posterior opening but clearly too small to come into contact with the androecium; 2) potential pollinators: insects leaving through the posterior openings that were observed to come in contact with the androecium, but not demonstrated to carry *Cypripedium* pollen, or those leaving through the central opening but carrying *Cypripedium* pollen; and 3) effective pollinators or pollen vectors: insects leaving through the posterior openings, touching the androecium and demonstrated to carry *Cypripedium* pollen.

**Table 1. Swedish localities of *Cypripedium calceolus* studied here (see text for references).**

<table>
<thead>
<tr>
<th>Province</th>
<th>Locality</th>
<th>No. of shoots</th>
<th>Coding in Fig. 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uppland</td>
<td>Rönntjärn</td>
<td>ca 100</td>
<td>A-I</td>
</tr>
<tr>
<td>Uppland</td>
<td>Göksnäre</td>
<td>ca 3000</td>
<td>A-II</td>
</tr>
<tr>
<td>Uppland</td>
<td>Holmskaten</td>
<td>ca 6000</td>
<td>A-III</td>
</tr>
<tr>
<td>Uppland</td>
<td>Raggårön</td>
<td>ca 100</td>
<td>A-IV</td>
</tr>
<tr>
<td>Uppland</td>
<td>Måsjön</td>
<td>ca 3000</td>
<td>A-V</td>
</tr>
<tr>
<td>Västergötland</td>
<td>Blomberg</td>
<td>&gt; 120</td>
<td>B-II</td>
</tr>
<tr>
<td>Västergötland</td>
<td>Rosenlund</td>
<td>&gt; 500</td>
<td>B-III</td>
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The pollinator fauna of mainland Sweden in relation to other regions

Results and discussion

In Västergötland, a total of 23 specimens from Hjärna and Rosenlund were collected between the 4 and 5 June 2003. Three of these were deposited in the Natural History Museum of Gothenburg and the other 20 in the Swedish Species Information Centre. The pollinator fauna of mainland Sweden in relation to other regions

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(Ishmuratova et al. 2006), this is their first report as pollen vectors in Scandinavia, where no other species of *Colletes* have been reported to pollinate *Cypripedium*.

Females of *A. haemorrhoa* were the most frequent pollen vectors of *C. calceolus* in our study, with six specimens positively identified as pollen vectors (Table 2). *Andrena haemorrhoa* was then followed by *A. nigroaenea* and *A. helvola*, each with four or more specimens positively identified as pollen vectors. It is worth noting, however, that these species were not necessarily the most abundant ones: for instance, *A. carantonica* was collected eleven times, but only one specimen in Västergötland and two in Uppland carried pollen. Females of *A. haemorrhoa* were also the dominant vectors in the Öland populations (Nilsson 1979), but apparently too large to serve as regular pollinators in the Danish populations where the only collected specimen was found dead in the posterior opening (Erneberg and Holm 1999). However, despite its absence from Uppland, the three studies indicate that this may be the only species that pollinates *Cypripedium* in mainland Sweden, Öland, Gotland and possibly Denmark (Table 3), even though with an apparently varying degree of importance.

Our study confirms earlier hypotheses of low pollination specificity in early diverging orchid lineages in general (Tremblay 1992), and in *C. calceolus* in particular (Nilsson 1979, Erneberg and Holm 1999). Other species of *Cypripedium*, however, appear to be more specialised in their pollination, e.g. *Cypripedium guttatum* being pollinated by a few species of *Lasioglossum* (Bänziger et al. 2005), *C. flavum* by two species of *Andrena*, and *C. yunnanense* by one species of *Lasioglossum* (Bänziger et al. 2008). If real, such specificity is puzzling as it is fairly unusual for food-deceptive plants of the non-mimicry type (Schiestl and Schlüter 2009). In *C. calceolus* it has been shown that the scent contains several compounds typical of insect pheromones (Nilsson 1979, Bergström et al. 1992), which may play a role in the deceptive pollination by attracting several closely related insect species.

Among the studied populations, the taxonomic composition of the pollinator fauna was fairly diverse, with each of the populations containing a different set of effective pollen vectors (Fig. 3). Such variation in the pollinator fauna among different sites could have several possible explanations (alone or in combination):

![Figure 3. Number of species identified as pollinators of *Cypripedium calceolus* in each of the main Scandinavian regions studied so far (details in Table 3). Numbers in intersectional areas denote species shared by two or more regions. No common species have been doubtlessly found in all regions.](image-url)
First, it could depend on the lack of some pollinators in some areas. The landscapes surrounding the localities of *C. calceolus*, the intrinsic characteristics of each locality, and the dispersal ability of each insect species may largely determine the pollinator fauna available. Species of *Andrena* often occur in forests and forest edges, while species of *Halictus* and *Lasioglossum* mainly occur in open landscapes. That may explain why in Uppland, where the localities are situated in areas dominated by coniferous forests, only *Andrena* species were identified as pollen vectors. In contrast, the localities in Denmark, Västergötland and Öland consist all of deciduous forests with open pastures nearby. Not surprisingly, species from *Lasioglossum*, *Halictus* and *Andrena* were among the pollinators at these sites.

Second, even if all insect species were present everywhere, in some regions they would be active while *Cypripedium* flowered, but in other regions their active period could come too early or too late in relation to the flowering period of *Cypripedium*.

Third, between-site variation in pollinator fauna could depend on pollinator selection by different flower sizes. Indeed, the variation in flower size among different populations of *C. calceolus* demonstrated in Denmark seems directly correlated to the finding that small bee species of *Halictus* and *Lasioglossum* were the dominating pollinators there (Erneberg and Holm 1999). This is in contrast with the results that the slightly larger bee species of *Andrena* were the most frequent pollen vectors in mainland Sweden, Öland and Gotland (Nilsson 1979).

Fourth, natural variations and/or insufficient sampling may have affected the overall results. For instance, the relatively few pollen vector species in Uppland could partly depend on the early and short flowering period of *Cypripedium* in 2007, triggered by warm weather with low precipitation in the late half of May to the early half of June. Insects that normally feed on early-flowering *Salix* pollen, such as certain species in the genera *Andrena* and *C. cunicularius*, were perhaps no longer active when the field work in Uppland began. Indeed, the sampled pollen vectors in Uppland belong to a group of solitary bees that gather pollen mainly from *Rosaceae* (Schmid-Egger and Scheuchl 1997). These species are active pollen collectors later than *Salix* collectors, from late spring to early summer. We found both early *Salix* pollen collectors and later *Rosaceae* and *Ericaceae* collectors in Västergötland, which could be explained by the average weather conditions and flowering period in 2003 (when field work took place in this province), as compared to 2007.

Another finding of this study concerns the deposition of pollen smear on the body of pollinators. Nilsson (1979) argued that the exact place for smear deposition depends mainly on the species, with all categories involving deposition on the dorsal part of the thorax (Fig. 6 in Nilsson 1979). In our study, however, *Cypripedium* pollen was also identified from smears on the abdomen, the middle and the posterior legs (specimens from Västergötland). This may be explained by the observation that effective pollen vectors seemed to encounter great difficulty in passing the posterior openings, being trapped by the column for several minutes. While setting themselves free, they turned around and pressed their bodies against the stamens several times and from various sides.

As many as 24 specimens from the family Syrphidae were collected. Specimens from two species (*Dasysyrphus venatus* and *Eristalis abutiva*) left through the posterior opening. Because no pollen was found on their body, and they did not seem to touch the androecium, we concluded they could not have acted as pollinators. Besides, these Syrphidae species have virtually no body hair, and could hardly function as effective pollen vectors. Species of Syrphidae have been reported from other pollination studies of *Cypripedium* (Nilsson 1979, Blinova 2002), and although they have never been demonstrated to act as pollinators this possibility cannot be excluded for all species (discussed by Blinova 2002).

**Implications for the conservation and management of *Cypripedium calceolus***

The survival of adult plants is most critical for preserving the remaining populations of *C. calceolus* in Europe. Considering the remarkable longevity of individual plants (60–100 years, Kull 1988) and other intrinsic features, extinction analyses have shown that for a population of about 20 individuals (characteristic of many European localities) there is no chance of survival if as few as two plants are removed at intervals of about five years, a level of aggression that is probably not atypical (Terschuren 1999). However, to make the conservation picture even worse, orchids are typically also limited in their reproductive success by pollination, and this is particularly true for deceptive species with often very low fruit set (Tremblay et al. 2005). In a long-term study with *Cypripedium acaule*, Gill (1989) showed that on average only 2% of the plants set fruit. This means that for the long-term survival of populations, the protection of pollinators and their habitats is also essential (Roberts 2003).

Pollinators require both suitable nesting sites and the occurrence of food sources. The various pollen preferences of the species discussed below have been gathered from Torp (1994), Schmid-Egger and Scheuchl (1997) and from personal observations. Most of the pollinators identified in this survey are thought to be generalists, in the sense that they visit a wide array of plant species in their search for food. To support generalist floral visitors, nearby meadows and pastures rich in flowering plants should be protected. However, three of the solitary bees found in Västergötland are probably more restricted in their pollen gatherings: *C. cunicularius* and *A. praecox* feeding on the genus *Salix*, and *Lasioglossum fraterellum* feeding on species of the family *Ericaceae* (Schmid-Egger and Scheuchl 1997). In Uppland, the pollinators identified are polylectic, but most of them prefer pollen from various trees and bushes in the family *Rosaceae*. One of the *Andrena* species found in Uppland also acted as a pollinator in Västergötland (Table 3). *Salix* species constitute an important food source for basically all pollinators, active early in the season. Similarly, *Rosaceae* species are an important food source for pollinators active later on. An optimal management of the areas with *C. calceolus* should therefore benefit plant species within both of these taxonomic groups, such as *Salix* spp., *Prunus spinosa* and *Crataegus* spp. Solitary bees have a radius of operation of around 500 m (Linkowski et al. 2004),
implying that the distance between these food source plants and the *Cypripedium* populations should remain within that limit. Many species in the genus *Salix* and in the families *Rosaceae* and *Ericaceae* occur in open or half-open habitats. A management favouring these plant groups should therefore include maintaining a canopy rich in light-gaps, well-developed forest edges, as well as maintaining or restoring existing pastures and meadows adjacent to the localities with *C. calceolus*. Suitable nesting sites for many *Andrena* and *Lasio lithoglossum* species is loose and open sand or soil with patchy, sparse vegetation (Linkowski et al. 2004). Nesting sites are typically found in sunny areas, outside the forest. Bees also preferentially visit sunny spots, and avoid dense forest.

Several studies have investigated the role of habitat loss, habitat quality, mycorrhizal associations and pollination on orchid conservation (Terschuren 1999, Shefferson et al. 2005, 2007, Janeckova, P. et al. 2006, Kull and Hutchings 2006, Brundrett 2007). For *C. calceolus*, one way to maintain suitable conditions for the pollinator fauna is to keep the forests half-open, and prevent the vegetation from becoming too dense in the nesting sites of the bees. In one Danish locality, this is done by regularly pruning trees (Erneberg and Holm 1999). Another way to achieve that condition is to create gaps in both tree- and scrub-layers by carefully and selectively cutting off trees and shrubs. Plant species that produce dense shadows should be dealt with in the first place. Many bee species, as well as a wide array of other organisms, are directly favoured by leaving dead wood on the forest floor whenever suitable.

**Conclusions**

At least 17 species of Hymenoptera are now known to pollinate Scandinavian populations of *C. calceolus*, including the four new species recorded in this study. Most of them belong to the bee genera *Andrena* and *Lasio lithoglossum*. In order to protect this orchid species, we clearly need to consider its ecological requirements as well as those of its pollinators. A heterogeneous forest is expected to benefit both: a half-open tree canopy allowing some light to reach the orchids, occurrences of *Salix* and Rosaceae shrubs nearby to feed the various pollinator species, and sun-exposed sandy soil to provide suitable places for insect nests.

*Acknowledgements* – We would like to thank Björn Cederberg at the Swedish Species Information Centre (Artdatabanken) for confirming the identification of the specimens collected; Robert Daun at Botaniska Analysgruppen, Göteborg for confirming our identification of pollen grains; Florian Schiestl and Jean Devillers-Terschuren for reading this manuscript and making several suggestions of improvement; Arne Anderberg at the Swedish Museum of Natural History and Marika Ström at Lantmäteriverket for allowing us to reproduce the underlying maps in Fig. 2; Lars-Thure Nordin and Hans Tibell for help during field work; Helena Persson and Erik LJungstrand for helpful discussions and advice; and finally the editor and two anonymous reviewers for many constructive comments. Part of this project, dealing with Uppland, was performed by KHIC as a BSc in Ecology and Biological Conservation at the University of Gothenburg. This project has received financial support from the Systematics Association and The County Administrative Board of Västra Götaland through the assistance by Ulf Wikander.

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