Dioecy and Pollination of *Morinda umbellata* subsp. *umbellata* (Rubiaceae) in the Ryukyu Islands

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Sex expression and pollination were examined in natural populations of *Morinda umbellata* subsp. *umbellata* (Rubiaceae), a woody climber distributed widely in southeastern Asia, including southwestern Japan. Sex expression of subsp. *umbellata* was confirmed as being functionally dioecious, with separate staminate and pistillate plants. Staminate flowers had four or five stamens and completely lacked a style and stigma, while pistillate flowers had a style with two stigmatic lobes and four or five abortive stamens, the anthers of which produced no functional pollen grains. The stigmas of pistillate flowers protrude beyond the corolla tube and are positioned above the anthers, whereas anthers of the staminate flowers protrude beyond the corolla tube, indicating that stigma and anther heights appear to be reciprocal between pistillate and staminate flowers. Both flowers produced nectar from the base of the corolla tube, and the nectar volume per flower did not differ significantly between the two sexual morphs. Various unspecialized, opportunistic insects, such as wasps, small bees, hover-flies, bee-flies, beetles, and butterflies, visited the flowers mainly to feed on nectar. Of these, small bees, wasps, hover-flies, and beetles appear to be effective pollinators of subsp. *umbellata*. The floral and reproductive characteristics of subsp. *umbellata* were compared with those of subsp. *boninensis*, which exhibits androdioecy.

Key words: androdioecy, dioecy, *Morinda umbellata* subsp. *boninensis*, *Morinda umbellata* subsp. *umbellata*, pollination, Rubiaceae, sex expression

Flowering plants are usually hermaphroditic and monomorphic in sex expression, but some species are sexually dimorphic. Information on sex expression is not only important for understanding the reproductive characteristics of individual species, but also for considering the evolutionary significance of floral variation (Bawa & Beach 1981, Richards 1997, Sakai & Weller 1999, Eckhart 1999). Consequently, although functional sexual expression has been examined in a variety of flowering plant species, numerous species remain unknown.

The genus *Morinda*, which contains approximately 80 species, is widely distributed in the tropical and subtropical regions of Central America, northern South America, Africa, Madagascar, the Mascarene Islands, and from India and Sri Lanka eastward to Southeast Asia, northern and northeastern Australia, and Melanesia and Polynesia (Peterson 1990, Johansson 1994). *Morinda umbellata* L. is widespread from Sri Lanka and India to southern China, Southeast Asia, including Taiwan and southwestern Japan, northern and northeastern Australia, and the Pa-
cific Islands (Wong 1984). It has long been regarded as a dioecious species (Wong 1984, Yamazaki 1986, 1993, Puff et al. 2005). It was recently reported, however, that *M. umbellata* L. subsp. *boninensis* (Ohwi) T. Yamaz., which is endemic to the Bonin (Ogasawara) Islands, Japan, is functionally androdiocious (Nishide et al. 2009). The sex expression of *M. umbellata* subsp. *boninensis* was regarded as being derived from disty- lous hermaphroditism, based on floral morphology (Nishide et al. 2009). Comparisons with the conspecific subsp. *umbellata*, the sex expression of which has been regarded as dioecy, is thus likely to further current understanding of the evolution of sexual systems in the species.

*Morinda umbellata* subsp. *umbellata* is widely distributed in eastern and southeastern Asia, but does not occur in the Bonin Islands. Our preliminary observations revealed subsp. *umbellata* to be functionally dioecious, as was suggested previously (Wong 1984, Yamazaki 1993, Puff et al. 2005), and clearly different from the system employed by subsp. *boninensis*. This is a rare case in which two different sexual systems, an- drodioecy and dioecy, co-occur among populations of the same species. In this paper, we report the morphological characteristics, nectar volume per flower, and pollinator visits to the flowers of subsp. *umbellata*. We also compared the sexual systems of subsp. *umbellata* and subsp. *boninensis*, reported previously (Nishide et al. 2009) and consider the evolutionary significance of two different sexual systems in populations of the same species.

**Materials and Methods**

**Study plants and sites**

*Morinda umbellata* L. subsp. *umbellata* is a perennial woody climber found near the edges of broad-leaved evergreen forests and flowers in June. Five to thirteen, condensed, umbellate inflorescences (referred to hereafter as compound umbels; Fig.1A, B) are apical on the shoots. All of the ovaries are fused into a compound structure, which we refer to here as a “capitulum” for this type of inflorescence, following Johansson (1994). The corollas are cup shaped, ca. 2 mm long, with four or five stamens, a single pistil and an inferior ovary (Figs. 1 & 2). Each ovary usually contains four ovules. Fruits mature in au- tumn and change color from green to orange. Each flower lasts only one day.

In the present study, we examined two populations located in southwestern Japan: Amami-Oshima, Ichiribaru (Amami-Oshima population) in Kagoshima Prefecture, and Iriomote-jima, Aira (Iriomote population) in Okinawa Prefec- ture.

**Floral morphology and pollen stainability**

To observe sexual and morphological differen- tiation, three to four capitula per plant were collected from 22 plants (12 pistillate and 10 sta- minate) in the Amami-Oshima population and from 18 plants (5 pistillate and 13 staminate) in the Iriomote-jima population, and preserved in FAA (formalin: acetic acid: 70 % ethanol = 1: 1: 18) or 70% ethanol. After fixing, two floral charac- ters were measured for each plant: (a) stigma height and (b) anther height, as illustrated in Fig. 2. The mean value of three flowers from three capita- lula was considered to be representative of each plant.

Pollen grains per flower were counted for 22 plants (12 pistillate and 10 staminate) from the Amami-Oshima population. Pollen stainability with aniline blue in lactophenol of more than 400 pollen grains per flower was then examined in 22 plants (12 pistillate and 10 staminate) from the Amami-Oshima population, and in 15 plants (5 pistillate and 10 staminate) from the Iriomote-jima population. To evaluate pollen stainability, two flowers per plant were examined and the mean value thus obtained was considered to be representative of each plant.

The number of flowers per capitulum was also examined in the Amami-Oshima population; three capitula from 15 plants (8 pistillate and 7 staminate) were randomly selected and the mean value was used as the representative flower number per capitulum.

To examine the ovules within ovaries, longi- tudinal sections of ovaries were observed using
Fig. 1. Flowers of Morinda umbellata subsp. umbellata growing on the edge of a broad-leaved evergreen forest on Amami-Oshima Isl., Kagoshima Pref. A: Pistillate plant with several inflorescences arranged in an apparent umbel. B: Staminate plant with several inflorescences arranged in an apparent umbel. C: Pistillate flower. D: Staminate flower. an, anther; co, corolla lobe; ov, ovary; stg, stigma; sty, style. Scale bar = 2 mm.

Fig. 2. Drawings of pistillate and staminate flowers from the Amami-Oshima population of Morinda umbellata subsp. umbellata. A: Pistillate flower. B: Staminate flower. a, stigma height; b, anther height; c, stigma length; d, anther length.
scanning electron microscopy (SEM). The samples from the two populations were dehydrated in an ethanol: t-butanol series, freeze-dried using a freeze-drying device (JFD-300, JEOL, Japan), mounted onto SEM stubs on double-sided carbon tape, coated with gold using an ion sputter coater (JFC-110E, JEOL), and observed using a scanning electron microscope (JSM-5600LV, JEOL).

**Nectary volume**

Nectar volume produced by flowers under open pollination was examined for 44 flowers from 6 plants (3 pistillate and 3 staminate) in the Amami-Oshima population. The nectar volume of each flower was collected and measured with 1- or 2-μL microcapillary tubes (EM minicaps, Hirschmann Laboratories, Germany) between 11:00 and 12:00 under fine weather condition.

**Flower visitors**

Insects visiting flowers were observed for two pistillate and two staminate plants growing close together along the edge of an evergreen broad-leaved forest in the Amami-Oshima population. Each plant bore more than 50 umbels. The observations were made intermittently during the day from 09:00 to 14:00 on June 21–24, 2007, June 21–22, 2008, and June 20–21, 2009 (approximately 30 hours in total).

**Results**

**Floral morphology and flower number per inflorescence**

Previously, Yamazaki (1993) reported *Morinda umbellata* subsp. *umbellata* to be sexually dimorphic, with stamine and pistillate plants, and that the style was rudimentary in stamine flowers. No mention, however, was made of the presence of abortive anthers in the pistillate flowers. Our observations of the floral morphology in natural populations of *M. umbellata* subsp. *umbellata* revealed this subspecies to be morphologically dimorphic, with pistillate and stamine plants (Fig. 1), as reported earlier. Morphologically, pistillate flowers had four or five stamens with apparent anthers and a style with two stigmatic lobes that extruded from the corolla tube (Figs. 1, 2). Stamine flowers had four or five stamens with anthers that extruded from the corolla tube, but completely lacked a style (Figs. 1, 2). While anthers from both pistillate and stamine flowers produced pollen grains, the pollen produced by pistillate flowers was significantly less than in stamine flowers (Mann-Whitney's U-test, *p* < 0.05; Fig. 3). Throughout flowering, the anthers of the pistillate flowers did not open, and pollen from those flowers did not stain with aniline blue in lactophenol, suggesting pollen sterility. Conversely, pollen from stamine flowers stained well with aniline blue in lactophenol, with stainability exceeding 90% (93.0 ± 4.2%, *N* = 10 in the Amami-Oshima population; 91.6 ± 6.1%, *N* = 10 in the Iriomote population). The

![Fig. 3. Number of pollen grains produced by pistillate (female) and stamine (male) flowers from the Amami-Oshima population of *Morinda umbellata* subsp. *umbellata*. Vertical lines indicate standard deviations. Numbers represent sample sizes.](image)
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FIG. 4. SEM micrographs of longitudinal sections of flowers, showing ovules (arrows) in the ovaries of Morinda umbellata subsp. umbellata. A: Pistillate flower, B: Staminate flower. Arrows indicate ovules.

Ovaries of both pistillate (Fig. 4A) and staminate flowers (Fig. 4B) consistently contained four ovules, with no significant difference in size and shape. Mature fruits, however, were observed only on pistillate plants. Taken together, these findings indicate that subspecies umbellata is obviously dioecious.

Comparisons of the stigma and anther heights from pistillate and staminate flowers are shown in Fig. 5. In the pistillate flowers, stigmas were significantly higher than the anthers (Fig. 5). Although the anthers of staminate flowers were significantly higher than those of the pistillate flowers, they were not significantly higher than the stigmas of the pistillate flowers (Fig. 5). These findings indicate that stigmas and anthers were reciprocally positioned between the two sexual morphs.

The mean number of flowers per inflorescence was higher (Mann-Whitney’s U-test, \( p < 0.01 \)) in staminate plants (9.5 ± 1.6, \( N = 8 \)) than in pistillate plants (5.4 ± 0.6, \( N = 7 \)).

FIG. 5. Stigma and anther heights of pistillate (F) and staminate (M) flowers of the Amami-Oshima and Iriomote-jima populations of Morinda umbellata subsp. umbellata. Vertical lines indicate standard deviations. Numbers represent sample sizes. Letters (a, b) indicate significant differences at \( p < 0.05 \) determined by Steel-Dwass’ multiple range test.
Nectar production

Nectar was produced inside the corolla tube and stored at its base (Fig. 1C, D). Occasionally, however, nectar appeared on the long, oblique-facing hairs of the corolla tube. Nectar volume (μL) per flower under open conditions varied considerably among flowers of the same plant. Mean nectar volume per flower in pistillate and staminate flowers was 1.52 ± 0.77 (N = 23) and 1.46 ± 1.03 (N = 21). No significant differences were observed between the two sexual morphs (Mann-Whitney U-test, p = 0.38).

Flower visitors

In Morinda umbellata subsp. umbellata, insects such as bees (Nomia pavonura, Xylocopa amamensis, Amegilla senhai, etc.), wasps (Sphex diabolicus, Scolia fascinata, etc.), hover flies (Milesia oshimaensis, Phytomia zonata, etc.), bee flies (Ligya tantalus), butterflies (Parantica sita, Graphium sarpedon, etc.), and beetles (Cerambycidae), visited the staminate and pistillate flowers during the observation periods (Fig. 6; see also Table 1) and usually fed on the nectar within the short corolla tube. In addition, bees and hover flies occasionally also collected pollen when visiting the staminate flowers. Of these visitors, bees, wasps, hover-flies and bee flies often came into physical contact with the anthers or stigmas of the different flowers they visited. Consequently, those insects are considered likely to be effective pollinators of subspecies. Conversely, butterflies and bee flies may not be effective pollinators since they only rarely come into physical contact with the stigmas or anthers.

Fig. 6. Insects visiting Morinda umbellata subsp. umbellata flowers from the Amami-Oshima population. A: Milesia oshimaensis (Syrphidae) visiting staminate flowers. B: Nomia pavonura (Halictidae) visiting staminate flowers. C: Eristalis tenax (Syrphidae) visiting pistillate flowers. D: Sphex diabolicus (Sphecidae) visiting pistillate flowers.

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<td><em>Cyphononyx fulvognathus</em> (♀)</td>
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<td><strong>Philanthidae</strong></td>
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<td><em>Cerceris okumurai</em> (♀)</td>
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<td><em>Polistes rothneyi</em> (♀)</td>
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<td><em>Eumerus sp.</em> (♀)</td>
<td><em>Milesia oshimaeensis</em> (♀ &amp; ♂)</td>
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<td><em>Milesia oshimaeensis</em> (♀ &amp; ♂)</td>
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<td><em>Eristalis tenax</em> (♀)</td>
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<td><strong>Conopidae</strong></td>
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<td><em>Conops santaroi</em> (♀)</td>
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<td><strong>Bombilyidae</strong></td>
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<td><em>Ligryrta tanta</em> (♀)</td>
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<td><strong>Cerambycidae</strong></td>
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<td><em>Leptura ochraceofasciata amamiana</em> (♀)</td>
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<td><strong>Papilionidae</strong></td>
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<td><em>Papilio memnon</em> (?)</td>
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<td><strong>Nymphalidae</strong></td>
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<td><em>Parantica sita</em> (?)</td>
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**Discussion**

The present study revealed that sex expression in *Morinda umbellata* subsp. *umbellata* is functionally dioecious, with both pistillate and staminate plants occurring separately in a natural population. Although Yamazaki (1993) did not mention it, the pistillate flowers have four or five abortive stamens as well as a style with two stigmatic lobes that extruded from the corolla tube. In comparison, the staminate flowers completely lack a style with bifurcated stigmas, but retain ovules within an ovary.

Although the sexual system of *Morinda umbellata* subsp. *umbellata* differs from that of subsp. *boninensis*, which is functionally androdioecious (Nishide et al. 2009), these two subspecies are closely allied and share the androdioecious floral morphology. Our unpublished phylogenetic analysis using the ITS sequences of 39 populations from 28 taxa, most of which are also included in Razafimandimbison et al. (2009), suggested that the Japanese populations of the two subspecies of *M. umbellata* were closely related and formed a monophyletic group. These data allow us to consider the evolutionary relationship between androdioecy and dioecy in the two subspecies of *M. umbellata*. In general, androdioecy has been regarded as being derived from dioecy (Pannell 2002), and reports of several species, such as *Datisca glomerata* (Datiscaceae; Liston et al. 1990), *Mercurialis annua* (Euphorbiaceae; Pannell 1997, Oubbard et al. 2006), and *Schizopepon bryoniiifolius* (Cucurbitaceae; Akimoto et al. 1999), support this hypothesis. However, it is occasionally suggested that androdioecy has been derived from hermaphroditism, as in *Sagittaria*
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lancifolia (Alismataceae; Muenchow 1998) and Fraxinus spp. (Oleaceae; Domme et al. 1999, Wallander 2001). If we compare the floral traits in the dioecious M. umbellata subsp. umbellata with those of the androecious subsp. boninensis, the fundamental difference between the two subspecies is whether the anthers produce stainable pollen grains or not. We therefore consider that the dioecy found in subsp. umbellata might have evolved from androecy by abortion of the anthers, because abortion of the anthers occurs more frequently in flowering plants. What still remains unclear, however, is the derivation of staminate flowers that contain ovules, but completely lack a style. To clarify the morphological differentiation of sexual systems in M. umbellata, further comparison with other related species, especially those in eastern Asia, will be needed.

In a previous study, Bawa (1980) reported that the majority of dioecious species in wet evergreen forests were entomophilous rather than anemophilous and were pollinated by small bees belonging to the Halictidae, Megachilidae, and Meliponini. He also suggested that the flowers of almost all insect-pollinated dioecious species are relatively small, unspecialized, and white, pale yellow, or pale green in color. The flowers of Morinda umbellata subsp. umbellata are small, actinomorphic and pale green in color, and they secrete nectar, which are floral traits considered to be typical of insect-pollinated, dioecious species in wet evergreen forests (Bawa 1980). The short tubed corolla (about 2 mm long) of subsp. umbellata allows for visits by small, short-tongued bees. In the Amami-Oshima population, a variety of opportunistic insects, such as wasps, short-tongued bees, hover flies, bee flies, beetles, and butterflies, were observed to feed mainly on the nectar of staminate and pistillate flowers of subsp. umbellata, and occasionally to collect the pollen from staminate flowers. As in other dioecious species from the wet evergreen forests, wasps, hover flies, and small, short-tongued bees are the main pollinators of subsp. umbellata, and the nectar secreted from the corolla tube is likely to be the primary floral reward for these insects.

In the androecious Morinda umbellata subsp. boninensis, staminate and hermaphroditic flowers have been reported to produce both pollen and nectar as rewards (Nishide 2006). In subsp. boninensis the corolla tube of the hermaphroditic flowers (N = 5) is 3.0–3.3 mm long and 1.5–2.2 mm wide and 2.5–3.3 mm long and 1.9–2.0 mm wide, in the staminate flowers (N = 5). In subsp. umbellata, on the other hand, the corolla tube of the pistillate flowers (N = 5) is 1.8–2.0 mm long and 2.0–2.2 mm wide, while in the staminate flowers (N = 5) the tube is 2.0–2.2 mm long and 2.1–2.4 mm wide. Corolla size is therefore slightly larger but nectar volume per flower is smaller in subsp. boninensis than in subsp. umbellata (Fig. 7). Meanwhile, since the pistillate flowers of the dioecious subsp. umbellata described here completely lack pollen as a reward, the production of nectar is considered to be important for the pollination of subsp. boninensis. We suspect that the difference in reward require-

![Fig. 7. Nectar volume per flower under open condition in pistillate (F) and staminate (M) flowers of Morinda umbellata subsp. umbellata (Amami-Oshima pop.) and in hermaphroditic (H) and staminate (M) flowers of Morinda umbellata subsp. boninensis (Chichi-jima and Haha-jima populations in the Bonin Islands). Data for subsp. boninensis are based on Nishide (2006). Vertical lines indicate standard deviations. Numbers represent sample size. Letters (a, b, c, d) indicate significant differences at p < 0.05 by Steel-Dwass' multiple range test.](image-url)
ments is responsible for the different sexual systems of the two subspecies of *M. umbellata*. Subsp. *boninensis* is largely pollinated by an introduced bee species, *Apis mellifera* (Abe 2006, Nishide et al. 2009), but no information is available on the native pollinators of this subspecies. Further detailed investigations of the native pollinators and their behavior on subsp. *boninensis* are therefore considered to be important for understanding the evolutionary significance of the observed differences in the sexual systems of the two subspecies.

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