Title:

Explosive wind-pollination in a monoecious plant, *Laportea bulbifera* (Urticaceae)

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Abstract

Laportea bulbifera (Ulticaceae) is a monoecious plant that has a unique sexual expression: female flowers form on the upper part and male flowers on the lower part on an individual shoot. Therefore, for the seed reproduction, pollen needs to be transferred from the lower (male) to the upper (female) flowers. Our observations of male flowers confirmed that pollen was dispersed upward by "explosive wind-pollination". A male flower has five stamens, and when the petals are open, the stamens were caught in a pistillode. With the growth of the stamens, they were released from the pistillode, and then straightened with a spring-like movement of the filament. At the same time, the anthers dehisced, and the pollen was dispersed. The explosive release of pollen from the anthers and light wind in the habitat (forest edge or gap) contributes to wind-pollination in *L. bulbifera*.

Keywords: anemophily, geitonogamy, monoecy, pistillode, self-pollination

INTRODUCTION

Most species of flowering plants are hermaphroditic. Among angiosperm species, only 5% of monoecious plants have separate sex flowers on the same individual (Barrett, 2002; Torices et al., 2011). Hermaphroditic flowers are more economical because the costs of the non-sexual parts of the flower, such as nectar, petals, and sepals, are shared by male and female functions. On the other hand, it has been argued that monoecy favors outcrossing, reduces pollen–stigma interference, allows a more flexible allocation of gender in a variable environment, and allows a more exact sex allocation in a constant environment (Chalesworth & Chalesworth, 1979; Lloyd, 1979). In a monoecious plant, male and female flowers are physically separated within an individual rather than having hermaphrodite flowers. Thus, a mechanism for pollen transfer between the separated flowers is required even in the case of geitonogamy.

Laportea bulbifera (Urticaceae) is a monoecious perennial herb growing on mesic forest edges. This plant forms female flowers on the upper part of the shoot and develops inflorescences of male flowers from the lower axils of the shoot (Fig. 1). Therefore, it is considered that for the seed reproduction, this plant should have a mechanism for pollen dispersal that allows pollen to move from the lower to upper part of the plant. High-speed pollen release from male flowers found in Moraceae can achieve such upward wind pollination (Taylor et al. 2006). Pederoli et al. (2019) clarified anatomical mechanisms of the explosive pollen release in the urticalean rosids (Cannabaceae, Moraceae, and Urticaceae), including a congeneric species, *L. aestuans*. The purpose of this study is to elucidate the mechanism that enables such pollen movement in *L. bulbifera* by careful observations and pollination experiments in the field

MATERIAL AND METHODS

Study species and study site

Laportea bulbifera (Siebold et Zucc.) Wedd. (Urticaceae) is a perennial herb that grows on the edge of forests. In Japan, this species is widely distributed from Hokkaido to Kyushu (Ohashi et al., 2016). This plant is monoecious and forms female flowers on the upper part (Fig. 1a, d) and male flowers in the lower part of the shoot (Fig. 1 b, e). Female flowers form on the shoot apex and have one ovule per stigma. Male flowers form in the axils with hundreds to thousands of small flowers with a diameter of 1 to 3 mm per shoot (Ohashi et al., 2016). The flowering period is from late July to late September, and seed formation occurs from late August to mid-October. In addition to seed production via female flowers, this plant forms bulbils, a vegetative propagation organ, on the axils (Fig. 1c).

Field studies (observations and experiments) were conducted on a population of *L. bulbifera* in Nopporo Forest Park (43°06'N, 141°51'E), Ebetsu City (vicinity of Sapporo City), Hokkaido.

Field observations and pollination experiments

To elucidate the mechanism of pollen dispersal, we made careful observations of the morphology of male flowers including anther dehiscence in the field. In addition, the dynamics of male flowers were recorded by using a camera, OLYMPUS STYLUS TG-2 Tough (Olympus Corporation).

We conducted emasculation, i.e., male flowers were removed from the shoot before flowering, to investigate the possibility of pollen transfer to female flowers from each shoot. If the female flowers of the emasculated shoot produced seeds, they must have received the pollen from other male flowers. We randomly selected 33 plants and carried out emasculation in 2021. We also randomly marked 55 plants with both male and female flowers as controls. At fruiting (September), we counted the total number of seeds and undeveloped female flowers. Female flowers have one ovule per flower, and the presence or absence of seed can be judged from the appearance. We calculated seed-setting rates (ratio of total number of seeds to total number of female flowers) for each shoot and compared between the two treatments.

In addition, we applied two artificial pollination treatments to evaluate the degree of self- compatibility, as follows: (1) self-pollination: female flowers were pollinated with using pollen of male flowers on the same shoot, (2) cross-pollination: female flowers were pollinated with pollen from male flowers on a different shoot (at least 100 m apart). After treatment, the flowers were covered by cellophane bags to prevent unintentional pollination. We used 20 plants randomly selected in the field for both self-pollination and cross-pollination experiments. At fruiting (September), we calculated seed-setting rates of each shoot and compared between the two treatments. A generalized linear model (GLM) was used to evaluate the effect of treatment on seed-setting rates (binomial distribution). The response variable was seed-setting rates, and the explanatory variable was the pollination treatment. GLM was performed with R version 4.0.2, using package “stats” (R Development Core Team, 2020).

RESULTS

Observation of male flowers

Figure 2 shows sequential photographs of the male flowers within 0.1 second. It can be readily recognized that pollen was released upwards from the male flowers like white dust. Additional detailed observation of male flowers clarified that each flower had five stamens, and when the petals were open, the stamens were caught in a pistillode (Fig. 3a, c-left). With the growth of the stamens, the stamens were released from the pistillode and straightened with a spring-like movement of the filament (Fig. 3b). At the same time, the anthers dehisced, and the pollen was released (Fig. 3c-right).

Pollination experiments

Figure 4 shows the results of pollination experiments. Of the individuals marked and treated for the experiments, some were damaged by insect feeding. Figure 4a illustrates the seed-setting rates of the emasculated shoots and controls. The average seed-setting rate of the emasculated shoots was 64.3 ± 1.6% (SD) (n = 21), and that of the control was 61.8 ± 2.2% (n = 44). A GLM showed no significant difference between the two treatments (P = 0.77). Therefore, it was confirmed that pollen transfer to other shoots can occur sufficiently.

Figure 4b illustrates the seed-setting rates of “self-pollination” and “cross-pollination”. The average seed-setting rate of self-pollination was 67.5 ± 5.5% (n = 10), and that of cross-pollination was 69.5 ± 4.9% (n = 13). A GLM showed no significant difference between the two treatments (P = 0.49), indicating that there was no difference in seed formation ability between self-fertilization and cross-fertilization. Therefore, high self-compatibility of this plant was confirmed.

DISCUSSION

Based on careful field observations of male flowers of *L. bulbifera*, it was clarified that the stamens were caught in a pistillode, and pollen was dispersed upward with a spring-like movement of the filament (Figs. 2 & 3). This pattern of pollen dispersal is called "Explosive wind-pollination” and has also been reported in several anemophilous herbaceous plants such as *Nanocnide japonica* Blume, *Boehmeria silvestrii* (Pamp.) W.T.Wang, and *Acalypha australis* L. (Knuth, 1906; Tanaka, 2000). Pedersoli et al. (2019) anatomically clarified the unusual synorganization of the staminate flower in wind-pollinated utricalean rosids including *Laportea aestuans* by using light and scanning electron microscopy. They detailed that the the pistillodes, stamens, and sepals form a floral apparatus that explosively release pollen to be carried by wind. The anthers dehisce when the stamens are still inflexed on the floral bud and an enveloped by the sepals and supported by an inflated pistillodes. The present study also confirmed that the same mechanisms operate in *L. bulbifera.*

In *L. bulbifera*, explosive wind-pollination functions well to enhance the impulse of pollen and successfully transfer pollen from lower (male) flowers to upper (female) flowers and to produce the seeds (Fig. 4). As shown in Fig. 4b, *L. bulbifera* has high self-compatibility, suggesting that seed formation is possible by geitonogamy within its own shoot. Further investigation is needed to elucidate how self-fertilized seed formation contributes to population maintenance in this plant. In addition, as the species name ‘*bulbifera*’ indicates, *L. bulbifera* produces bulbils on the axils (asexual propagules) that are much larger in size than the seeds (Fig. 1c). Thus, *L. bulbifera* is a species suitable for evaluating the contribution of sexual and asexual reproductive options to population dynamics (Tsujimoto et al., in preparation).

ACKNOWLEDGEMENTS

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Figure Legends

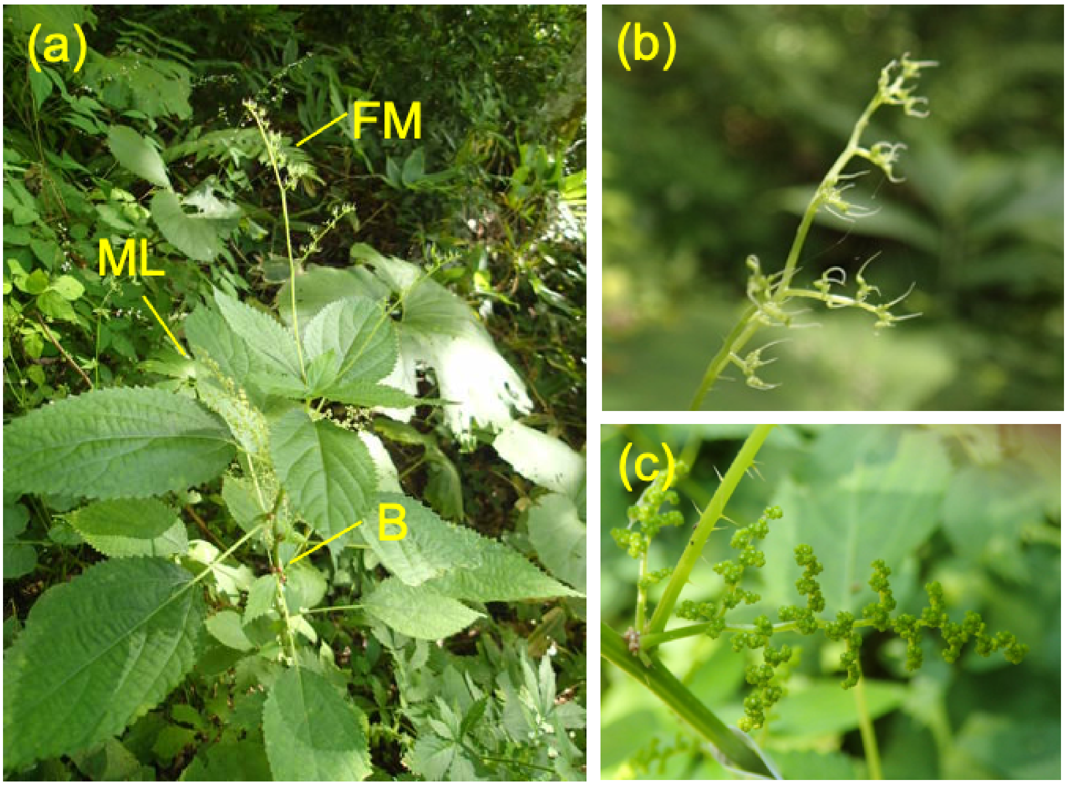

Figure 1: Flowering individual of *Laportea bulbifera*. (a) FM, female flowers; ML, male flowers; B, bulbils, (b) enlarged photo of female flowers, (e) buds of male flowers.

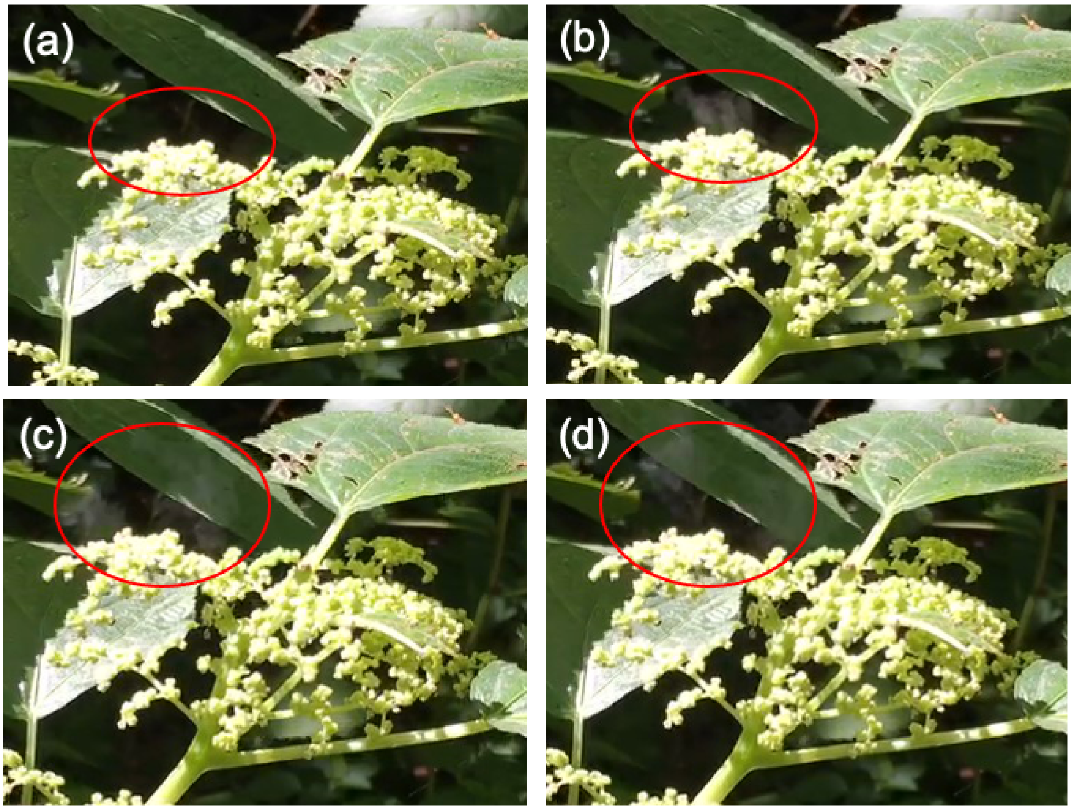

Figure 2: High-speed consecutive photographs of pollen dispersal from male flowers. Photos taken by a digital camera: OLYMPUS STYLUS TG-2 Tough (Olympus Corporation). Photos shown at (a) 0.00 second, (b) 0.04 s, (c) 0.09 s, (d) 0.10 s. The red color ellipses show the diffusion states at each timing.

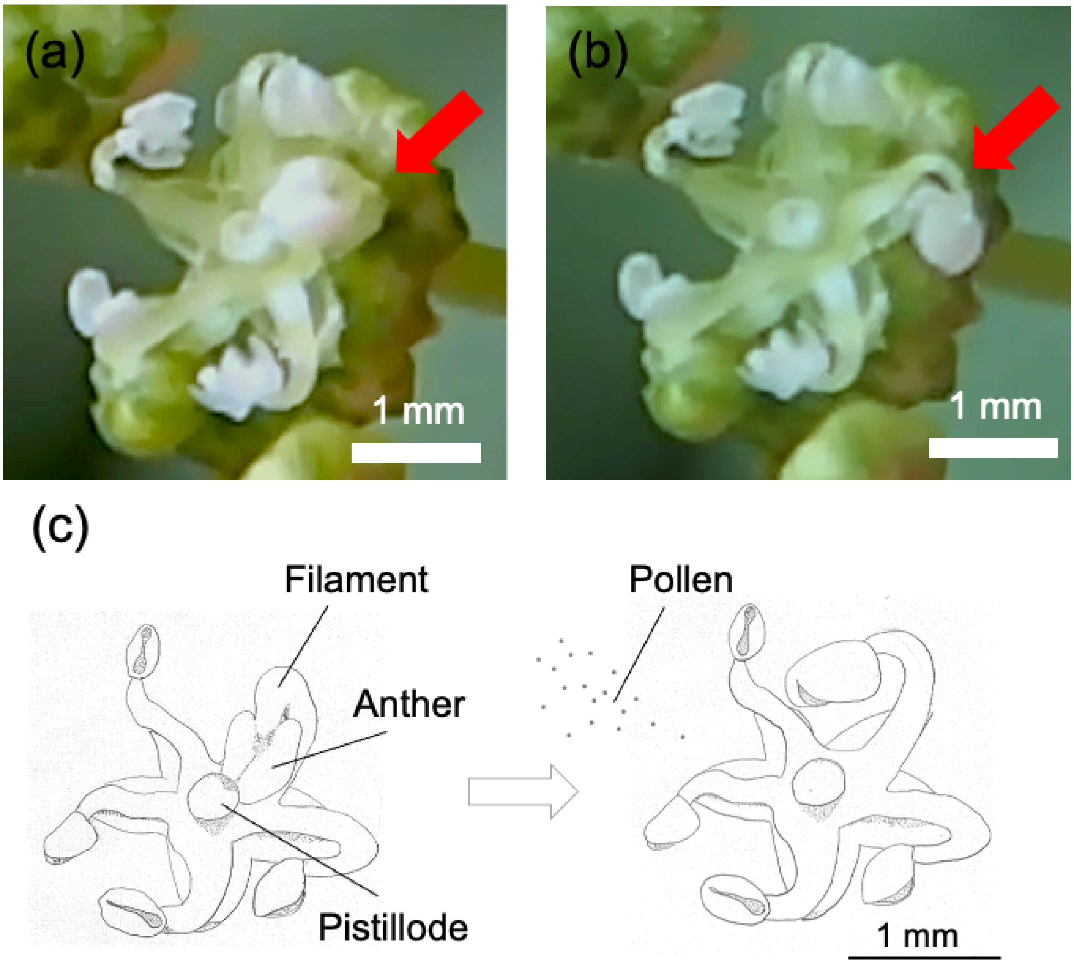

Figure 3: Mechanisms of pollen dispersal of a male flower. (a) inflexed stamen (red allow), (b) dehiscence of anthers with a spring-like movement of filament, (c) illustrations showing the mechanisms of pollen dispersal.

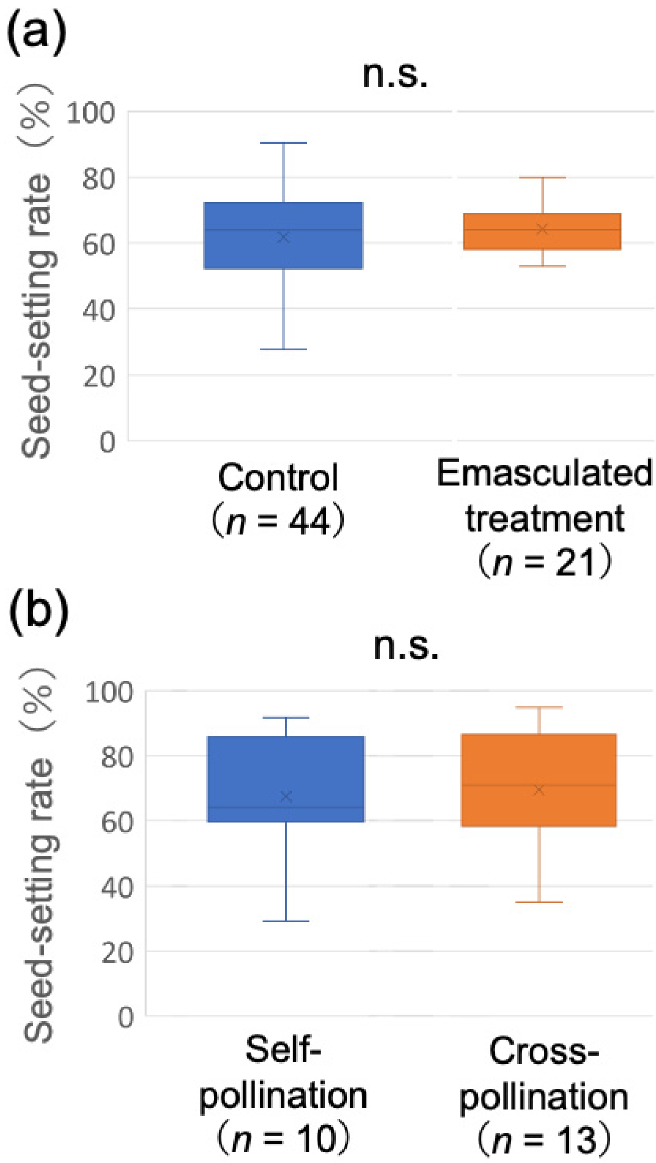

Figure 4: (a) Comparison of seed setting rates between control and emasculated shoots. (GLM, *P* = 0.77). (b) Comparison of seed setting rates between self-pollination and cross-pollination. (GLM; Generalized Linear Model, *P* = 0.49). The numbers in the parentheses are sample sizes. “n.s” means that there are no significant differences between the treatments.