

Andromonoecious sex expression of flowers and pollinia delivery by insects in a Japanese milkweed *Metaplexis japonica* (Asclepiadaceae), with special reference to its floral morphology

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Abstract

Pollination ecological studies of *Metaplexis japonica* Makino were carried out in central Japan. We found that the flowers were andromonoecious; the proportion of hermaphrodite flowers was 61%. Unlike other Asclepiadaceae flowers, *M. japonica* has a unique and outstanding long protrusion on the pistil end, which looks like a stigma. However, we confirmed that the true stigma is present on the lateral surface of the style, as it is in other Asclepiadaceae flowers. Various insects visited the flowers for nectar and the most effective pollinators were scoliid wasps. The pollen/ovule ratio was 21 and the fruit set of the hermaphrodite flowers was 11%.

Keywords: andromonoecy, Asclepiadaceae, floral morphology, insect pollination, *Metaplexis japonica*.

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Introduction

The asclepiad flower has attracted the attention of flower ecologists for a long time because of its peculiarity in having a united column of stamens and a pistil and a package of pollen delivered in the form of a pollinium, making it a dicot counterpart to the well-known orchid flower. Since Sprengel (1793) described the detailed floral structure and the pollination mechanism of *Asclepias* species, extensive investigations have been made on flowers of Asclepiadaceae (Müller 1873; Knuth 1899; Proctor & Yeo 1973). More recently, quantitative and morphological observations of the pollen delivery of *Asclepias* have been made, and factors affecting the fruit set and a unique late-acting self-incompatibility system have been reported (Wyatt & Broyles 1990, 1994). Asclepiad flowers have two free ovaries, but in most cases only one of the two develops to a mature fruit, and the total fruit set can be as low as 0.33–5.0% (Wyatt & Broyles 1990, 1994). Electron

microscopic studies of *Asclepias amplexicaulis* revealed that pollen tubes penetrating the stigmatic surface were transmitted to one of the two ovaries (Tammy *et al.* 1990).

Metaplexis japonica Makino is a climbing perennial herb of Asclepiadaceae, native throughout East Asia. It is common in open habitats in central Japan. The flowers are described as hermaphrodite (Murata 1981; Fu *et al.* 1999), flowering typically in July to September. In contrast to well studied *Asclepias*, however, little is known about the mechanisms of its pollination. Scoliid wasps (Tanaka 1984) and honeybees (Tanaka 1990) are reported to visit *M. japonica* flowers, but their contribution to pollination remains uncertain. Recently, Sugiura and Yamazaki (2005) reported that various moths visited the flowers of *M. japonica* after sunset and that seven species of moths carried out the pollinia on their proboscis, but pollination by other insects in the daytime has not been carefully observed. Moreover, little is known about the function of the floral morphology unique to *M. japonica*; an unusual shape with a long protrusion projecting out of the corolla, which makes a striking morphological difference from the flowers of *Asclepias*. In the taxonomic literature of Japan

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Fig. 1 Japanese milkweed *Metaplexis japonica*. (a) A hermaphrodite flower, (b) a male flower, (c) a mature fruit, (d) a young fruit and (e) a seed with hairs. Illustrated by S. Kawachino.

and China, this protrusion on the pistil end has been described as a stigma (Ohwi 1953; Jia & Jia 1955; China Institute of Botany 1974; Murata 1981; Osada 1981; Fu *et al.* 1999). However, other asclepiad flowers lack such a protrusion and the stigma is present on the lateral surface of the style (Müller 1873; Knuth 1899; Proctor & Yeo 1973). Thus, both the morphological feature of the protrusion and its role in pollination remain entirely obscure. Therefore, we attempted to elucidate the floral morphology and pollination biology of *M. japonica*. As a result, we found that *M. japonica* has male flowers in addition to hermaphrodite ones. In the present paper, we report our findings with special reference to the following questions.

- 1 Where is the position of the stigma: Is it on the pistil protrusion or not?
- 2 How are the flowers pollinated by insects?
- 3 How do male flowers differ from hermaphrodite ones?
- 4 How high are the fruit set and pollen/ovule ratio and is there any difference in these traits between andromonoecious *M. japonica* and other asclepiads?

Materials and methods

Overall structures of *M. japonica* and its flowers are illustrated in Figures 1 and 2. The flowers in racemes have

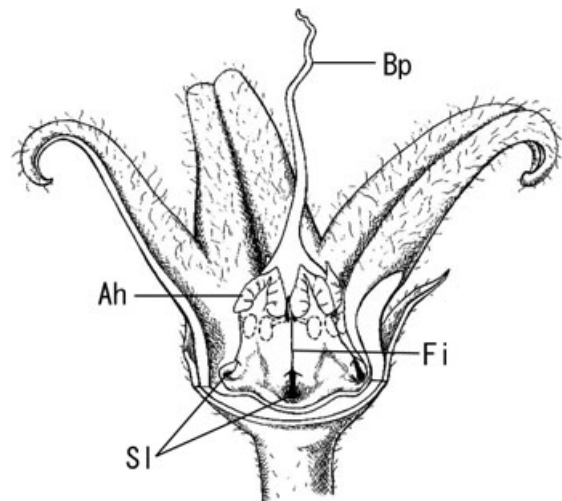


Fig. 2 A flower of *Metaplexis japonica*. Corolla lobes of the front side have been removed. Ah, anther hood; Bp, beak-like protrusion of the pistil end; Fi, fissure; Sl, slit. Illustrated by S. Kawachino.

five-lobed, pale violet or white corollas measuring approximately 1 cm in diameter with a number of white hairs inside. Follicles are fusiform, 6–10 cm long, and have hundreds of hairy seeds inside. The flower has two free, superior ovaries. Two styles unite together in the

upper part and fuse with the outer walls of five surrounding anthers. Each anther is covered with a cordate membranous hood. Five stamens unite together and surround two ovaries. Unlike *Asclepias*, the corona at the bottom of the stamens is small and the corolla forms a tube.

Observations of floral characteristics and pollinators, and experiments examining pollination and pollen germination were carried out from August to October in 2002, 2003 and 2004 in the following locations of central Japan; Setagaya Ward and Machida City of Tokyo Metropolis, Yokohama City of Kanagawa Prefecture and Saitama City of Saitama Prefecture.

Results

Morphology of pollinia and the number of pollen grains

Five anthers have paired pollinia, and two pollinia in adjacent anthers are joined by arms to a clip (corpusculum). A pollinium is yellow-brown, elliptic, and measures approximately 550 μm × 300 μm. A clip is dark brown and approximately 0.5 mm long, with a narrow vertical crack (Fig. 3a). Pollinia contained irregular shaped pollen grains approximately 50 μm in size (Fig. 3b). The number of grains per pollinium was approximately 800.

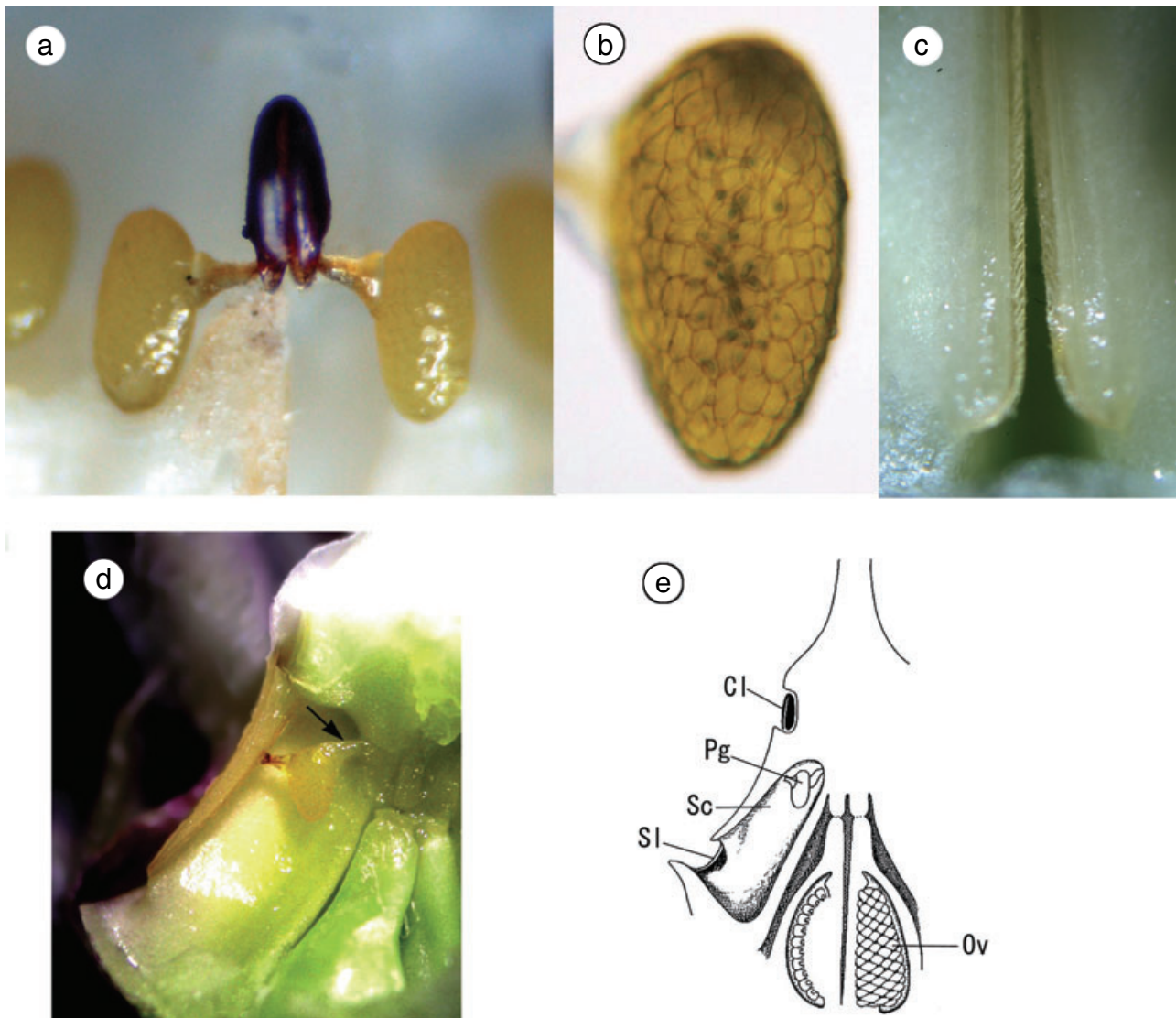


Fig. 3 (a) A pair of pollinia in the *Metaplexis japonica* flower (stamens in the front have been removed). (b) Pollen grains in a pollinium. A pollinium was made partially transparent in 10% sucrose solution and examined under a microscope. The number of pollen grains in a pollinium was determined to be approximately 800 on the basis of this photograph. (c) Upwardly pointed comb-like bristles on the edges of a fissure between the stamens. (d) Germinating pollinium in a stigmatic chamber. The arrow indicates a bundle of pollen tubes. (e) Longitudinal section of the column of a *M. japonica* flower. Cl, clip; Ov, ovary and ovule; Pg, germinating pollinium in a stigmatic chamber; Sc, stigmatic chamber; Sl, slit. Photos by O. Kitamura (a), Y. Yaoi (b,c) and H. Tanaka (d). Illustration (e) by S. Kawachino.

Floral morphology and pollination mechanisms

The pistil has a crown-like capital, the end of which elongates into a long, white protrusion approximately 5–8 mm long. Its tip is beak-like, two-cleft or entire, and it looks like a stigma (Figs 1,2). However, the tip has no mucilage or papilla, and pollinia rarely attached themselves to it. We examined more than 1000 flowers in natural fields, but pollinia attached to the protrusion were hardly ever found and germination on it was never observed. We also attempted to attach pollinia to the tips on approximately 50 occasions, but failed in most cases, and even if the pollinia were artificially attached they never germinated there. Thus, we believe that the tip of the long protrusion might not be a functional stigma that receives pollinia.

The stamen column has five V-shaped narrow fissures between each anther filament, and at their basal parts five vertical slits open into the cavity between the stamens and ovaries, leading to the stigmatic chambers just beneath the capital end of the pistil (Figs 2,3e,4b). These floral structures of *M. japonica* are similar to those reported for *Asclepias*. Therefore, we assumed that the slits might be the entrances used by pollinia to the stigmatic chambers, as in *Asclepias*, and tried to insert pollinia artificially into the slits.

A thin filament, as a substitute for the proboscis of insects, was inserted into the bottom of the corona, where nectar is pooled, and then the filament was pulled up along a fissure. The tip of the filament was easily caught by the crack of the clip of pollinia and the pollinia were carried out from the flower. The fissures had a number of upwardly pointed comb-like minute bristles on both edges that became progressively narrower, preventing the backward movement of the filament (Fig. 3c). When a filament carrying pollinia was inserted into a slit and pulled up along the fissure, the pollinia were cut away from the arms and left in the cavity of the column (Fig. 4b), and finally lodged in the stigmatic chambers. Pollen tubes then emerged from the convex surface of the pollinia, which is usually facing the stigma (Fig. 3d,e).

Sex expression of flowers

We noticed that plants of *M. japonica* have two types of flowers that are morphologically distinguishable by the size of stamen columns and ovaries (Fig. 4a–c). We called them large flowers and small flowers, respectively. Stamen columns of large flowers were approximately 4 mm × 4 mm, with vertical slits opening at their bottoms, to which pollinia are inserted. Ovaries are 3 mm long and contain hundreds of ovules. Stamen columns of small flowers are 3 mm × 3 mm, and the slits at the bottom are closed. Ovaries are 2 mm long and ovules are immature.

The slits of the larger flowers are open throughout the stages of flowering from bud to fade, whereas those in the smaller flowers are always closed.

Germination of pollen grains began within 3 h in 10% sucrose solution at approximately 27°C, without complete loosening of the pollinia. When examined in Saitama City on 20 September 2004, 27 out of 44 pairs of pollinia (61%) germinated within 13 h. Pollinia of both larger and smaller flowers germinated at a similar rate.

The ability of ovaries to attract pollen tubes was compared between the larger and smaller flowers. Ovaries from which styles had been cut off were placed on 1% agar plates that contained 10% sucrose, and pollinia were placed at 1–2 mm from the ovaries. The agar plates were left to stand at room temperature (approximately 28°C) for 18 h. As shown in Figure 4d, pollen tubes were strongly attracted to an ovary of a larger flower, but less distinctly to a smaller flower (Fig. 4e). Thus, we concluded that large flowers are hermaphrodite, while small flowers function as males. Pollinia could easily be removed from the anthers of both types of flowers at every stage, so dichogamy was not evident.

The proportion of hermaphrodite flowers was examined in Saitama City on 30 September 2004. Out of 102 flowers, 62 (61%) were hermaphrodite. The number of ovules in 14 ovaries of seven hermaphrodite flowers was 276–380 ($n = 14$, 313 ± 30.7 [mean \pm standard deviation]). Because hermaphrodite flowers have two ovaries and 10 pollinia, but male flowers lack ovules, the pollen/ovule ratio of the total flowers was calculated to be 21.0.

Insect visitors

The flowers have a sweet and strong smell, with plenty of nectar at the bottom of the corona, and various insects frequently visited the flowers in the daytime (Fig. 4f,g). Table 1 shows the insect visitors observed at four sites in central Japan from September to November 2004. A number of large insects carried pollinia on their ligula, labial palp or on the hairs on their legs (Fig. 4h,i). However, some small insects tended to be trapped by the flowers because their tongues or legs were pinched in the fissures between the stamens.

Pollination and fruit set

Pollinia of two male and 18 hermaphrodite flowers were examined in Saitama City on 2 October 2004. Twenty-six pairs of pollinia (26%) had already been removed from these flowers. Ten pollinia were found in seven out of 90 stigmatic chambers of hermaphrodite flowers, and all pollinia in the stigmatic chambers had already germinated there.

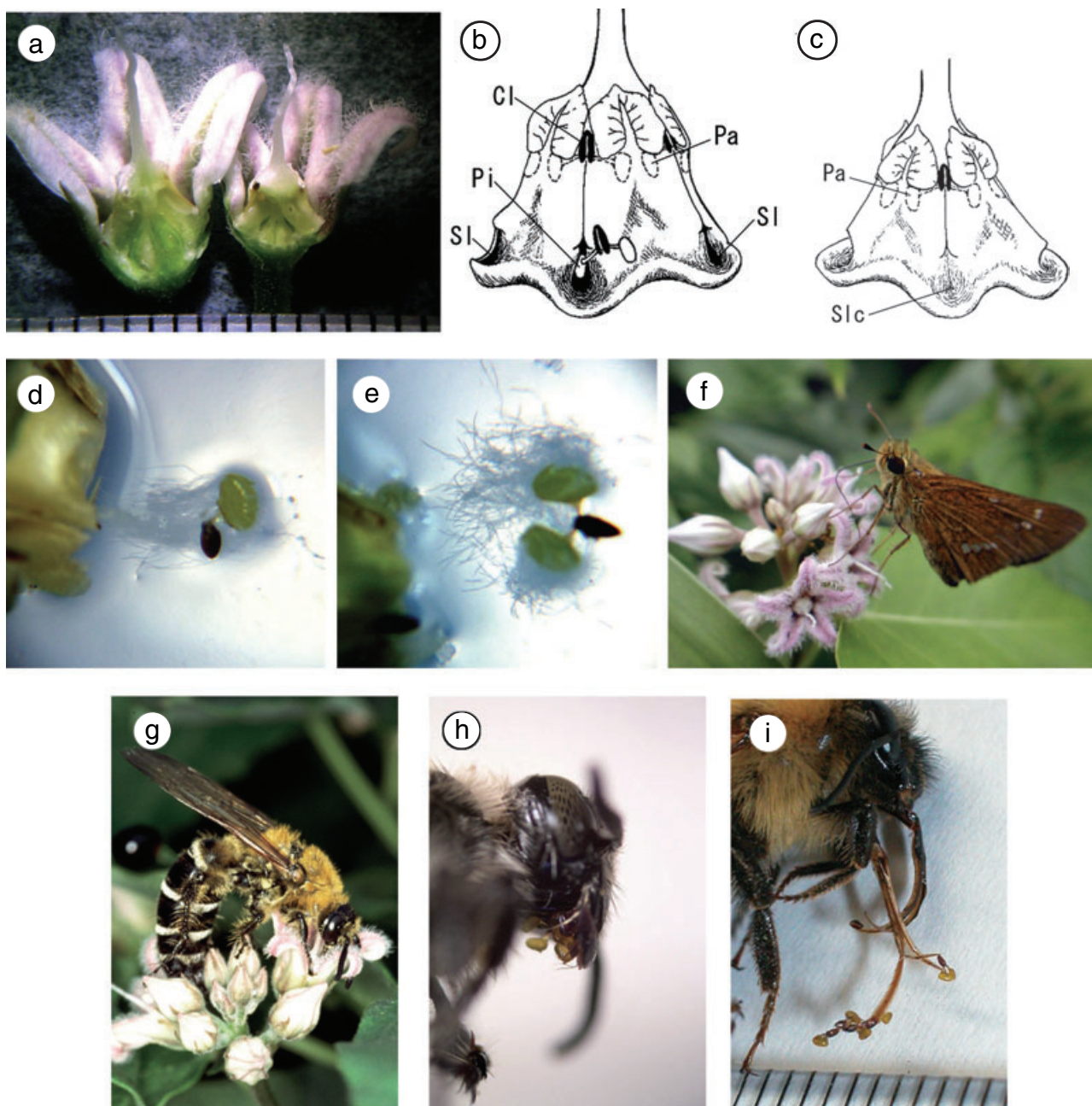


Fig. 4 (a) Comparison of the longitudinal sections of hermaphrodite (left) and male (right) *Metaplexis japonica* flowers. (b) Column of a hermaphrodite and (c) a male flower. Cl, clip; Pa, pollinium in the anther; Pi, pollinium being inserted into the slit; Sl, slit of hermaphrodite flower; Slc, closed slit of male flower. (d) Attraction of pollen tubes by an ovary of a hermaphrodite flower compared with (e) the activity of a male flower on agar plates. (f) *Parnara guttata* visiting a *M. japonica* flower. (g) *Megacampsomeris grossa* visiting a *M. japonica* flower. (h) *Lasioglossum occidentale* with pollinia on its mouthpart. (i) *Bombus diversus* with many pollinia on its proboscis. Photos by H. Tanaka (a,f,h), Y. Yaoi (d,e), T. Tada (g,i). Illustrations (b,c) by S. Kawachino.

The numbers of flowers per raceme observed on 24 and 30 September 2004 in Saitama City were 6–40 ($n = 25$, 19.9 ± 7.5 [mean \pm standard deviation]) and 2–37 ($n = 26$, 16.3 ± 7.9 [mean \pm standard deviation]), and the number of flowers in anthesis was 1–15 and 0–8, respectively. Ten

young fruits were found on 25 randomly selected racemes; usually one fruit per raceme, but in two cases a raceme had two young fruits. Traces of fallen flowers were 146. From these data, we determined the fruit set to be 11% for hermaphrodite flowers and 6.5% for total

Table 1 Insect visitors to the flowers of *Metaplexis japonica*

Insects	Study sites	Pollinia attached
Hymenoptera		
<i>Megacampsomeris grossa</i>	SA, SE	+
<i>Campsomeriella annulata</i>	SA	+
<i>Xylocopa appendiculata circumvolans</i>	SA	
<i>Bombus diversus</i>	SA	+
<i>Lasioglossum occidens</i>	SA	+ or – and c
<i>Polistes snelleni</i>	SA	–
Paper wasp	SA	–
Vespid wasp	SA	
<i>Lasius spathepus</i>	YO	c
<i>Monomorium intrudens</i>	MA	c
Diptera		
<i>Stomorhina obsoleta</i>	SA	–, c
Flies	SA	–, c
Lepidoptera		
<i>Pseudozizeeria maha</i>	SA	–, c
<i>Parnara guttata</i>	SA, SE	+ or –
<i>Pelopidas mathias oberthueri</i>	SA	–
<i>Gurelca himachala sangaica</i>	SA	–
Coleoptera		
<i>Oxyctonia jucunda</i>	MA, SE	+
<i>Acanthoscelides obtectus</i>	SA	+
Beetle	MA	+

MA, Machida City; SA, Saitama City; SE, Setagaya Ward; YO, Yokohama City; +, attached; –, not attached; c, insects caught by the fissures between stamens or by the cracks of clips.

flowers. Among 566 fruits examined in November at Setagaya Ward, Tokyo, in 14 cases (2.5%) a single flower produced double fruits.

Discussion

In the past, flowers of Asclepiadaceae have been described as hermaphrodites (Honda & Sakisaka 1930; Ohwi 1953; Jia & Jia 1955; Murata 1981; Fu *et al.* 1999), and the presence of male flowers has not been known. However, our observations and experiment showed that 39% of *M. japonica* flowers were morphologically and functionally male. Stamen columns of the male flowers lacked the slits at the bottom and pollinia could not be inserted into the stigmatic chambers. Their ovules were always immature and did not attract pollen tubes. In contrast, the morphology and germinating activity of the pollinia of the male flowers were normal. To our knowledge, *M. japonica* is the first case in Asclepiadaceae that has been confirmed to have both hermaphrodite and male flowers.

In flowers of representative asclepiad genera, such as *Asclepias* and *Vincetoxicum*, pistil ends are covered with crown-like capitals, but the top surfaces of the capitals are non-receptive for pollinia. Studies on the pollination

mechanisms of asclepiad flowers have well established that the stigma is present on the lateral surface of the style beneath the capital. That is, pollinia germinate in five stigmatic chambers, and the stigmatic surfaces in the chambers receive pollen tubes (Müller 1873; Knuth 1899; Procter & Yeo 1973). These facts were correctly cited in some old Japanese publications (Honda & Sakisaka 1930; Hayata 1935). Nevertheless, the shape of the beak-like protrusion in the center of the corolla of *M. japonica* is so outstanding and misleading that the protrusion has, in general, been described as a stigma in more recently published Japanese and Chinese articles. For this reason, the true stigma in flowers of *M. japonica* has not been exactly located to date. A few plants of Asclepiadaceae found in China, for example *Metaplexis hemsleyana* and *Toxocarpus villosus*, have similar protrusions, although their length is variable (China Institute of Botany 1974).

Our observations and experiment showed that the stigma-like tip of the protrusion of *M. japonica* was non-receptive; pollinia neither attached themselves to it nor germinated there. Using a pollination experiment and microscopic observation, we confirmed that the stigma of *M. japonica* was present on the lateral surface of the style just beneath the capital, as in *Asclepias*.

Ikuse (2001) reported that pollinia of *M. japonica* are 300–320 µm × 510–570 µm, and the pollen grains are 22–70 µm. We determined that the number of pollen grains per pollinium was approximately 800, and that the number of ovules per ovary was 313. Wyatt *et al.* (2000) reported that across four tribes of the Asclepiadaceae, the number of pollen grains per pollinium varied from 14 to 445, and the number of ovules per ovary varied from 4 to 229. The numbers of pollen grains per pollinium and ovules per ovary in *M. japonica* are greater than the range reported by Wyatt *et al.* (2000). The proportion of hermaphrodites was 61% of total *M. japonica* flowers. On the basis of our data, the pollen/ovule (P/O) ratio of *M. japonica* is 21.0. The average P/O ratio in hermaphrodite plants is 3450 (Gruden 2000), but the P/O ratio of Asclepiadaceae and Orchidaceae, in which pollen grains are packaged in pollinia, is known to be lower. Gruden (2000) reported that the P/O ratio of 10 Asclepiadaceae plants ranges from 3.8 to 18.4. Our results showed that the P/O ratio of *M. japonica* was slightly higher than the other Asclepiadaceae plants, probably because of the presence of the male flowers.

Sugiura and Yamazaki (2005) recently reported that the pollinia of *M. japonica* were transported on the tips of the proboscis of nocturnal moths, and that diurnal insects were not considered to be important pollinators. However, we observed that many insects visited the flowers in the daytime and transported the pollinia. Pollinia were born by the most frequently visited insects, *Megacampsomeris grossa* and *Campsomeriella annulata*, indicating that

they are effective pollinators. A beetle, *Oxycentonia jucunda*, visited frequently, and *Bombus diversus*, which was observed once on a flower, also appear to be effective pollinators. In contrast, attachment of pollinia was rarely observed on Lepidoptera, such as *Parnara guttata* and *Gureluca himachala sangaica*, which suckle nectar with a long proboscis and appear to be nectar thieves. Small, powerless insects, such as ants, flies, a bee *Lasioglossum occidens* and a butterfly *Pseudizeeria maha*, often could not escape from the flowers after their mouthparts or legs were trapped by the cracks of clips or by the fissures between the stamens, and these species might disturb the pollination by other insects. Thus, we conclude that *M. japonica* is pollinated by relatively large, irregularly visiting insects.

The fruit set of the total flowers was 6.5%, slightly higher than the 0.33–5.0% recorded in *Asclepias*, in which all flowers are hermaphrodite (Wyatt & Broyles 1990). The proportion of flowers producing double fruits was 2.5%, lower than the 5.0–24.4% reported in *Asclepias* (Wyatt & Broyles 1990).

The present study elucidated several fundamental aspects of the mechanisms of pollination of *M. japonica*. These include the floral morphology, sex expression of flowers, insect visitors, P/O ratio and fruit set. However, many problems remain to be solved. The physiological function of a long and outstanding protrusion on the pistil, the possibility of self-incompatibility, and the sources of nectar and smell are interesting areas requiring further study.

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