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Diurnal hawk moth pollination of *Melampyrum koreanum* (Orobanchaceae) and the origin of this endemic Korean species

SANGJIN JO, DONG-KEUN YI & KI-JOONG KIM*
Division of Life Sciences, Korea University, Seoul 136-701, Korea

Abstract

Melampyrum koreanum (Orobanchaceae) was described recently as an endemic species in Korea. This study examined the distribution range, relationship with the host plant, flower developmental pattern, pollinator, pollination mechanism, and seed development of *M. koreanum* (Orobanchaceae) on the island of Somaemul-do, Korea. *M. koreanum* is hemiparasitic and a strong candidate for its host plant is *Pinus thunbergii,* with which it is distributed sympatrically. The flower of *M. koreanum* represents a typical hawk moth pollination syndrome. *Macroglossum pyrrhostictum* Butler (Sphingidae, Lepidoptera), a diurnal hawk moth, is the only efficient pollinator. The proboscis length of *M. pyrrhostictum* and the floral tube length of *M. koreanum* are well coordinated, and the positions of the anther and stigma of *M. koreanum* are mechanically conformed to hawk moth pollination. The results suggest that adaptation to the pollinator is the main factor that has affected the evolution of the longer floral tube of *M. koreanum* compare to other species. Pollinator conservation in the native range of *M. koreanum* is an important issue for the conservation of endemic plant species, and it is necessary to establish a conservation plan for the host plant, *Pinus thunbergii,* as well as *M. koreanum.*

Key words: hawk moth pollination syndrome, hemiparasite, coevolution, conservation of endemic species

Introduction

Melampyrum koreanum Kim and Yun (2012: 48) is a species recorded on Somaemul-do, Hansan-myeon, Tongyeong-si, Gyeongsangnam-do, which is a small island in the South Sea of Korea (Kim & Yun 2012). This species has the longest floral tube and pistil (about 30 mm in length) in the genus *Melampyrum* Linnaeus (1753: 605) (Orobanchaceae). In addition, the species also has taller and more branched stems, fewer spines on the bract, and larger fruit, which can easily be distinguished from the closely related species, *Melampyrum roseum* Maximowicz (1859: 210) complex. In particular, the length and the shape of the corolla and the length of the pistil easily distinguish *M. koreanum* from other *Melampyrum* species. Most *Melampyrum* species, including three previously recognized Korean species, have floral lips with wide entries and short floral tube lengths (<15 mm), which allow effective pollination by wasps or bees, whereas it has been reported a hawk moth may be the pollinator of *M. koreanum* because its corolla is thin and long (Kim & Yun 2012).

Hawk moths are typically nocturnal and flowers pollinated by hawk moths normally bloom at night where the flowers are white or light pink. These flowers have a long and thin corolla, which secretes nectar and releases a scent (Grant 1983). Well-known hawk moth pollination systems include *Manduca quinquemaculata* (Sphingidae) on *Mirabilis longiflora* Linnaeus (1755: 176) (Nyctaginaceae) flowers, which has a long floral tube, in the southeast area of the US (Grant & Grant 1983), and *Sphinx* and *Eumorpha* (Sphingidae) pollination of the long-spurred *Aquilegia chrysantha* Gray (1873: 1335) (Ranunculaceae) in the southeast area of the US (Miller 1985). Species belonging to Solanaceae and Martyniaceae have been reported as feeding plants for hawk moth caterpillars in these areas (Mechaber & Hildebrand 2000). *Aerangis* Reichenbach (1865: 190), an African long spurred orchid (Orchidaceae), is pollinated by a hawk moth (Maritns & Johnson 2007) and several species of *Zaluzianskya* Schmidt (1793: 11) (Scrophulariaceae, *s.l.*) have also been reported to be pollinated by hawk moths (Johnson *et al.* 2002). These previous reports were focused on nocturnal hawk moth pollination.

There are 52 species of hawk moths (Sphingidae) belonging to 34 genera in Korea (Park 2012, Shin 2007). Among these, the five species of *Macroglossum* (Sphingidae) are diurnal. The flowers of *M. koreanum* open in the early morning, so the pollinating hawk moths should be diurnal rather than nocturnal. Among the Scrophulariaceae

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and Orobanchaceae, it appears that *M. koreanum* is the only flower with a hawk moth syndrome in Korea. Therefore, this study aimed to analyze the pollination behaviors of diurnal hawk moths and the evolution of *M. koreanum* flowers. Measures to conserve *M. koreanum* are also presented with the findings of this study.

Materials and Methods

This study was conducted on Somaemul-do, Hansan-myeon, Tongyeong-si, Gyeongsangnam-do (N 34° 36-41', E 128° 31-34'), which is the native range of *M. koreanum*, between September and October in 2012 and 2013. The numbers of individuals in each *M. koreanum* population were counted. The dominant species and the frequency of each plant species were also recorded in the associated vegetation within a 3 m radius of each *M. koreanum* population to identify the possible host plant(s) of the hemiparasitic *M. koreanum.* Figure 1 shows the location of the study and the population distribution in Somaemul-do. To characterize the development of the flower and the nectar secretion patterns, 100 flowers in population 5 were observed and measured, and 100 flowers from all nine populations were tagged to calculate the seed formation rate. The number of flowers visited by one hawk moth during 10 min was recorded in populations 3, 5 and 9, and the counts were repeated three times in each population.

Two pollinating hawk moths and other insects present around *M. koreanum* flowers were collected and prepared as specimens, and the species were identified by an entomologist at the School of Life Sciences, Korea University. In addition, the lengths of the body, wing, and proboscis were measured to analyze their correlations with the lengths of the floral tube and pistil. Token samples of insects and plants collected during this study are housed in the Insect Museum of Korea University and Korea University Herbarium (KUS), respectively. In addition, the leaf samples used for DNA analysis are housed in Plant DNA Bank of Korea (PDBK, Acc. no. 2012-1610~1619 and PDBK 2013-1616, 1623).

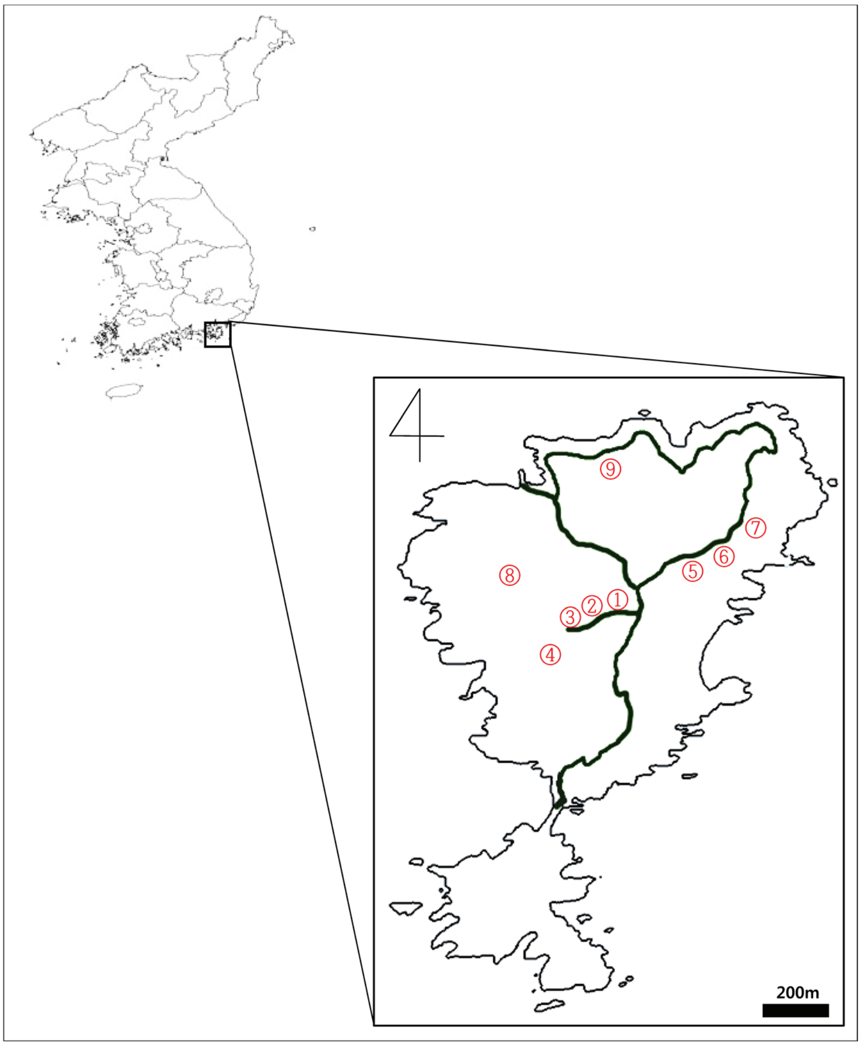

FIGURE 1. The location of Somaemul-do (insert), Hansan-myeon, Tongyeong-si, Gyeongsangnam-do, Korea. The nine studied *M. koreanum* populations are indicated by the red circles.

Results and Discussion

Host species and the number of individuals

All *Melampyrum* species are known to be hemiparasitic. To determine the host plant(s), all nine populations of *M. koreanum* were subjected to vegetation analysis. The plant species within a 3 m radius of the clumped distribution of *M. koreanum* were recorded and the dominant species were determined based on the vegetation coverage (Table 1). The only species present in all nine populations with *M. koreanum* was *Pinus thunbergii* Parlatore (in de Candolle 1868: 388). However, *Ligustrum japonicum* Thunberg (1780: 207) was present in eight populations, *Camellia japonica* Linnaeus (1753: 698) in six populations. *Eurya japonica* Thunberg (1783: 68) and *Miscanthus sinensis* Anderson (1855: 166) were recorded in five populations. The frequency of these plants co-occurring was: *Pinus thunbergii* > *Ligustrum japonicum* > *Camellia japonica* > *Eurya japonica/Miscanthus sinensis.* Thus, *Pinus thunbergii* is considered to be the most likely host plant. Around the Somaemul-do area, *Pinus thunbergii* is the dominant plant species. In addition, *Camellia japonica, Ligustrum japonicum, Eurya japonica*, and *Castanopsis sieboldii* (Makino 1910: 232) Hatusima (1971: 223) are locally dominant on some seaside slopes of the island, whereas *Carpinus turczaninovii* Hance (1868: 203) is common in the summit area of island. However, *M. koreanum* has difficulty surviving in shady forests and it is distributed at the margins of *Pinus thunbergii* forest. The general habitat of *M. koreanum* was partially open sunny clearings or the forest trail areas near the *P. thunbergii* forest. Therefore, the sunny spots around *Pinus thunbergii* forest are considered to be the optimum habitat for *M. koreanum* in Somaemul-do (Fig. 2A).

TABLE 1. Population size, pollinator and seed sets of *M. koreanum* in Somaemul-do.

	No. of Individuals	No. of observed hawk moth(30min)	Seed sets per 100 flowers
Population 1	51	1	25
Population 2	64	2	27
Population 3	107	3	38
Population 4	22	no	26
Population 5	70	2	33
Population 6	257	2	30
Population 7	21	no	25
Population 8	125	3	27
Population 9	28	2	20
Total	745	15	251

In total, nine *M. koreanum* populations were found on Somaemul-do. The numbers of individuals from each population ranged from 21 to 257 (Table 2). In total, 745 individuals were recorded from the nine populations. In addition, a few individuals were scattered throughout the open area of Somaemul-do. Therefore, approximately 800 *M. koreanum* individuals were counted on Somaemul-do. The size of each individual varied among the habitats from small (30 cm) with a few branches to tall (up to 80 cm) with many branches (Fig. 2B). Therefore, the number of individuals counted was based on the main stems. Populations 1–3 and 5–7 were located close to each other, so the same hawk moths may have visited flowers from each of these populations. Populations 1–7 were examined twice in 2012 and 2013, whereas populations 8 and 9 were added to the study in 2013. In addition to Somaemul-do, an authority at Hallyeo Marine National Park also reported that small numbers of *M. koreanum* were distributed on Maemul-do, which is an island close to Somaemul-do (B.-B. Kim, personal communication).

Flowering season, flower development, and seed formation

On Somaemul-do, the flowers of *M. koreanum* begin to bloom on the 10th of September and they last for about 50 days until the end of October. Each individual plant normally blooms for about 30 days and flowering progresses from the base to top of each branch. Each branch develops 10–15 layers of leaf axils, where usually two (occasionally one) new flowers bloom every day for 10–15 days in total (Fig. 2C). As a flower develops, the calyx opens and a white and light purple corolla appears. The development of floral tube is completed within 4 days when the upper and lower lips of the corolla open. After the flower opens (fourth day), pollen is discharged from each anther when it is touched. On the fifth day, the pistil can accept pollen. Finally, the corolla fades and falls to ground in the late afternoon on the fifth day. During this process, the first 48 h (days 1 and 2) of floral tube development is very slow, but the tube length extends rapidly between 48 and 72 h (day 3). On the fourth day, the extension of the floral tube slows down and the upper and lower floral lips open to accept pollinators (Fig. 3). Scent and nectar are secreted from the space between the superior ovary and the floral tube base. Each flower secretes 10–15 μl of nectar at two times, i.e., in the morning on days 4 and 5, which condenses and dries out by around 3 pm.

After the pollen dispersal and pollination processes are complete, seed development is a rapid process. Within 1 month of fertilization, the plant bears mature capsules at the leaf axil, where the immature seed is white and egg-shaped. As the capsule dehisces, the seeds become light yellow to light brown in color, but after the capsule dehisces completely, it becomes black (Fig. 2D). The proportion of successful seed formation in each population was 20–38% with slight variations among populations (Table 2).

TABLE 2. Plant species communities occurring with 3 m diameter circle from the *M. koreanum* population.

population / Species	1	2	3	4	5	6	7	8	9	Numbers of Population.
Trees										
Pinus thunbergii	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	+(D)	9
Camellia japonica	+(D)	+(D)	+(D)	+(D)		+	+(D)			6
Mallotus japonicus		+			+				+	3
Platycarya strobilacea		+	+	+						3
Carpinus turczaninovii				+(D)		+				2
Albizia julibrissin			+							1
Castanopsis sieboldii									+	1
Shrubs										
Ligustrum japonicum	+	+	+(D)	+	+(D)	+(D)		+	+	8
Eurya japonica	+	+	+(D)					+	+(D)	5
Ficus erecta	+	+	+		+					4
Rubus ribisoideus	+	+	+						+	4
Ardisia japonica	+	+						+		3
Smilax china	+	+(D)						+		3
Cocculus trilobus		+								1
Euscaphis japonica		+								1
Ligustrum obtusifolium	+									1
Parthenocissus tricuspidata	+									1
Rhamnus yoshinoi			+							1
Rhus javanica					+					1
Rosa wichuraiana		+								1
Herbs										
Miscanthus sinensis var. *purpurascens*	+	+		+	+	+				5
Artemisia keiskeana	+	+	+	+						4
Pueraria lobata	+	+			+					3
Asparagus schoberioides			+	+						2
Dendranthema boreale				+		+				2
Dioscorea batatas		+						+		2
Eupatorium japonicum	+	+								2
Imperata cylindrica var. *koenigii*						+		+		2
Isodon japonicus	+		+							2
Leibnitzia anandria		+	+							2
Liriope platyphylla		+		+						2
Oplismenus undulatifolius	+	+								2
Persicaria hydropiper	+		+							2
Pteridium aquilinum var. *latiusculum*	+	+								2
Setaria viridis		+		+						2
Achyranthes japonica	+									1
Arisaema ringens		+								1
Corchoropsis tomentosa	+									1
Crepidiastrum lanceolatum									+	1
Dicranopteris pedata								+		1
Dryopteris erythrosora								+		1
Farfugium japonicum									+	1
Humulus japonicus	+									1
Solidago virgaurea subsp. *asiatica*								+		1
Spodiopogon cotulifer						+				1
Themeda triandra var. *japonica*								+		1

Symbol (D) indicate 1-4 dominant species based on the coverage.

FIGURE 2. Habitat, flower and seed development of *Melampyrum koreanum*. A) A *M. koreanum* population in the Somaemuldo (Island), Maejuk-ri, Hansan-myeon, Tongyeong-si, Gyeongsangnam-do, Korea. *Pinus thunbergii*, *Camellia japonica* and *Ligustrum japonica* grow together with *M. koreanum.* B) An individual of *M. koreaunm*. It is almost 1 m tall and branched many times. C) Two opened flowers at the bottom are four days old since flower bud opening. The central flower is three days old and the floral tube already elongated almost completely but the corolla lips are not open yet. D) Capsule open approximately one month after pollination. There are 2-3 seeds per capsule. Seed color is pale yellow at the capsule opening and it turn to black at a couple of days after.

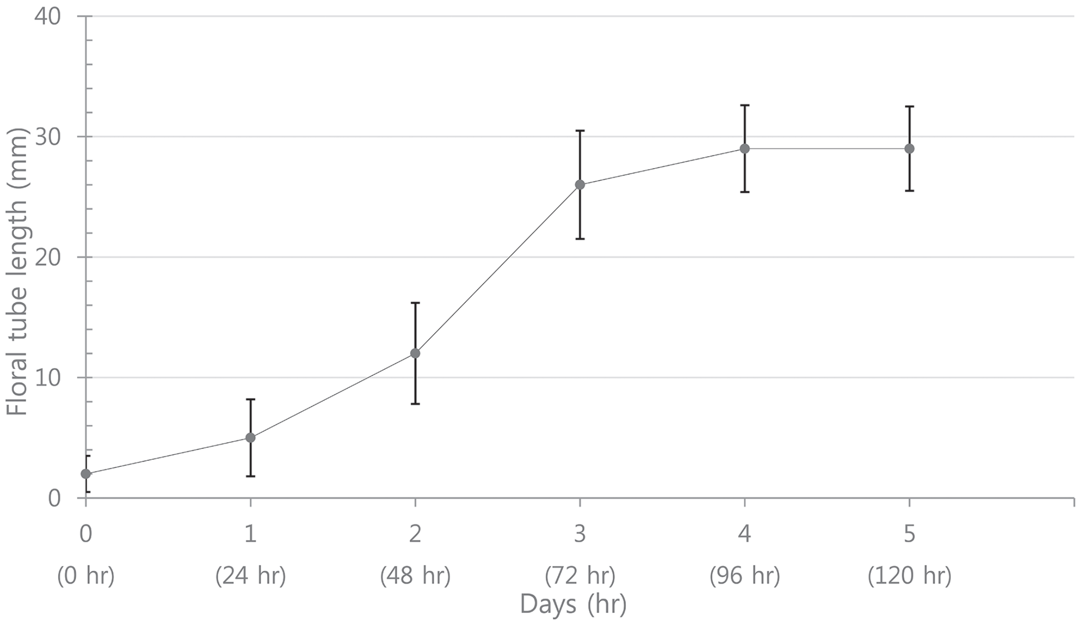

FIGURE 3. Elongation pattern of the floral tube of *M. koreanum* after calyx opening. The floral tube is expended rapidly at the 3rd day and the flower open at the 4th and 5th days for two days.

Pollinator and its process

Two hawk moths were collected and the lengths of their body parts were measured at the study location, i.e., body length = 30–36 mm, wing length = 25–30 mm, and proboscis length = 30–35 mm. The hawk moth was confirmed as *Macroglossum pyrrhostictum* Butler (Park 2012, Shin 2007).

Hawk moths fly very fast and they hover over flowers like hummingbirds. They approach the flowers of *M. koreanum* with a coiled proboscis, which is then extended into the flowers (Fig. 4A). The unfurled lengths of the proboscises of the two hawk moths were 30 and 35 mm. The proboscis lengths were slightly longer than the lengths of *M. koreanum* flowers (Fig. 4B). However, the hawk moth bends its proboscis when sucking nectar from a flower, so the lengths of the proboscis and the floral tube are similar. The hawk moth approaches the *M. koreanum* flowers until its head area touches the upper flower lips and it sucks nectar by hanging close to the upper and lower flower lips with its two antennae stretched out at either side. It contacts the flowers very tightly so there is no gap between the pollinator and the flower. The nectary is located in a gap between the base of the floral tube and the ovary, and each flower secretes approximately 10–15 μl of nectar each day for 2 days. The hawk moth places its head in flowers to suck the nectar and its head touches the tips of the pistil and stamen immediately inside the upper lips of the flower, which coats the head, antennae, and proboscis areas of the hawk moth with pollen grains. White pollen grains were often observed on the heads of hawk moths (Figs 4C and 4D).

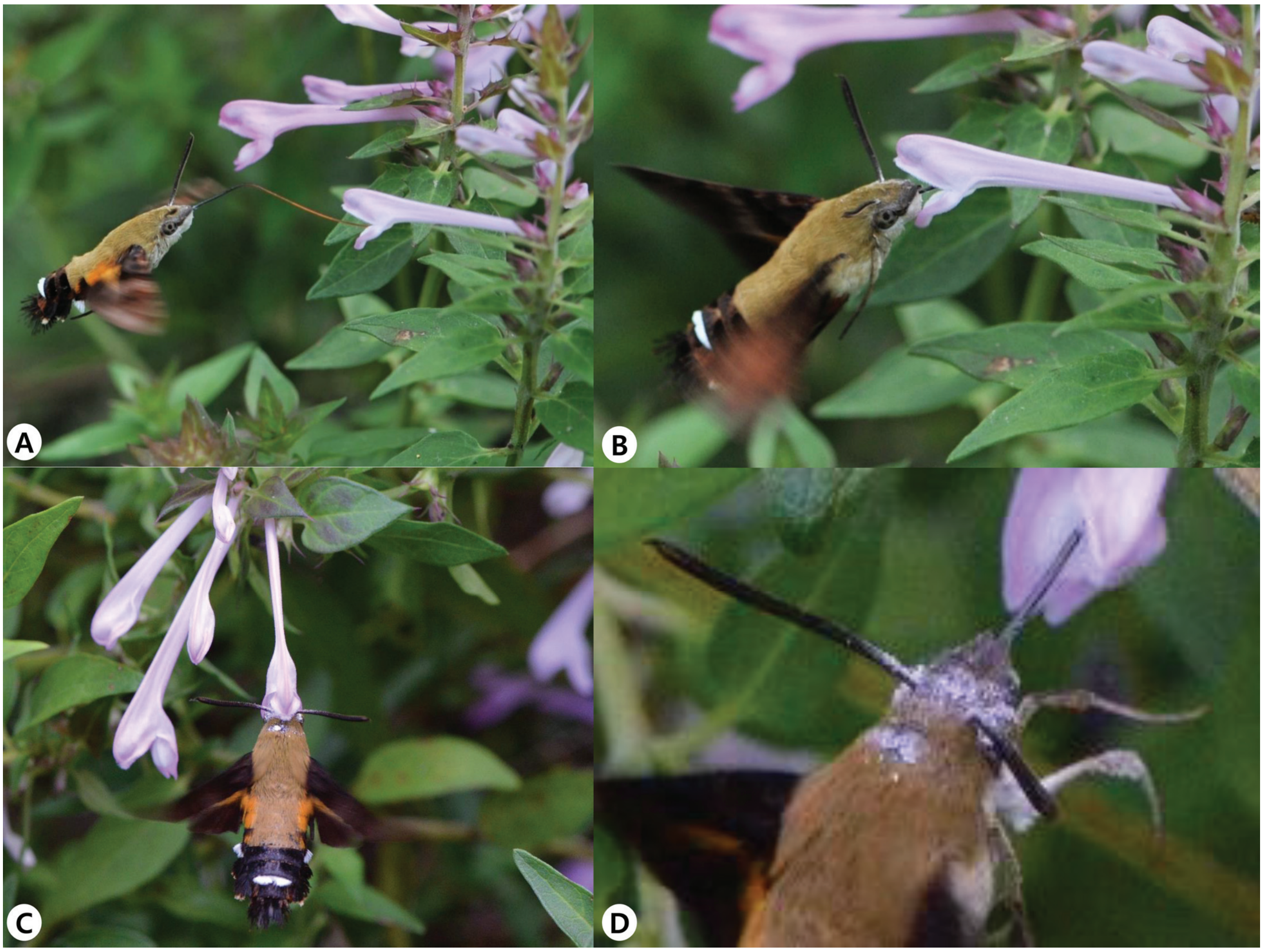

FIGURE 4. Diurnal hawk moth pollination on the *M. koreanum* Flowers. A) A hawk moth with long proboscis approaching to the flower of *M. koreanum*. B) A hawk moth close contact to the nectary on the floral tube. C) The head of hawk moth touch to the stigma and anthers which are located on the inside of upper lips of flower. White pollen grains are deposited over the head areas of the hawk moth at this stage of pollination. D) The hawk moth visiting another flower with a lot of white pollen grains over his (her) head area including antenna and proboscis.

M. pyrrhostictum visited 15–20 flowers each minute and it took 1 s to find a flower, place its proboscis in the flower, and move to another flower. In general, it visited flowers for about 10 min during one flight. *M. pyrrhostictum* visited around 150–200 flowers during one flight (per 10 min) in *M. koreanum* populations (the range was measured three times in three different populations). This varied among populations, but it is estimated that 1–3 hawk moths can visit 1,800–2,400 flowers in 1 h. Assuming that the ratio of mature stamens and pistils is 1:1, it is estimated that approximately 900–1,200 flowers can be pollinated in 1 h. *M. pyrrhostictum* is diurnal and it actively visited the *M. koreanum* population from 9 am to 3 pm. Thus, the number of flowers that could be pollinated in a population during 6 h each day is approximately 5,400–7,200. This suggests that *M. pyrrhostictum* is a very effective pollinator. However, it is not expected that all visits by *M. pyrrhostictum* will mediate pollination and the visitation frequency may differ depending on the weather conditions. Therefore, the actual numbers of pollinated flowers would be smaller than the estimated figures, although most flowers would be pollinated by *M. pyrrhostictum.*

The flowers of *M. koreanum* were also visited by wasps, flies, *Parnara* butterflies, and butterflies other than *M. pyrrhostictum* (Fig. 5A–5D). However, the wasps and flies are not pollinators and they robbed nectar from the floral base of *M. koreaunum* after making a hole. *Parnara guttata* and other butterflies (Kim 2002) visited *M. koreanum* flowers but they never carried pollen grains (Fig. 5A–5D).

It was also observed that *M. pyrrhostictum* visited *Dendranthema zawadskii* (Herbich 1831: 43) Tzvelev (1961: 376), *Aster yomena* (Kitamura 1937: 51) Honda (1952: 139)*, Clinopodium chinense* Kuntze (1891; 515)*,* and *Crepidiastrum lanceolatum* Nakai (1920: 150), in addition to *M. koreanum*. However, given the length of its proboscis and head structure, it would not be an effective pollinator of these flowers. Therefore, *M. koreanum* is the only plant that *M. pyrrhostictum* pollinates efficiently during September to October on Somaemul-do. In other seasons, *M. pyrrhostictum* also visited *Clerodendrum trichotomum* Thunberg (1780: 208) (Verbenaceae), *Scutellaria* Linnaeus (1753: 598) (Lamiaceae) species, and *Trachelospermum asiaticum* Nakai (in Mori 1922: 293) (Apocynaceae) on the island of Somaemul-do. The feeding plants of the larvae of *M. pyrrhostictum* were not identified in this study.

FIGURE 5. Ineffective and effective pollinators to the flower of *M. koreanum*. A) Moth, an ineffective but a possible pollinator of *M. koreanum*. B) Wasp robbing the nectar of *M. koreanum*. C) Fly also attracted to the *M. koreanum* flower to get nectar. D) Effective pollinator, hawk moth with white pollen grains on the head area.

Conclusion

M. koreanum has a thin and long floral tube, which distinguishes it from other closely related species, and this characteristic appears to have developed as an adaptation for hawk moth pollination. In contrast, *M. laxum* Miquel (1865; 123) and

M. roseum, closely related species to *M. koreanum* have wider floral tubes and shorter floral lengths. Their flowers are usually pollinated by wasps and bees, so the long and thin corolla tube of *M. koreanum* is assumed to be an adaptation to hawk moth pollination. Therefore, it is assumed that coadaptation between the plant and pollinator had an important role in the origin of *M. koreanum*. The hawk moth pollinator is distributed widely in the southern islands, so *M. koreanum* may be present more widely on the islands and inland areas of the South Sea, Korea, although our investigations around Geoje-si, Tongyeong-si did not confirm this theory. Our study also confirmed that the most likely host plant for hemiparasitic *M. koreanum* is *Pinus thunbergii,* or less likely *Ligustrum japonicum* and *Camellia japonica*.

M. koreanum is readily observed on Somaemul-do. However, the rapidly increasing number of tourists on Somaemul-do has caused many environmental issues, including contamination of trail areas, garbage incineration, and power plant extension due to power shortage. Therefore, the challenges for *M. koreanum,* an endemic species in Korea, may include: 1) decreased numbers of hawk moths, the pollinator; 2) habitat loss around the mountain trails; 3) thoughtless collection by plant lovers; 4) the vulnerability of *Pinus thunbergii*, the host plant, to the pine tree parasitic *Bursaphelenchus xylophilus* (Nematoda). The present study did not identify the feeding plants during the caterpillar period for the hawk moth. Therefore, further studies must be conducted to better understand the biology of hawk moths in order to maintain sufficient numbers for pollination. To conserve the endemic *M. koreanum* on Somaemul-do, future studies must examine all aspects of conservation, including maintenance of the correct hawk moths as pollinators, the hawk moth feeding plants, *M. koreanum* populations, and healthy host plant communities. In addition, it will also be necessary to study the relationships between *M. koreanum* and the host plant with respect to seed germination and juvenile growth.

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