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Breeding system in *Mussaenda shikokiana* (Rubiaceae)

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ヒロハコンロンカ（アカネ科）の繁殖様式

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抄録: ヒロハコンロンカの繁殖様式の調査を静岡県浜松市横山で行った．この植物には主に3種のチョウ類が訪花していた．自然状態，人工的に自家受粉させた場合，袋掛けをして放置した場合の結実率は，それぞれ78.0±15.5%，97.1±7.6%，90.6±12.6%であった．これらの実験から，ヒロハコンロンカの花は自家和合性を持ち，自動自家受粉もしうることが示された．非常に近縁なコンロンカは雌雄異株であることから，ヒロハコンロンカは繁殖様式に関して異なる進化を遂げてきている．

Abstract: The reproductive ecology of a shrub, *Mussaenda shikokiana* (Rubiaceae), was studied at Yokoyama, Hamamatsu City, Shizuoka Prefecture, Japan. The flowers were visited mainly by three butterfly species in the daytime. Fruit set of the natural controls, self-pollinated inflorescences, and bagged and intact inflorescences of *M. shikokiana* was 78.0 ± 15.5%, 97.1 ± 7.6%, 90.6 ± 12.6%, respectively. These experiments revealed that this species is self-compatible and autogamous. The direction of the evolution in the mating system of *M. shikokiana* is different from a closely related, dioecious *M. parviflora*.

Key Words: Lepidoptera; pollinator; self-compatibility; autogamy

Floral display, the number of open flowers on a plant and their arrangement within and among inflorescences, is one of the most important traits of the flowering plants for pollination efficiency (Galen, 1999). In the amazingly diverse floral display of the flowering plants, flower-like inflorescences are among the most fascinating ones; some plants bear condensed inflorescences which consist of many small flowers and are associated with colored petal-like structures derived from calyx lobes, bracts, leaves or other structures (Weberling, 1989; Claßen-Bockhoff, 1996).

Mussaenda is one of the well-known genera with flower-like inflorescences. One of the calyx lobes of some flowers in an inflorescence enlarges into a white, yellow or red petal-like lobe (Claßen-Bockhoff, 1996). Butterflies and hawkmoths are the major pollinators of *M. parviflora* (Naiki and Kato, 1999). The enlarged calyx-lobes effectively attract butterflies as proved by the calyx-lobe removal experiments in *M. frondosa* (Borges et al., 2003).

Two species of *Mussaenda* are distributed in Japan; *M. parviflora* grows in the Southwest

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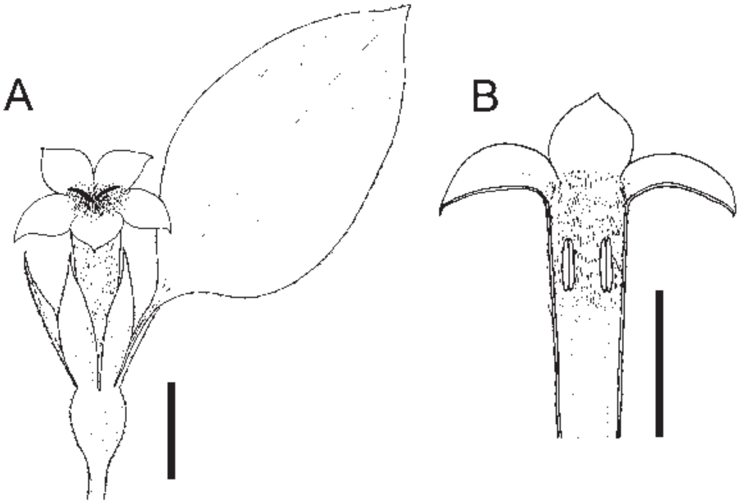

Fig. 1. Illustrations of *Mussaenda shikokiana*. A. flower, B. sectioned corolla tube. Bar 5mm.

islands of Japan, and *M. shikokiana* grows in Tokai, South Kinki, Shikoku and Kyushu. *Mussaenda parviflora* is dioecious (Naiki and Kato, 1999), but the mating system of *M. shikokiana* has not yet been elucidated. In this study, the mating system of *M. shikokiana* was investigated by bagging and crossing experiments and observing the insect visits to flowers. The main goal was to compare the breeding system and pollinators of *M. shikokiana* with those of *M. parviflora* because the two species are closely related (Alejandro et al., 2005).

Materials and Methods

Study plant

Mussaenda shikokiana Makino is a shrub about 1 to 5 m tall. In some areas, *M. shikokiana* is treated as an endangered plant (e.g. Kagoshima Prefecture, 2003; Mie Prefecture, 2005). The plant is usually found in low densities at the edges, or in gaps, of forests. It flowers from June to July. Flowers of *M. shikokiana* are illustrated in Fig. 1. Inflorescences consist of about 10 to 50 small, long-tubed, deep yellow flowers, and one to five flowers open per day in an inflorescence. Some peripheral flowers in an inflorescence have one enlarged white calyx-lobe (Fig. 1A). Flowers of *M. shikokiana* are deep yellow and radially symmetrical with a salverform corolla. The upper half of the inner corolla tube has abundant upward-facing hairs 1 to 1.5 mm long (Fig. 1B). Anthers are inserted on the corolla tube among the hairs, and filaments are adnate to the corolla tube. Bilobed stigma is slightly exerted from the corolla tube. The conspicuous calyx-lobe is about 2 to 5 cm long and 1 to 2.5 cm wide. The other calyx-lobes are lanceolate, and about 7 to 10 mm long and 2-3 mm wide.

Study site

Field studies were carried out at Yokoyama, Hamamatsu City, Shizuoka Prefecture, Japan

Table 1. Fruit set in the two experiments and the control in *Mussaenda shikokiana.*

Treatments	N	Fruit set* (%)
(a) Self hand-pollinated	48 (7)	97.1 ± 7.6[a]
(b) Bagged-intact	96 (4)	90.6 ± 12.6[ab]
(c) Open pollinated (control)	185 (12)	78.0 ± 15.5[b]

N: number of flowers examined (inflorescences (=individuals) examined).
*: per inflorescence.
a, b: not significantly different between treatments sharing the same letter.

(34° 56′ 43″ N, 137° 48′ 55″ E). The vegetation on this site was mostly occupied by cultivated forests of *Cryptomeria japonica* and *Chamaecyparis obtusa*, and some evergreen broad-leaved trees remained along the edge of the forests.

Pattern of nectar secretion

Nectar volume of *Mussaenda shikokiana* was measured with 2 μL micro capillary tubes (Drummond, U.S.A.) for bagged and open flowers every four hours from 6: 00 to 22: 00 on 7, July, 1998. Sugar concentration of the nectar was measured using a pocket refractometer (Bellingham & Stanley, England). Inflorescences were bagged with fine nylon mesh (φ0.25 mm, Teijin, Japan) before and after measurements of nectar volume and sugar concentration to prevent insect visitors from harvesting the nectar. Each flower was emptied every 4 h, for a total of five samples. On each three plants, three flowers were bagged and three were left unbagged. On one plant, one replication was damaged and not used in the analysis.

Pattern of insect visits

Insect visits to one individual of *Mussaenda shikokiana* were observed continuously from 5 : 00 to 22 : 00 on 6 July, 1998. To observe nocturnal flower visitors, a headlamp covered with red filter was used. The next day, some visitors were collected for identification.

Bagging and hand-pollination experiments

The mating system of *Mussaenda shikokiana* was investigated by bagging and hand-pollination experiments from 6-8 July, 1998. Eleven inflorescences on the plants were bagged with fine nylon mesh before anthesis. When flowers opened, 48 flowers on seven plants were selfed by hand pollination and re-bagged immediately after the treatment in order to prevent insects from visiting the flowers. Ninety six flowers on four plants were bagged only and not treated further (Table 1). The fruit : flower ratio (fruit set) of each inflorescence of each treatment was investigated three months later.

Results

Anthesis and nectar production

Usually one to five flowers opened in each inflorescence per day. Most flowers began to open before dawn, and corolla tubes started to fall from inflorescences late at night on the day of flowering. Flowers secreted nectar throughout flowering. The nectar secretion peaked at 6 : 00 (Fig. 2A), and the sugar concentration of nectar was 10-40% and peaked at 14 : 00 (Fig. 2B). The nectar volume in unbagged flowers was always significantly lower than that of bagged flowers (Fig. 2A; one-way analysis of variance (ANOVA), $F = 48.1$, df = 1, $p < 0.01$).

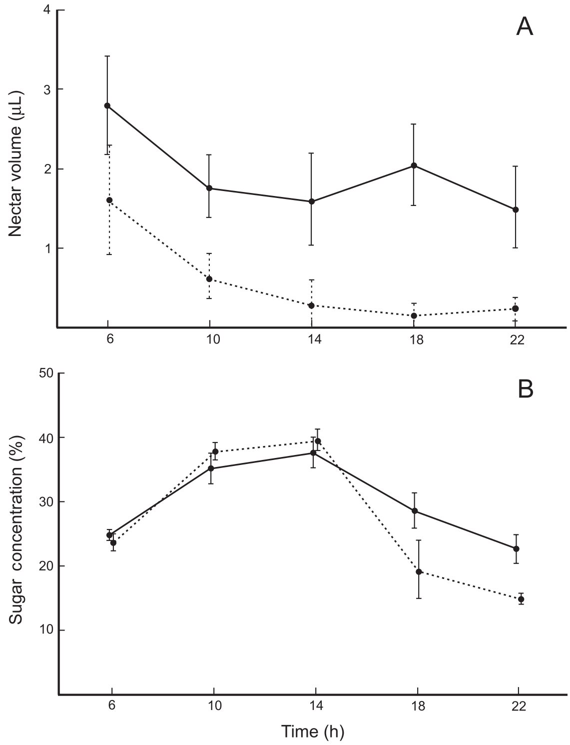

Fig. 2. A. Nectar volume secreted in successive 4-h periods in flowers of *Mussaenda shikokiana*. B. Nectar sugar concentration of *M. shikokiana*. Solid line (—) represents bagged flowers, dashed line (---) shows unbagged flowers.

Insect visits

Five insect species were observed to visit the flowers of *Mussaenda shikokiana*: three butterfly species, *Papilio helenus nicconicolens* Butler, *Graphium sarpedon nipponum* (Fruhstorfer) (Papilionidae), *Parnara guttata* (Bremer et Grey) (Hesperiidae), one hawkmoth species, *Macroglossum saga* Butler (Sphingidae), and one bee species, *Xylocopa appendiculata circumvolans* Smith (Apidae). Of all flower visits, 79% were by Lepidoptera. The pattern of the number of insect visits was bimodal; during 6 : 00－8 : 00, and 14 : 00－16 : 00 more insect visits were observed than other periods (Fig. 3). All insects except for *Macroglossum saga* visited the flowers of *M. shikokiana* throughout the day time. *Macroglossum saga* was observed only in the morning. No insect visits were observed after 18 : 00.

Fruit set

All treatments (a) to (c) set fruits are shown in Table 1. Fruit set of the natural controls of *Mussaenda shikokiana* (c) was 78.0 ± 15.5 (SD) %. Self-pollinated inflorescences (a) set more fruits (97.1 ± 7.6%). Even bagged and intact inflorescences (b) showed high fruit set (90.6 ± 12.6%). The fruit sets were significantly different among the treatments (Kruskal-Wallis test, df = 2, $\chi^2 = 10.98$, $p < 0.005$), and the fruit sets between self-pollinated inflorescences (a) and the natural controls (c) were only significantly different by the Steel's nonparametric multiple comparison test ($p < 0.05$).

Discussion

Flowers of *Mussaenda shikokiana* were monomorphic, i.e., stamens were slightly shorter than a pistil. High rate of fruit sets by the bagging and selfing experiments shows that *M. shikokiana* is self-compatible, and even autogamous, although actual selfing rate should be examined. Lower part of the stigmas and upper part of the anthers were closely situated to each other in a corolla tube of *M. shikokiana*. Upper part of the stigmas can even be touched by the anthers when the corolla tube falls. For the one-day habit of *M. shikokiana* flowers, self-compatibility and autogamy could compensate for the fruit set reduction when the number of pollinators is few caused by bad weather or other reasons.

The breeding system of *Mussaenda* is thought to be predominantly distylous, i.e., flowers of one morph have a long style and short stamens, and flowers of the other morph have a short style and long stamens (Alejandro et al., 2005). Some *Mussaenda* species are morphologically distylous but functionally dioecious (Baker, 1958; Naiki and Kato, 1999). Monomorphic, self-compatible flowers of *M. shikokiana* are probably a rare case in *Mussaenda*, and thought to have evolved from a distylous ancestor by the collapse of distyly. *Mussaenda shikokiana* is closely related to dioecious *M. parviflora* (Alejandro et al., 2005). The direction of the evolution in the mating system *M. shikokiana* is different from *M. parviflora*.

Observation of insect visits suggests that main pollinators of *M. shikokiana* are butterflies. Several observations suggest that *Mussaenda* is pollinated mainly by Lepidoptera. *Mussaenda parviflora* is pollinated mainly by both long-tongued butterfly and hawkmoth species (Naiki and Kato, 1999). *Mussaenda frondosa* is also thought to be pollinated by butterflies, shown by the calyx-lobe removal experiments (Borges et al., 2003). Baker (1958) reported that a West African *M. elegans* was visited by butterflies.

The amount of nectar secretion seems to correspond to the number of insect visits to flowers of *M. shikokiana* (Fig. 2 and 3). Nectar volume of the unbagged flowers was significantly lower

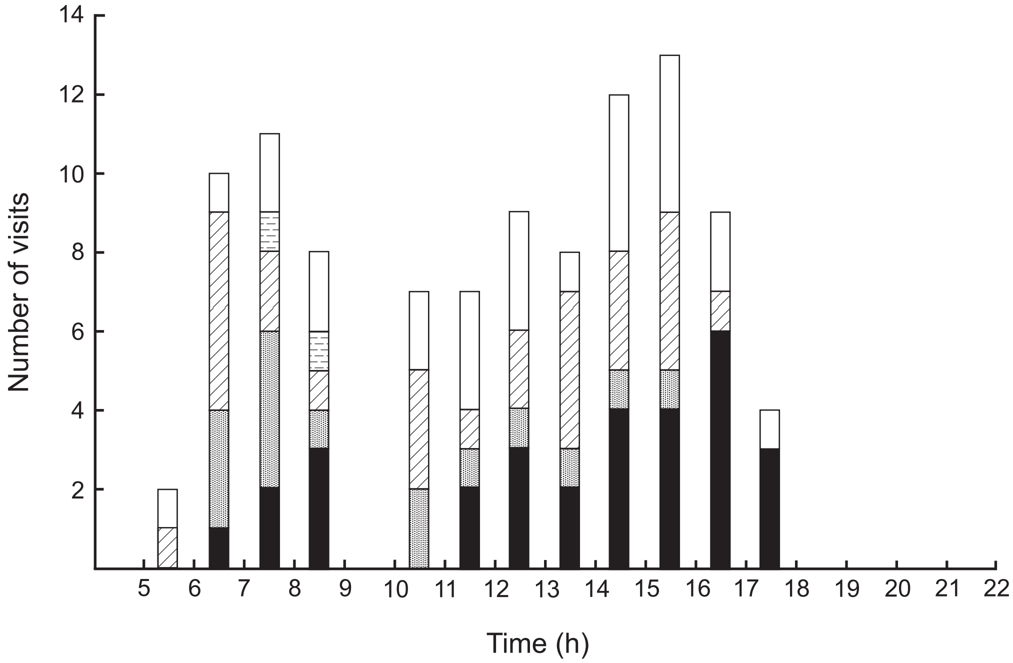

Fig. 3. Number of insect visits to *Mussaenda shikokiana* per hour.
( ) *Papilio helenus nicconicolens*, ( ) *Graphium sarpedon nipponum*, ( ) *Parnara guttata*, ( ) *Macroglossum saga*, ( ) *Xylocopa appendiculata circumvolans*.

than that of the bagged flower during 18 : 00 – 22 : 00 on 7, July (Fig. 2). This result suggests that more than one insect visited the flowers of *M. shikokiana* during this period, although no insect visits were observed after 18 : 00 on the previous day (Fig. 3).

Carpenter bees, *Xylocopa appendiculata* subsp. *circumvolans* seemed to be a nectar robber, not a pollinator because some flowers of *M. shikokiana* had a slit on the lower part of the corolla tube after they were visited by *X. appendiculata* subsp. *circumvolans*. In *M. parviflora*, pollen attachment to the proboscides of *Xylocopa amamiensis* was much fewer than that of effective pollinators such as butterflies and hawkmoths (Naiki and Kato, 1999).

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