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Potential pollinator of *Vanda falcata* (Orchidaceae): *Theretra* (Lepidoptera: Sphingidae) hawkmoths are visitors of long spurred orchid

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Abstract. *Vanda falcata* is a species of orchid native to China, Korea and Japan. While it is arguably one of the most celebrated orchids in Japan there is no information on its pollinators. Although most species of the subtribe Aeridinae, to which *V. falcata* belongs, have a short spur, *V. falcata* has a long spur. The results of the current study provide strong evidence that *V. falcata* is pollinated by long-tongued hawkmoths (*Theretra* spp.), which indicates that the evolution of long spurs in *V. falcata* could be an adaptation to pollination by long-tongued moths.

INTRODUCTION

The unique morphology of orchid flowers has always been a source of fascination and their floral diversity has inspired many biologists since the 19th Century, including Charles Darwin (Darwin, 1862). From a cultural point of view, the aesthetic beauty and spectacular floral diversity of orchids has long been appreciated and Eastern Asian countries, such as Korea, China and Japan, have a long history of cultivating these plants. The earliest record of orchid cultivation in China dates back to 500 BCE, the time of Confucius (Cribb, 1997).

One species with a long history of cultivation is *Vanda falcata* (Thunb.) Beer, records of which date to as early as 1665 (Reinikka, 1995). The 11th shogun (ruler of Japan), Ienari Tokugawa, greatly admired this species and as a result Japanese feudal lords tried to find interesting wild specimens to present to him in order to obtain favours (Reinikka, 1995). During this period there were frequently exhibitions of *V. falcata* collections, and prized specimens were placed on expensive stands and wrapped in silver or gold nets. As a consequence, *V. falcata* is often referred to as the "Fuukiran," which means "the orchid of wealth and nobility" (Duttke et al., 2012). Although *V. falcata* is arguably one of the most esteemed orchids in Japan over-collection has resulted in only a few individuals remaining in the high canopies of trees in their natural habitat. The probability of this species becoming extinct within the next 100 years is estimated to be 98% (Environment Agency of Japan, 2000), and *Vanda falcata* is now listed as a vulnerable species in Japan (Environment Agency of Japan, 2000). The successful propagation of *V. falcata* from seed for horticultural purposes has therefore been the subject of intense study (Chung, 1979; Ichihashi & Islam, 1999; Islam & Ichihashi, 1999). However, detailed information on its life history and reproduction in its natural habitat, including the identity of typical pollinators, is still poorly understood. Such information could be crucial for the success of future conservation efforts.

While *V. falcata* has been treated as *Neofinetia falcata*, recent molecular phylogenetic analyses (e.g. Topik et al., 2005; Kocyan et al., 2008) indicate that *Neofinetia* should be included in the genus *Vanda* to preserve monophyly (Gardiner, 2012). The genus *Vanda* is comprised of approximately 70 epiphytic or lithophytic species and is widely distributed throughout East Asia in China, Korea and Japan, and in Southeast Asia, from India and Nepal southwards through the Philippines and Indonesia, all the way to northern Australia and the Solomon Islands (Govaerts, 2012; Gardiner et al., 2013). The genus is globally one of the five most important horticultural orchid genera, exhibiting a great diversity in colour and floral shape, particularly the structure of its labellum (Gardiner et al., 2013). Many *Vanda* species are characterized by having large, showy, colorful flowers, often with open, spreading tepals that attract bees and butterflies, at least in cultivated situations (Gardiner et al., 2013). Consequently, it has been concluded that typical *Vanda* spp. are pollinated by bees (Holttum, 1953, but also see Gardiner et al., 2013).

However, despite a lack of direct evidence, it is suggested that the presence in *V. falcata* of nectar, white floral colouration and long spurs (ca. 3–5 cm) are adaptations consistent with pollination by moths (Gardiner et al., 2013; Topik et al., 2005). In addition, the afternoon and evening emission of its floral scent, the constituents of which include methyl benzoate and cis-3-hexenyl tiglate, support the idea of nocturnal pollination of this species by moths (Kaiser, 1993; Ono & Miyazawa, 1999; Taneda et al., 2009). The current study was initiated to test this hypothesis by observing the nocturnal floral visitors of *V. falcata* by interval photography using a digital camera.

MATERIAL AND METHODS

We mainly used a waterproof digital camera's interval-programming function (Optio WG-1, Pentax, Japan) because pollination events in orchids are very rare (Neiland & Wilcock, 1995) and recent studies confirmed the effectiveness of this technique as a substitute for direct observation (Suetsugu & Tanaka, 2013a, b; Suetsugu & Tanaka, 2014). We investigated the floral visitors of *V. falcata* at one site in Tsukaba City, Ibaraki Prefecture and two sites in Kitakyushu City, Fukuoka Prefecture, Japan. The Tsukuba site was cultivated plants in Tsukuba Botanical Garden. The two Kitakyushu populations were located in a broad-leaved evergreen forest dominated by *Machilus thunbergii* and *Castanopsis sieboldii*. Preliminary investigations revealed that pollen removal oc-

Fig. 1. Flowers and a potential pollinator of *V. falcata*. A – flowering plants exhibiting elongated spurs; B – *Theretra japonica* with pollinaria (indicated by arrow) attached to its proboscis.

curs mainly at night, so we set the cameras mainly at night to investigate the insect visitors that might pollinate these plants.

We mounted the camera on a flexible tripod (gorillapod, Joby, San Francisco) and placed the camera in front of an individual plant (ca. 30 cm away) so the entire inflorescence (4–28 open flowers) was in focus. Because the automatic focus mode significantly shortens the photo opportunity, we manually focused the camera on the inflorescences. We set our camera to automatically take pictures at 120-second intervals (Tsukuba) in mid-July 2010 or 10- to 20-second (Kitakyushu) intervals in early to mid-July 2012–2013, with an internal flash and batteries (D-LI92 Pentax, Japan; Appendix 1). The PEG-format 7 MG images were automatically recorded onto an 8 GB SD card. After each investigation, the existence of floral visitors was checked by visual inspection of each photograph on a PC monitor. Pollinators were defined as those species that not only visited flowers, but also carried pollinaria.

RESULTS AND DISCUSSION

The photographic evidence obtained in the current study indicated a great variety of insect species, including members of the Araneae, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera and Neuroptera, visited *V. falcata.* However, most did not appear to exhibit typical pollinator behaviour, merely alighting or resting on the flowers or piercing the plant tissues and sucking out the juices (Table 1). Although the spider *Clubiona* sp. and moth *Mabra charonialis* showed nectar-seeking behaviour as with the similar reports on relationship between *Clubiona* spider and nectariferous orchid *Neottianthe* (Suetsugu et al., 2014) and *Mabra charonialis* and nectarferious orchid *Platanthera* (Suetsugu &

Table 1. List of floral visitors recorded using interval photography. Numbers given are the total numbers of frames recording visitors and times visited. The same species occurring in consecutive frames were counted as one visit.

No.	Photograhed species	Order	Frames with visitors captured	Times visited	Pollinia attached	Visiting time
1	*Clubiona* sp.	Araneae	1	1	No	23:54
	Drepanopteryx phalaenoides	Neuroptera	8	2	No	1:29–1:41, 2:33
2	*Drepanopteryx phalaenoides*	Neuroptera	1	1	No	20:03
3	*Ceresium* sp.	Coleoptera	1	1	No	20:54
4	*Theretra nessus*	Lepidoptera	1	1	Yes	19:27
5	*Halyomorpha halys*	Hemiptera	6	1	No	22:58–23:00
6	*Ceresium* sp.	Coleoptera	52	1	No	01:07–01:24
10	*Theretra japonica*	Lepidoptera	1	1	Yes	20:06
12	Mordellidae sp.	Coleoptera	20	1*	No	23:27–23:54*
13	*Polistes* sp.	Hymenoptera	2	1	No	09:53–09:54
17	*Mabra charonialis*	Lepidoptera	221	1	No	22:45–23:52
18	*Mabra charonialis*	Lepidoptera	36	1*	No	00:39–00:52*
20	*Geisha distinctissima*	Hemiptera	34	1	No	20:47–20:55
23	*Mabra charonialis*	Lepidoptera	4	1	No	21:29–21:30

Observations at which no floral visitor was recorded are not shown. *We observed these floral visitors intermittently at very short intervals and thus considered it to be the same visitor.

Hayamizu, 2014), respectively. However, they were unable to carry the pollinaria because they were too small, and it was therefore assumed that they were not true pollinators of *V. falcata*.

The hawkmoths *Theretra nessus* and *Theretra japonica* also inserted their proboscises into the nectar spur. While some hawkmoths, such as pollinators of the nectariferous orchid *Habenaria epipactidea,* use their forelegs to grab the flowers to stabilize themselves while feeding (Peter et al., 2009), the hawkmoths visiting *V. falcata* did not use their forelegs in this way (Fig. 1). Instead, they hovered over the flowers and extended their proboscises to forage for nectar, behaviour that has also previously been reported for other hawkmoths such as *Hyloicus pinastri*, which is a pollinator of rewarding *Platanthera chlorantha* (Steen, 2012). In contrast to *V. falcata* other floral visitors, the hawkmoths *T. nessus* and *T. japonica* visiting *V. falcata* were observed with pollinaria attached to their proboscises (Fig. 1; Table 1). It should be noted that we could not be absolutely certain how the pollinaria became attached to their proboscis and from where they originated because each hawkmoth was not photographed consecutively (Table 1). However, we considered the possibility that the pollinaria were originated from other species to be very minor, given that the morphology of the pollinaria appeared identical to those of *V. falcata*, and that there were no other flowering species of orchid with a similar floral architecture at the localities studied. In addition, although the fruit set was not scored precisely, several of the flowers investigated at both the Tsukuba and Kitakyushu sites had developed fruit when the sites were revisited at the end of the fruiting season. Taken together, these results provide strong evidence that hawkmoths do pollinate *V. falcata* in the areas investigated.

Although the Tsukuba population is an artificial one, it is located just 50 km away from a natural habitat in Kashima and *T. nessus* also occurs in the natural habitats of *N. falcata.* The observation of pollinaria attached to the proboscis of *Theretra* spp. in two *V. falcata* populations separated by approximately 1,000 km, provides further evidence that *V. falcata* utilizes *Theretra* spp. as pollinators. During the current investigation, the *Theretra* hawkmoths were recorded visiting both the Tsukuba and Kitakyushu populations of *V. falcata* just after sunset (19:27 and 20:06, respectively), the peak period of floral fragrance emission (Taneda et al., 2009), which is likely to be another adaptation facilitating pollination by hawkmoths.

The subtribe Aeridinae, to which the genus *Vanda* belongs, is a large and diverse group of orchids with approximately 1,350 species in as many as 100 genera (Topik et al., 2005). Although the pollination strategy of the Aeridinae is poorly understood, it could be an important factor accounting for the morphological and evolutionary diversification of this group (Topik et al., 2005; Kocyan et al., 2008; Stpiczyńska et al., 2011). Orchids belonging to the Aeridinae appear to employ a wide range of pollination strategies and a diverse spectrum of pollinators, including beetles (van der Cingel, 2001; Pedersen et al., 2013), carpenter bees (Xylocopinae; Carr, 1928; Dressler, 1993; van der Cingel, 2001; Kocyan et al., 2008; Suetsugu & Tanaka, 2013b), bumblebees (Suetsugu & Tanaka, 2013b), stingless bees (van der Cingel, 2001) and birds (Dressler, 1993; van der Cingel, 2001). However, despite this range of pollination strategies, to date, there have been no reports of Lepidopteran pollinators. The discovery that hawkmoths visiting *V. falcata* have pollinaria attached to their proboscises is therefore the first example of this pollination strategy in any Aeridinae species. A recent molecular phylogenetic analysis of the *Vanda* alliance has shown that *V. falcata* may belong to one of the earliest diverging lineages in the genus *Vanda* sensu lato, which forms a sister group with the genus *Holcoglossum* (Gardiner et al., 2013) or *Papilionanthe* (Zhang et al., 2013). Most of *Holcoglossum* and *Papilionanthe* have whitish flowers and relatively long spurs (ca. 1–1.5 cm; Topik et al., 2005; Kocyan et al., 2008; Xiang et al., 2012), which may be pre-adaptations to pollination by moths.

Flower spurs in particular are of great adaptive significance, increasing the efficiency of pollination in species that target long-tongued insect pollinators (e.g., Anderson & Johnson, 2009; Sletvold & Ågren, 2010). Our demonstration of the pollination of *V. falcata* by hawkmoths confirms that the evolution of long spurs in the subtribe of Aeridinae could be an adaptation to pollination by moths and it might be useful to extrapolate the present findings to the pollinators of other species with a similar morphology. Because *Amesiella*, which also belongs to the Aeridinae, also has white flowers with long spurs, it is worth investigating its pollinators in a future study. *Vanda falcata* also shows intraspecific variation in morphology, such as spur length and floral coloration (Duttke et al., 2012). In addition, its closest relatives, *V. richardsiana and V. xichangensis,* have a much shorter spur length (i.e. 1–1.6 cm; Gardiner, 2012). More detailed investigations of the relationships between pollinators and floral characters in these three species could greatly enhance our understanding of the evolution of pollination strategies in the subtribe Aeridinae.

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Appendix 1. Details of time-lapse photography: dates and when recorded, number of observed flowers and time interval.

No.	Study site	Date	Time	Number of flowers	Time interval (s)
1	Tsukuba	2010/7/14	16:25 ~ 10:27	4	120
2	Tsukuba	2010/7/15	18:23 ~ 09:27	5	120
3	Tsukuba	2010/7/17	17:06 ~ 13:18	11	120
4	Tsukuba	2010/7/18	17:55 ~ 04:33	15	120
5	Kitakyusyu A	2012/7/12	20:49 ~ 23:59	9	20
6	Kitakyusyu A	2012/7/13	0:22 ~ 04:53	8	20
7	Kitakyusyu A	2012/7/13	23:33 ~ 00:28	14	20
8	Kitakyusyu A	2012/7/13	0:00 ~ 02:28	10	20
9	Kitakyusyu A	2012/7/13	20:19 ~ 0:27	4	20
10	Kitakyusyu A	2012/7/14	19:38 ~ 01:11	11	20
11	Kitakyusyu A	2012/7/14	22:31 ~ 02:42	11	20
12	Kitakyusyu A	2012/7/15	20:10 ~ 00:17	5	20
13	Kitakyusyu A	2012/7/16	00:18 ~ 10:37	5	20
14	Kitakyusyu A	2012/7/18	03:02 ~ 06:26	8	20
15	Kitakyusyu A	2012/7/18	20:00 ~ 21:48	5	20
16	Kitakyusyu A	2012/7/20	19:54 ~ 22:29	2	10
17	Kitakyusyu A	2013/7/6	22:45 ~ 23:52	8	15
18	Kitakyusyu A	2013/7/7	00:39 ~ 00:52	8	15
19	Kitakyusyu A	2013/7/8	03:50 ~ 06:36	7	15
20	Kitakyusyu A	2013/7/8	20:46 ~ 20:55	7	15
21	Kitakyusyu B	2013/7/11	19:59 ~ 22:09	8	20
22	Kitakyusyu B	2013/7/11	20:03 ~ 00:20	11	20

Appendix 1 (continued).

No.	Study site	Date	Time	Number of flowers	Time interval (s)
23	Kitakyusyu B	2013/7/12	19:53 ~ 22:34	17	20
24	Kitakyusyu B	2013/7/12	00:00 ~ 00:51	10	20
25	Kitakyusyu B	2013/7/12	19:47 ~ 00:39	16	20
26	Kitakyusyu A	2013/7/14	06:56 ~ 09:48	13	10
27	Kitakyusyu A	2013/7/14	20:28 ~ 23:23	14	15
28	Kitakyusyu A	2013/7/14	20:55 ~ 21:18	25	20
29	Kitakyusyu A	2013/7/16	19:42 ~ 20:03	28	15
30	Kitakyusyu A	2013/7/16	19:48 ~ 19:54	23	20
31	Kitakyusyu A	2013/7/20	23:31 ~ 03:46	10	20