Moth floral visitors of the three rewarding *Platanthera* orchids revealed by interval photography with a digital camera

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We investigated the insect visitors of *Platanthera ussuriensis*, *Platanthera sachalinensis* and *Platanthera florentii*, which might contribute to pollination, using a digital camera with automatic interval photography. Monitoring was conducted for a total of five nights (45 hours), and during every night, at least one moth species visited. The total number of pictures taken and pictures taken of moths were 3607 and 152, respectively. We concluded, based on our findings, that Geometridae and Noctuidae moths probably pollinated *P. florentii* and *P. sachalinensis*, respectively, whereas no pollinia attachment or removal was observed in *P. ussuriensis* during the intensive visit of the Crambidae moth.

**Keywords:** interval photography; moth pollination; Orchidaceae; *Platanthera*; pollinator

**Introduction**

*Platanthera* is a genus of photosynthetic and mycoheterotrophic orchids within the tribe Orchideae and comprised of 150 species (Karasawa 2003). These species are found in a range of environments, from grasslands to forest understories. *Platanthera* has seemingly gone through an extreme diversification in both floral form and pollination systems (Hapeman and Inoue 1997). The flower ranges in colour from green and white to orange or purple, and may have elaborate dissections of the labellum and petals, lending the flowers a “fringed” appearance. All *Platanthera* species have a nectar spur on the lip, whose length may exceed 4 cm in some taxa, such as *Platanthera praeclara* (Summers 1996; Hapeman and Inoue 1997).

Although the majority of species are known to be pollinated by noctuid and pyralid moths, other insects, such as butterflies, beetles, bees, flies and mosquitoes, have also been recorded as the pollinators (Nilsson 1983; Inoue 1985; Hapeman and Inoue 1997). *Platanthera’s* wide variety of pollinators represents the majority of non-deceptive pollination types within the Orchidaceae family (van der Pijl and Dodson 1966). The diverse pollinators found in the genus *Platanthera* would provide solid examples of how orchid–pollinator interactions might influence orchid diversification, whereas the information on the floral visitors in many *Platanthera* have been poorly documented.

Direct observations of orchid pollination events are difficult because the proportion of flowers visited per flowering season is often low in orchid species (Neiland and...
Wilcock 1998; Tremblay et al. 2005; Suetsugu and Fukushima 2014 a,b). Both the long lifespans of individual flowers and the low population densities make direct observations time-consuming and difficult (Widmer et al. 2000). Here we investigated the insect visitors of three Japanese orchids (*Platanthera florentii*, *Platanthera ussuriensis* and *Platanthera sachalinensis*) that could contribute to pollination, using a digital camera with automatic interval photography to overcome the difficulty in observing floral visitors in the orchidaceous species.

**Material and methods**

In July and August of 2012, we investigated the floral visitors of nectariferous *P. florentii*, *P. ussuriensis* and *P. sachalinensis* in Hokkaido, the northern part of Japan, which is situated in a cool-temperate area (Table 1). In all species, individual flowers remain open for c. 6-10 days, unless the flowers are pollinated (Suetsugu, pers. obs.). All of the investigated populations were in deciduous broad-leaved forest dominated by *Acer mono* var. *glabrum*, *Quercus crispula*, *Fraxinus lanuginosa* and *Alnus hirsuta*, while some coniferous trees such as *Abies sachalinensis* also occasionally grew. Relative importance of daytime and night-time visitors for pollination was preliminarily evaluated by walking around the population and checking pollinia in the morning and evening. As a result, we observed no pollen removal during the day, but some pollinia removal occurred during the night. Narrow spurs with deeply concealed nectar and white coloration observed in all the investigated species may also correspond with nocturnal moth pollination syndrome. Therefore, we set up waterproof digital cameras (Optio WG-1, Pentax, Japan) at night to capture nocturnal floral visitors.

We fixed the hardwood stick on the ground and mounted the camera with the GorillaPod flexible tripod (Joby, San Francisco, CA, USA). We placed the camera in front of the chosen individual (c.30 cm away) so as to capture the entire inflorescence.

| Plant species            | Site       | Study date     | Time          | Number of flowers photographed | Individuals/ha |
|--------------------------|------------|----------------|---------------|-------------------------------|----------------|
| *Platanthera ussuriensis*| Yuhutsu    | 26–27/7/2012   | 20:30–04:43   | 12                            | 30             |
| *Platanthera sachalinensis* (Pop. 1)| Eniwa | 3–4/8/2012     | 20:00–05:36   | 14                            | 50             |
| *Platanthera sachalinensis* (Pop. 2)| Sapporo | 9–10/8/2012   | 20:00–05:38   | 9                             | 5              |
| *Platanthera florentii*  | Tomakomai  | 26–27/8/2012   | 20:00–05:05   | 8                             | 50             |
| *Platanthera florentii*  | Tomakomai  | 27–28/8/2012   | 20:00–04:43   | 12                            | 50             |
We manually focused on inflorescences because the automatic focus mode significantly shortened the photo opportunity. We set the cameras to automatically take pictures at 45-second intervals with an internal flash. By using batteries (D-LI92 Pentax, Japan), observations were recorded with a maximum of 9.5 hours determined by battery capability. The JPEG-format 7-MB images (3072 × 2304 pixels) were automatically recorded onto the 8-GB SDHC card. After each investigation, the existence of floral visitors was checked by visual inspection of each photograph on a PC monitor.

Results

A total of five nights (45 hours) of monitoring was conducted, and at least one moth species visited each night (Table 2). The total numbers of pictures taken and pictures taken of moths were 3607 and 152, respectively. The crambid moth species *Mabra charonialis*, observed in *P. ussuriensis*, may not work as a pollinator, because in spite of an intensive visit across the entire inflorescence to forage for nectar (two visits; 132 pictures), there was no pollinia attachment or removal observed. Attachment of *P. sachalinensis* pollinaria on the proboscis of Noctuidae sp. (Eniwa population) and *Polychrysa splendida* (Noctuidae; one visit; one picture, Sapporo population) was observed. In addition, in the Sapporo population, *Paratalanta* sp. (Crambidae) foraging nectar was also observed in *P. sachalinensis* (one visit; one picture). Attachment of *P. florentii* pollinaria to the eyes of *Lampropteryx* sp. (Geometridae) was witnessed, (one visit; three pictures). Furthermore, *Paratalanta* sp. (Crambidae) and Scopariinae sp. (Crambidae) were captured on the *P. florentii* inflorescence, but there was no pollinia attachment or removal observed (Figure 1).

Discussion

It is time-consuming to describe species interactions, particularly regarding those occurring at night and any that are rare (Steen 2012). Direct pollinator observations of orchids with low visitation rates in the wild are even more demanding of time

| Plant species     | Insect species        | Frames captured | Times visited | Pollinia attached | Visting time          |
|-------------------|-----------------------|-----------------|---------------|-------------------|-----------------------|
| *P. ussuriensis*  | *Mabra charonialis*   | 132             | 2             | No                | 21:00–21:31 22:47–23:56 |
| *P. sachalinensis*| Noctuidae sp.         | 1               | 1             | Yes               | 3:27                  |
|                   | *Polychrysa splendida*| 1               | 1             | Yes               | 3:59                  |
|                   | *Paratalanta* sp.     | 1               | 1             | No                | 3:59                  |
| *P. florentii*    | *Lampropteryx* sp.    | 3               | 1             | Yes               | 20:37–20:39 |
|                   | *Paratalanta* sp.     | 3               | 1             | No                | 21:19–21:21 |
|                   | Scopariinae sp.       | 11              | 1             | No                | 3:18–3:25  |
(Steen 2012). To alleviate the paucity of pollinator information, a molecular approach has been conducted for orchid–pollinator relationships based on the analysis of DNA recovered from pollinaria found on insects collected from other flowering plants, light traps or taken from museum collections (Widmer et al. 2000; Cozzolino et al. 2005; Nakase and Kato 2012). Recent studies have also used a video-monitoring system to obtain the data on orchidaceous floral visitors (e.g. Micheneau et al. 2008, 2010; Steen and Aase 2011; Steen 2012; Steen and Mundal 2013). In contrast, interval photography has rarely incorporated methods to investigate floral visitors of orchids (Suetsugu and Tanaka 2013, 2014). This study successfully demonstrated that moths visited all three Platanthera species and confirmed the effectiveness of an interval photography technique (Table 2).

We observed P. sachalinensis pollinaria attachment on the proboscis of Noctuidae spp. in two investigated populations separated by approximately 30 km. Inoue (1983) investigated the pollinators of P. sachalinensis in Niigata Prefecture, Japan, which is about 600 km away from the current study area, and also observed attachment of its pollinaria to the proboscis of Noctuidae spp. Considering that P. sachalinensis are pollinated by Noctuidae spp. in distant habitats, it is highly possible that P. sachalinensis uses the Noctuidae spp. as the main pollinators.

Platanthera florentii pollinia attachment to the eyes of Lampropteryx sp (Geometridae) was also observed. This was the first report of probable pollinators in P. florentii. Platanthera is one of several genera in which eye transfer of pollinia
occurs (Inoue 1983; Nilsson 1983; Hapeman and Inoue 1997). Within *Platanthera*, a floral construction that places the pollinaria on the eyes of the pollinator is a derived character state and has arisen from tongue-attachment (Nilsson 1983; Maad and Nilsson 2004). Attachment of the pollinia onto the eyes of *Lampropteryx* sp. was observed once (Table 2), so we cannot conclude that eye transfer of pollinia is the main pollination system of *P. florentii*. However, more detailed observation of *P. florentii* would help us to understand the complexity and diversity of the *Platanthera* pollination system.

Overall, our study suggested that *P. florentii* and *P. sachalinensis* would be likely to use Geometridae and Nocturidae moths as pollinators, respectively. It should be noted that pollinia attachment may have occurred before visiting the orchid that was being monitored. Hence, a single image of pollinia attachment in the present study is not true evidence of successful pollinia transfer from the orchid species being monitored. Importantly, the pollinia may have its origin from other orchid species. However, we considered the possibility to be very low because captured pollinia morphology seems identical to investigated species and there are no co-flowering orchidaceous species with similar floral architecture that can potentially deposit pollinia in the investigated populations. However, the limited amount of observation does not allow for a confirmation with regards to whether other species of moths also visit these *Platanthera* species. Interactions between orchids and pollinators are not always species-specific, and some nectariferous and food-deceptive orchids use diverse, locally available pollinators (Cozzolino and Widmer 2005). For example, some floral visitors may be quick in their approach and resource collection, and so may have been missed with a frame rate interval of 45 seconds. In particular, the system may have underestimated hawkmoths (Sphingidae), as they are known to just hover in front of the inflorescence and their visit is quite fast (Steen 2012).

Overall, interval photography would be useful to register and identify floral visitors. Our study system had some advantages in terms of cost and compactness compared with video monitoring, as the whole cost per unit (a digital camera and a flexible tripod, made with wood sticks easily found in the field) was less than US$ 200. In addition, digital cameras with flexible tripods can be attachable in almost any environment, making lithophytic and epiphytic orchid observation easy. Furthermore, our study system was light (c.250 g with a digital camera and a tripod) compared with video monitoring, and so would be ideal for investigations in roadless, deep mountain areas and in large-scale community level analyses. However, interval photography with equipped batteries has some disadvantages. For example, there are limitations associated with the batteries lasting for only 9.5 hours. According to the specification stated in Steen and Aase (2011), when using a battery with 80 Ah (20 kg), event-triggered video will last for 8–12 days. Similar external power supply will be needed to increase monitoring time. In addition, interval photography may underestimate some groups of visitors, and it is not capable of registering precise flower visiting rates and detailed behaviour. It is worth testing the interval frequency and species assemblage of captured floral visitors using differential time-interval photography (e.g. 10 seconds, 30 seconds, 1 minute and 2 minutes) and video (continuous monitoring and event-triggered movie) for future study.
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