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3	Explosive wind-pollination in a monoecious plant, <i>Laportea bulbifera</i> (Urticaceae)
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# 24 Abstract

25	Laportea bulbifera (Ulticaceae) is a monoecious plant that has a unique sexual
26	expression: female flowers form on the upper part and male flowers on the lower part
27	on an individual shoot. Therefore, for the seed reproduction, pollen needs to be
28	transferred from the lower (male) to the upper (female) flowers. Our observations of
29	male flowers confirmed that pollen was dispersed upward by "explosive wind-
30	pollination". A male flower has five stamens, and when the petals are open, the stamens
31	were caught in a pistillode. With the growth of the stamens, they were released from the
32	pistillode, and then straightened with a spring-like movement of the filament. At the
33	same time, the anthers dehisced, and the pollen was dispersed. The explosive release of
34	pollen from the anthers and light wind in the habitat (forest edge or gap) contributes to
35	wind-pollination in <i>L. bulbifera</i> .
36	

- 37 Keywords: anemophily, geitonogamy, monoecy, pistillode, self-pollination
- 38

### **39 INTRODUCTION**

40 Most species of flowering plants are hermaphroditic. Among angiosperm species, only 41 5% of monoecious plants have separate sex flowers on the same individual (Barrett, 42 2002; Torices et al., 2011). Hermaphroditic flowers are more economical because the 43 costs of the non-sexual parts of the flower, such as nectar, petals, and sepals, are shared 44 by male and female functions. On the other hand, it has been argued that monoecy 45 favors outcrossing, reduces pollen-stigma interference, allows a more flexible 46 allocation of gender in a variable environment, and allows a more exact sex allocation 47 in a constant environment (Chalesworth & Chalesworth, 1979; Lloyd, 1979). In a 48 monoecious plant, male and female flowers are physically separated within an 49 individual rather than having hermaphrodite flowers. Thus, a mechanism for pollen 50 transfer between the separated flowers is required even in the case of geitonogamy. 51 Laportea bulbifera (Urticaceae) is a monoecious perennial herb growing on mesic 52 forest edges. This plant forms female flowers on the upper part of the shoot and 53 develops inflorescences of male flowers from the lower axils of the shoot (Fig. 1). 54 Therefore, it is considered that for the seed reproduction, this plant should have a 55 mechanism for pollen dispersal that allows pollen to move from the lower to upper part 56 of the plant. High-speed pollen release from male flowers found in Moraceae can 57 achieve such upward wind pollination (Taylor et al. 2006). Pederoli et al. (2019) 58 clarified anatomical mechanisms of the explosive pollen release in the urticalean rosids 59 (Cannabaceae, Moraceae, and Urticaceae), including a congeneric species, L. aestuans. 60 The purpose of this study is to elucidate the mechanism that enables such pollen 61 movement in L. bulbifera by careful observations and pollination experiments in the 62 field

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### 65 MATERIAL AND METHODS

# 66 Study species and study site

67 *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

69 Kyushu (Ohashi et al., 2016). This plant is monoecious and forms female flowers on the

vul upper part (Fig. 1a, d) and male flowers in the lower part of the shoot (Fig. 1 b, e).

71 Female flowers form on the shoot apex and have one ovule per stigma. Male flowers

72 form in the axils with hundreds to thousands of small flowers with a diameter of 1 to 3

73 mm per shoot (Ohashi et al., 2016). The flowering period is from late July to late

74 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 propagationorgan, 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.

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# 82 Field observations and pollination experiments

To elucidate the mechanism of pollen dispersal, we made careful observations of themorphology of male flowers including anther dehiscence in the field. In addition, the

85 dynamics of male flowers were recorded by using a camera, OLYMPUS STYLUS TG-

86 2 Tough (Olympus Corporation).

87 We conducted emasculation, i.e., male flowers were removed from the shoot before 88 flowering, to investigate the possibility of pollen transfer to female flowers from each 89 shoot. If the female flowers of the emasculated shoot produced seeds, they must have 90 received the pollen from other male flowers. We randomly selected 33 plants and 91 carried out emasculation in 2021. We also randomly marked 55 plants with both male 92 and female flowers as controls. At fruiting (September), we counted the total number of 93 seeds and undeveloped female flowers. Female flowers have one ovule per flower, and 94 the presence or absence of seed can be judged from the appearance. We calculated seed-95 setting rates (ratio of total number of seeds to total number of female flowers) for each 96 shoot and compared between the two treatments.

97 In addition, we applied two artificial pollination treatments to evaluate the degree 98 of self- compatibility, as follows: (1) self-pollination: female flowers were pollinated 99 with using pollen of male flowers on the same shoot, (2) cross-pollination: female 100 flowers were pollinated with pollen from male flowers on a different shoot (at least 100 101 m apart). After treatment, the flowers were covered by cellophane bags to prevent 102 unintentional pollination. We used 20 plants randomly selected in the field for both self-103 pollination and cross-pollination experiments. At fruiting (September), we calculated 104 seed-setting rates of each shoot and compared between the two treatments. A 105 generalized linear model (GLM) was used to evaluate the effect of treatment on seed-106 setting rates (binomial distribution). The response variable was seed-setting rates, and 107 the explanatory variable was the pollination treatment. GLM was performed with R 108 version 4.0.2, using package "stats" (R Development Core Team, 2020). 109

110 **RESULTS** 

#### 111 **Observation of male flowers**

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Figure 2 shows sequential photographs of the male flowers within 0.1 second. It can be 113 readily recognized that pollen was released upwards from the male flowers like white 114 dust. Additional detailed observation of male flowers clarified that each flower had five 115 stamens, and when the petals were open, the stamens were caught in a pistillode (Fig. 116 3a, c-left). With the growth of the stamens, the stamens were released from the 117 pistillode and straightened with a spring-like movement of the filament (Fig. 3b). At the 118 same time, the anthers dehisced, and the pollen was released (Fig. 3c-right). 119 120 **Pollination experiments** 121 Figure 4 shows the results of pollination experiments. Of the individuals marked and 122 treated for the experiments, some were damaged by insect feeding. Figure 4a illustrates 123 the seed-setting rates of the emasculated shoots and controls. The average seed-setting 124 rate of the emasculated shoots was  $64.3 \pm 1.6\%$  (SD) (n = 21), and that of the control 125 was  $61.8 \pm 2.2\%$  (*n* = 44). A GLM showed no significant difference between the two 126 treatments (P = 0.77). Therefore, it was confirmed that pollen transfer to other shoots 127 can occur sufficiently. 128 Figure 4b illustrates the seed-setting rates of "self-pollination" and "cross-129 pollination". The average seed-setting rate of self-pollination was  $67.5 \pm 5.5\%$  (n = 10), 130 and that of cross-pollination was  $69.5 \pm 4.9\%$  (*n* = 13). A GLM showed no significant 131 difference between the two treatments (P = 0.49), indicating that there was no

132 difference in seed formation ability between self-fertilization and cross-fertilization.

133 Therefore, high self-compatibility of this plant was confirmed.

### **135 DISCUSSION**

136 Based on careful field observations of male flowers of L. bulbifera, it was clarified that 137 the stamens were caught in a pistillode, and pollen was dispersed upward with a spring-138 like movement of the filament (Figs. 2 & 3). This pattern of pollen dispersal is called 139 "Explosive wind-pollination" and has also been reported in several anemophilous 140 herbaceous plants such as *Nanocnide japonica* Blume, *Boehmeria silvestrii* (Pamp.) 141 W.T.Wang, and Acalypha australis L. (Knuth, 1906; Tanaka, 2000). Pedersoli et al. 142 (2019) anatomically clarified the unusual synorganization of the staminate flower in 143 wind-pollinated utricalean rosids including Laportea aestuans by using light and 144 scanning electron microscopy. They detailed that the the pistillodes, stamens, and sepals 145 form a floral apparatus that explosively release pollen to be carried by wind. The 146 anthers dehisce when the stamens are still inflexed on the floral bud and an enveloped 147 by the sepals and supported by an inflated pistillodes. The present study also confirmed 148 that the same mechanisms operate in L. bulbifera. 149 In L. bulbifera, explosive wind-pollination functions well to enhance the impulse of 150 pollen and successfully transfer pollen from lower (male) flowers to upper (female) 151 flowers and to produce the seeds (Fig. 4). As shown in Fig. 4b, L. bulbifera has high 152 self-compatibility, suggesting that seed formation is possible by geitonogamy within its 153 own shoot. Further investigation is needed to elucidate how self-fertilized seed 154 formation contributes to population maintenance in this plant. In addition, as the species 155 name 'bulbifera' indicates, L. bulbifera produces bulbils on the axils (asexual 156 propagules) that are much larger in size than the seeds (Fig. 1c). Thus, L. bulbifera is a 157 species suitable for evaluating the contribution of sexual and asexual reproductive 158 options to population dynamics (Tsujimoto et al., in preparation).

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# 194 Figure Legends



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



206	Figure 2: High-speed consecutive photographs of pollen dispersal from male flowers.
207	Photos taken by a digital camera: OLYMPUS STYLUS TG-2 Tough (Olympus
208	Corporation). Photos shown at (a) 0.00 second, (b) 0.04 s, (c) 0.09 s, (d) 0.10 s. The
209	red color ellipses show the diffusion states at each timing.
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218 Figure 3: Mechanisms of pollen dispersal of a male flower. (a) inflexed stamen (red

- 219 allow), (b) dehiscence of anthers with a spring-like movement of filament, (c)
- 220 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.