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PLANT SPECIES BIOLOGY

Floral Biology of *Heterotropa tamaensis* (Aristolochiaceae) in Japan

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Abstract The floral biology of *Heterotropa tamaensis* (Makino) F. Maekawa was investigated in native populations. The flowers were protogynous, and the stigmas and anthers were distant from one another. These floral characters are significant for cross-pollination, but breeding experiments showed that the flowers are fairly self-compatible and their pollination is obligately dependent upon animal pollen vectors. The most efficient pollinator for *H. tamaensis* is a fungus gnat, a member of Mycetophylidae. The fungus gnat visited the flowers relatively frequently and often carried many pollen grains on their hairy bodies. Furthermore, their eggs were frequently found within flowers collected from native habitats. The fungus gnats actively moved about within the flowers and often touched the stigmas and anthers with their bodies. Therefore, *H. tamaensis* seems to be predominantly self-pollinated, though cross-pollination may occur rarely. The presence of fungus gnat eggs in many flowers strengthened Vogel's hypothesis that the flowers of *Heterotropa* species mimic the basidiomycetes, in which fungus gnats usually oviposit.

Key words: Aristolochiaceae, *Asarum*, floral biology, *Heterotropa*, Mycetophilidae.

The genus *Heterotropa* is an evergreen perennial herb endemic to eastern Asia ranging from China to Japan (Maekawa, 1933, 1953; Cheng and Yang, 1983). The genus comprises approximately 70 species. Most species grow in evergreen, broad-leaved forests and the rest in deciduous, broad-leaved forests. Each plant bears a few cup-shaped flowers which lie on the ground or under fallen leaves. The large, dark-violet flowers produce a characteristic foul odor and possess peculiar glandular trichomes on the inner surface of the calyx-tube (Sugawara, unpubl.). From this floral morphology, *Heterotropa* species appeared to have an entomophilous pollination system (Hiura, 1978).

Earlier studies of the floral biology of the Aristolochiaceae are limited to a few species of *Asarum*, which is closely related to the genus *Heterotropa*. In *Asarum canadense, A. caudatum, A. caulescens* and *A. europaeum*, automatic self-pollination has been reported by Kugler (1934), Wildman (1950), Werth (1951), Daumann (1972), Lu (1982) and Tanaka and Yahara (1987). Very little information on the breeding system of *Heterotropa* species is available. Elucidation of this subject is very critical for an understanding of the adaptive significance and evolution of peculiar floral characteristics in *Heterotropa* species and between *Heterotropa* and other closely related genera. In this paper, I report on the pollination and breeding systems of *Heterotropa tamaensis* (Makino) F. Maekawa in central Japan.

Materials and Methods

Heterotropa tamaensis (Makino) F. Maekawa is native to the Tama Hill and its adjacent regions in Tokyo and Kanagawa Prefectures (Maekawa, 1933; Takahashi, 1971). Field observations were carried out at six localities: Komiya, Nishi-ikuta, Nishiya-A and -B, Manpukuji-A and -B. All populations occur in deciduous broad-leaved forests largely composed of *Quercus serrata* Murray, *Viburnum erosum* Thunberg and *Cornus controversa* Hemsly with exception of the Manpukuji-B population in a mixed conifer forest associated with shrubs.

To determine pollinators and frequency of their visitation, I observed more than 120 flowers in total from the two selected populations of Nishi-ikuta and Nishiya-A during April in 1986 and 1987. Flowers were checked at various times during daylight and night for visitors. The animals which visited the flowers were caught for identification and for examination of the presence of *Heterotropa* pollen loads on their bodies.

In order to estimate the fruit and seed set percentages under natural conditions, flowers were randomly collected about one month later after anthesis from the above two and additional four populations in 1983 and 1986. Additional populations are Komiya, Nishiya-B, Manpukuji-A, and -B. About one month later after anthesis, swollen seeds are recognizable and sterile fruits are not yet decayed. Thus both fruit and seed sets can be examined at this stage.

To test for self-compatibility and self-pollination ability, flowers prior to anthesis on plants transplanted from the field into the experimental garden of Makino Herbarium (MAK), Tokyo Metropolitan University, Tokyo, were bagged with perforated polyethylene envelopes and after anthesis were artificially self-pollinated.

The voucher specimens of plants and insects and other animals were deposited in MAK.

Observations

1. *Floral Morphology and Flowering*

Heterotropa tamaensis flowered from early April to the beginning of May, and fruits matured in June. The large, dark-violet flowers usually lie on the forest floor or under fallen leaves (Fig. 1). When flowering they produce a characteristic foul odor. The flower is actinomorphic and has three sepals, 12 stamens and a six-carpelled, compound ovary (Fig. 2). The sepals form a cup-shaped calyx-tube spreading above the gynoecium. It possesses an orifice ring above the gynoecium and its inner surface is reticulated by longitudinal and transverse ridges. The 12 stamens form an outer and an inner whorl of six stamens each. The stigma is positioned on top of the inverted boot-like style. The stamens are below the gynoecium, and the flowers are protogynous. One or two days later after anthesis, the six inner stamens, alternating with the styles, dehisce. One or two days later, the outer stamens dehisce. Anther dehiscence is extrorse in the inner stamens and laterorse in the outer stamens. Most of the pollen grains remained in anthers for two or more days when insects were excluded from the flowers. The pollen grains are non-sticky.

2. *Pollination Experiments and Fruit Set under Natural Conditions*

Results of the pollination experiments are shown in Table 1. None of the flowers bagged produced fruits and seeds, but 66 % of self-pollinated and 83 % of artificially cross-pollinated flowers set fruit.

There is no evidence for apomixis; none of the bagged flowers produced fruits and seeds.

Fruit and seed sets in six natural populations are shown in Table 2. Fruit set percentages in the six populations were notably higher than that of a con-

Fig. 1. *Heterotropa tamaensis* habit. Dark-violet flowers lie on the ground. ×0.3.

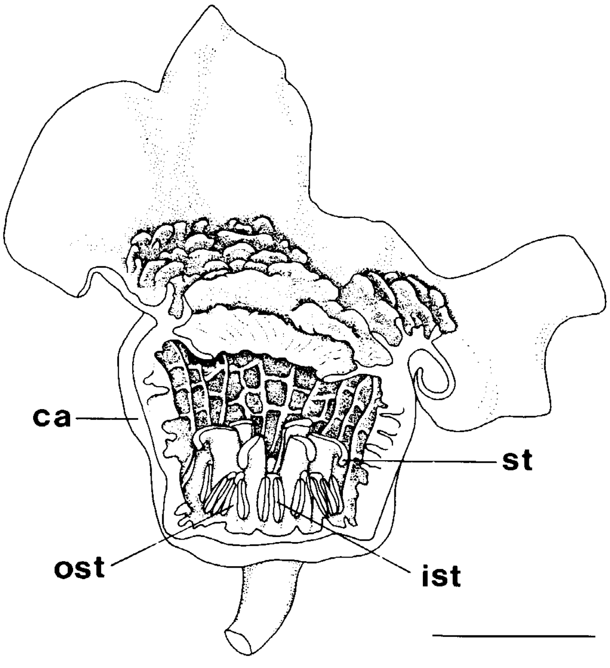


Fig. 2. Flower of *Heterotropa tamaensis* with one third of calyx removed. st, stigma; ca, calyx-tube; ist, inner stamen; ost, outer stamen. Bar: 10 mm.

Table 1. Pollination experiments with *Heterotropa tamaensis*.

	self-pollination						cross-pollination		
	bagged			pollinated			pollinated		
	No. of flowers sampled	Fruit set %	No. of seeds/ fruit	No. of flowers sampled	Fruit set %	No. of seeds/ fruit	No. of flowers sampled	Fruit set %	No. of seeds/ fruit
1983	14	0	0	16	75.0	9.5	—	—	—
1984	6	0	0	8	62.5	4.6	3	100.0	18.7
1985	—	—	—	14	71.4	10.2	3	100.0	12.3
1986	16	0	0	—	—	—	—	—	—
1987	53	0	0	12	50.0	9.8	6	66.7	19.2
Total	89	0	0	50	66.0	8.5	12	83.3	16.7

(mean ovules/ovary 45.7)

trol in Gard. MAK. and were considerably different among the populations and between seasons.

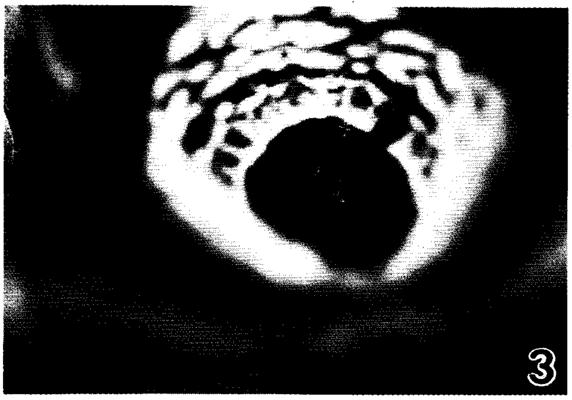
3. *Flower Visitors*

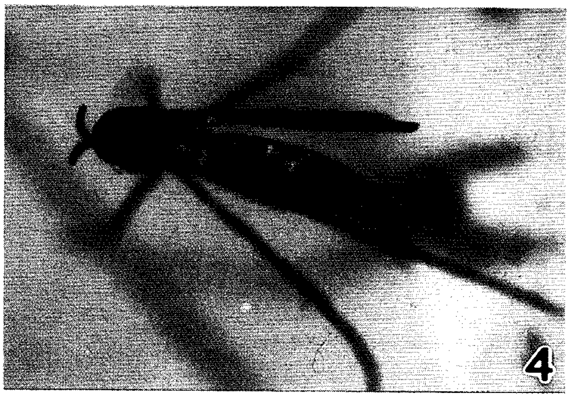
Although frequency of their visitation was relatively low, various unrelated animals visited the flowers of *Heterotropa tamaensis* (Table 3), and most of them can be regarded as potential pollinators.

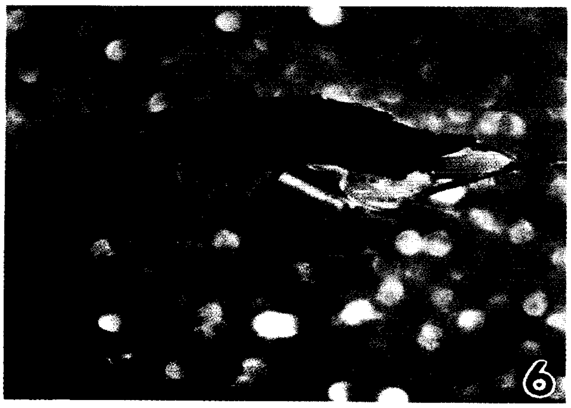
The most abundant animals visiting the flowers were fungus gnats in the family Mycetophilidae (Figs. 3 and 4). They visited flowers during the day and even at night. A fungus gnat had no sooner alighted on the limb of a calyx than it entered the calyx-tube in which it was very activity and often touched the anthers and stigmas. It usually stayed within the calyx-tube more than 15 min. The fungus gnats caught within the flower had many pollen grains on the body, especially on the back of head, thorax, and the base of wings (Fig. 4). Because of their hairiness, they can bear numerous pollen grains on their bodies. Although details of behavior of the fungus gnats within a calyx is uncertain, they probably oviposit within the calyx-tubes since all the fungus gnats caught in the flowers were female and their eggs, as described below, were frequently observed within the calyx-tube (Figs. 5 and 6). Fungus gnat eggs found within the calyx-tube are elliptical, 640 μm long and 280 μm wide. The eggs were randomly deposited in small chambers formed by longitudinal and transverse ridges on the inner surface of the calyx-tube. In order to estimate flower percentages with eggs, I checked more than 150 flowers from Nishiikuta, Nishiya-A and Manpukuji-B in 1987. In this case the flowers which had opened more than a week before were sampled and checked microscopically. The results are shown in Table 4. From this table it can be seen that the fungus gnat eggs are commonly found in the flowers

Table 2. Fruit and seed set percentages of *Heterotropa tamaensis* in six natural populations. Gard. MAK is a control.

Population (year)	No. of individuals examined	No. of flowers examined	Fruit set %	No. of seeds/ fruit
Gard. MAK (1986)	44	150	14.0	13.1
Nishi-ikuta (1986)	47	80	81.3	24.3
Nishiya-A (1983)	23	40	45.0	16.5
Nishiya-A (1986)	44	98	67.3	20.1
Nishiya-B (1986)	12	23	47.8	10.7
Manpukuji-A (1983)	48	68	27.9	18.8
Manpukuji-B (1986)	24	39	41.0	17.9
Komiya (1983)	75	109	60.6	14.7







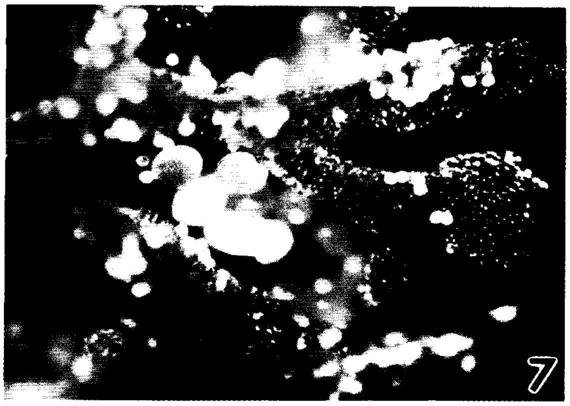


Fig. 3. *Cordyla* pollinator landing on the stigma of *Heterotropa tamaensis*. ×2.
Fig. 4. *Cordyla* pollinator, body loaded with pollen grains of *Heterotropa tamaensis*. ×10.
Fig. 5. *Cordyla* eggs deposited in inner surface of the calyx-tube. ×25.
Fig. 6. Oviposition of *Cordyla* and its eggs. ×15.
Fig. 7. Unidentified insect eggs deposited in inner surface of the calyx-tube. ×25.
Fig. 8. *Oxidus gracilis* intruding into the calyx-tube of *Heterotropa tamaensis*. ×0.4.

of three populations.

In several flowers an another type of egg was found (Fig. 7), which is 280 μm long and 170 μm wide. I could not identify these eggs.

Members of the Strongylosomidae, Machilidae, Carcinophoridae, and wood lice were infrequently observed in the flowers. Millipedes of the Strongylosomidae are relatively frequently around *Heterotropa* plants during the night, but millipedes intruding into flowers are rare (Fig. 8). These animals seem to serve occasionally as pollinators for self-pollination but probably play no part in cross-pollination because they are generally sedentary in their wingless form, and their bodies are smooth or relatively hairless.

Visitation by small slugs was rarely observed, and it appears that slugs do not play a part in pollination. They often eat the flowers.

Discussion

From the present study it is clear that the flowers

Table 3. Number of animals visiting flowers of *Heterotropa tamaensis*.

Visitor	Population	
	Nishi-ikuta[1]	Nishiya-A[2]
Mycetophilidae *Cordyla* (female)	11	2
Strongylosomidae *Oxidus gracilis*	5	1
Machilidae	2	—
Carcinophoridae *Carcinophora marginalis*	1	—
Wood lice	—	1
Slugs	2	—

[1] Apr. 15, 23, 1986; Apr. 9, 11, 16, 22, 1987. 43 hr.
[2] Apr. 16, 20, 25, 1987. 15 hr.

of *Heterotropa tamaensis* are protogynous; stigmas appear to be receptive for pollination prior to anther dehiscence. This floral nature may be as an adaptation for cross-pollination, but this species shows high self-compatibility when hand self-pollinated. Automatic self-pollination does not occur due to spatial separation of stigmas and anthers; none of the bagged flowers produce fruits and seeds. Furthermore, percentages of fruit set in native populations are considerably variable between populations and even between seasons. These facts suggest that the Japanese species *H. tamaensis* obligately depends upon animal vectors for pollination.

Members of the Mycetophilidae, Strongylosomidae, Machilidae and others visit flowers of *H. tamaensis*. Although all of them can be regarded as possible pollinators, fungus gnats (Mycetophilidae) are considered the most efficient pollinators for *H. tamaensis*, because they frequently visit the flowers, often carry many pollen grains on their hairy bodies, and because many flowers have their eggs on the inner surface of the calyx-tube. Fungus gnats usually stay in the flower for a long time (usually more than 15 min) and actively move about touching the anthers and stigmas thus providing a greater chance for self-pollination. Based on these facts and its high self-compatibility, *Heterotropa tamaensis* seems to be predominantly self-pollinated by fungus gnats. Cross-pollination may rarely occur, however, because these pollinators are capable of transporting pollen grains to different individuals.

In several species of *Asarum*, a genus closely related to *Heterotropa* but different from it in chromosome number and karyotype (Ono, 1960; Sugawara, 1981), predominant self-pollination has been reported. Compared to that of *Heterotropa tamaensis*, the pollination of these *Asarum* species occurs independently of the pollen vectors. This difference in breeding systems seems to be reflected in some of their floral characters. In *Asarum* species, for example, the stamens are nearly as long as the gynoecium, while in *Heterotropa tamaensis*, as well as in other *Heterotropa* species, they are shorter than the gynoecium (Sugawara, 1987).

Pollination by fungus gnats of the Mycetophilidae or Sciaridae has also been reported in several unrelated genera: *Arisaema* (Proctor and Yeo, 1972), *Aristolochia* (Stebbins, 1971), and *Listera* (Ackerman and Mesler, 1979; Mesler et al, 1980). In these sapromyophilous flowers, however, none of the fungus gnat eggs or larvae have been observed. In general, it has been known that fungus gnats usually oviposit in basidiomycetes and resulting larvae feed on them (Vockeroth, 1981). Thus, the presence of fungus gnat eggs in the flowers of *Heterotropa tamaensis* is very unusual. Based on his observation on pollination of *Asarum caudatum* in cultivation, Vogel (1978) speculated that the flowers of *Asarum* sensu lato, especially of East Asian species (i.e., *Heterotropa* species) which possess particular floral ridges on the inner surface of the calyx-tube, may function in mimicry for deceptive attraction of fungus gnats. Lu (1982), however, found no evidence supporting this idea from observ-

Table 4. Flowers with fungus gnat eggs in three populations.

Population (year)	No. of individuals examined	No. of flowers examined	Flower percent with eggs	Mean eggs/ flower
Nishi-ikuta (1987)	71	90	46.7	6.6
Nishiya-A (1987)	56	95	24.2	3.9
Manpukuji-B (1987)	27	47	23.4	5.5

ing pollination in the North America *Asarum caudatum*; the species is predominantly autogamous and fungus gnats and other insects hardly play a part in pollination. In Japanese *Heterotropa tamaensis*, it is certain from the present study that the primary flower pollinators are fungus gnats. The flowers often contain their eggs within the calyx-tube, especially on its inner surface where the particular ridges apparently appear to provide the place for ovipositing of the fungus gnats. These facts strengthen the above-mentioned hypothesis of Vogel.

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