



Title	Explosive wind-pollination in a monoecious plant, <i>Laportea bulbifera</i> (Urticaceae)
Author(s)	Tsujimoto, Ryutaro; Ohara, Masashi
Citation	Plant Species Biology, 37(5), 327-331 https://doi.org/10.1111/1442-1984.12384
Issue Date	2022-09
Doc URL	http://hdl.handle.net/2115/90453
Rights	This is the peer reviewed version of the following article: Tsujimoto, R., & Ohara, M. (2022). Explosive wind-pollination in a monoecious plant, <i>Laportea bulbifera</i> (Urticaceae). <i>Plant Species Biology</i> , 37(5), 327– 331, which has been published in final form at https://doi.org/10.1111/1442-1984.12384 . This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions. This article may not be enhanced, enriched or otherwise transformed into a derivative work, without express permission from Wiley or by statutory rights under applicable legislation. Copyright notices must not be removed, obscured or modified. The article must be linked to Wiley 's version of record on Wiley Online Library and any embedding, framing or otherwise making available the article or pages thereof by third parties from platforms, services and websites other than Wiley Online Library must be prohibited.
Type	article (author version)
File Information	HUSCAP_Tsujimoto & Ohara_PSB(2022).pdf



[Instructions for use](#)

1 **Title:**

2

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

4

5

6 **Authors:**

7 **Ryutaro Tsujimoto and Masashi Ohara***

8

9

10

11 **Affiliation:**

12 Graduate School of Environmental Science, Hokkaido University, Kita-10 Nishi-5,

13 Sapporo 060-0810, Japan

14

15

16 **Correspondence:**

17 Masashi Ohara, Graduate School of Environmental Science, Hokkaido University, Kita-

18 10 Nishi-5, Sapporo 060-0810, Japan

19 Email: ohara@ees.hokudai.ac.jp

20 Orchid ID: <https://orcid.org/0000-0003-3277-5743>

21

22

23

24 **Abstract**

25 *Laportea bulbifera* (Urticaceae) 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 *L. bulbifera*.

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

63

64

65 **MATERIAL AND METHODS**

66 **Study species and study site**

67 *Laportea bulbifera* (Siebold et Zucc.) Wedd. (Urticaceae) is a perennial herb that grows
68 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
70 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
75 seed production via female flowers, this plant forms bulbils, a vegetative propagation
76 organ, on the axils (Fig. 1c).

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

80

81

82 **Field observations and pollination experiments**

83 To elucidate the mechanism of pollen dispersal, we made careful observations of the
84 morphology 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**

112 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.

134

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).

159 **ACKNOWLEDGEMENTS**

160 We thank Mr. Abe and Mr. Naruse for their in the field work and Hokkaido Prefectural
161 Government for permission to carry out this study in Nopporo Forest Park. This study
162 was supported by Grants-in Aid for Scientific Research from the Japan Society for the
163 Promotion of Science (JSPS) (19H03294, 20K06821).

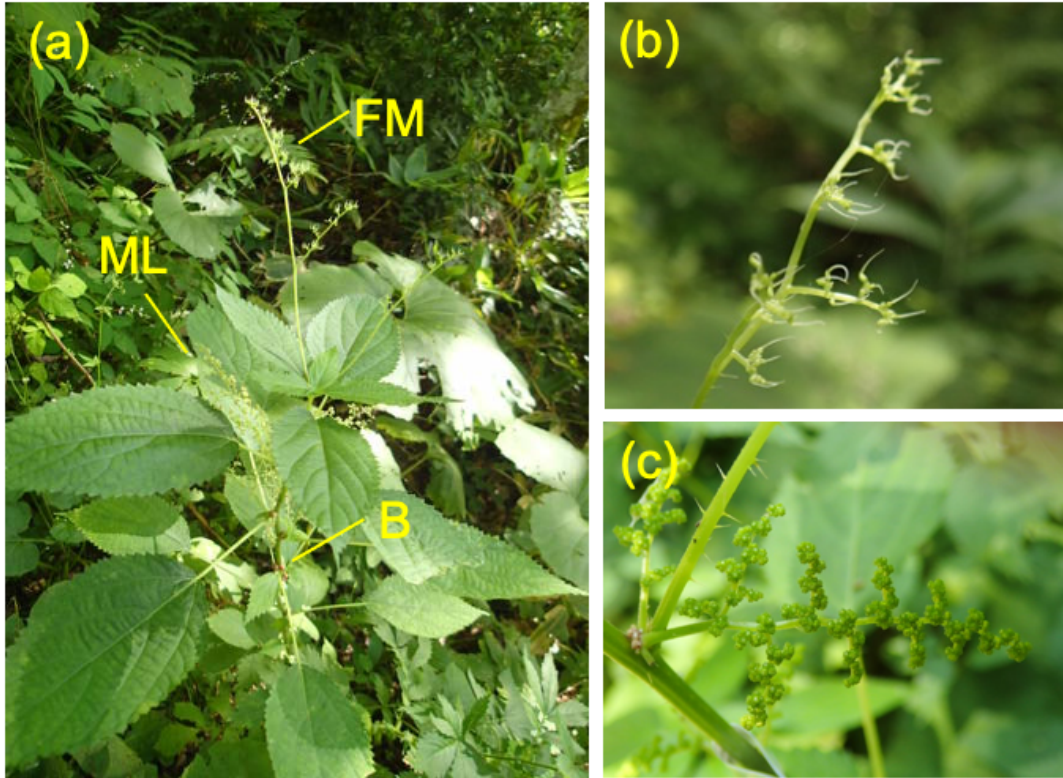
164

165 **REFERENCES**

- 166 Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews*
167 *Genetics*, 3, 274–284. <https://doi.org/10.1038/nrg776>
- 168 Chalesworth, D. & Chalesworth, B. (1979). The evolutionary genetics of sexual systems
169 in flowering plants. *Proceedings of the Royal Society B. Biological Sciences* 205,
170 513–530. <https://doi.org/10.1098/rspb.1979.0082>
- 171 Knuth, P. (1906). Handbook of Flower Pollination: based upon Hermann Müller's work
172 'The Fertilization of Flowers by Insects'. Oxford, Clarendon Press.
- 173 Lloyd, D. G. (1979) Evolution towards dioecy in heterostylous populations. *Plant*
174 *Systematics & Evolution*, 131, 71–80. <https://doi.org/10.1007/BF00984123>
- 175 Ohashi, H., Kadota, Y., Murata, J., Yonekura, K. & Kihara, H. (2016). Wild Flowers of
176 Japan. Vol. 2. Heibon-Sha, Japan. (In Japanese)
- 177 Pedersoli, G. D. Leme, F. M., Leite, V. G. & Teixeira, S. P. (2019). Anatomy solve the
178 puzzle of explosive pollen release in wind- pollinated urticalean rosids. *American*
179 *Journal of Botany*, 106, 489–506. <https://doi.org/10.1002/ajb2.1254>
- 180 R Development Core Team (2020). *R: A Computer Language for Statistical Data*
181 *Analysis*. The R Project for Statistical Computing <http://www.R-project.org/>

- 182 Tanaka, H. (2000). Size and dispersal of pollen grains in anemophilous angiosperms.
183 *Journal of Japanese Botany*, 75, 116-122. (In Japanese with English summary)
- 184 Taylor, P.E., Card, G., House, J., Dickinson, M.H. & Flagan, R.C. (2006). High-speed
185 pollen release in the white mulberry tree, *Morus alba* L. *Sex Plant Reproduction* 19,
186 19–24. <https://doi.org/10.1007/s00497-005-0018-9>
- 187 Torices, R., Méndez, M. & Gómez, J. M. (2011). Where do monomorphic sexual
188 systems fit in the evolution of dioecy? Insights from the largest family of
189 angiosperms. *New Phytologist*, 190, 234–248. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2010.03609.x)
190 [8137.2010.03609.x](https://doi.org/10.1111/j.1469-8137.2010.03609.x)
- 191
- 192
- 193

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, (c) buds of male
197 flowers.

198

199

200

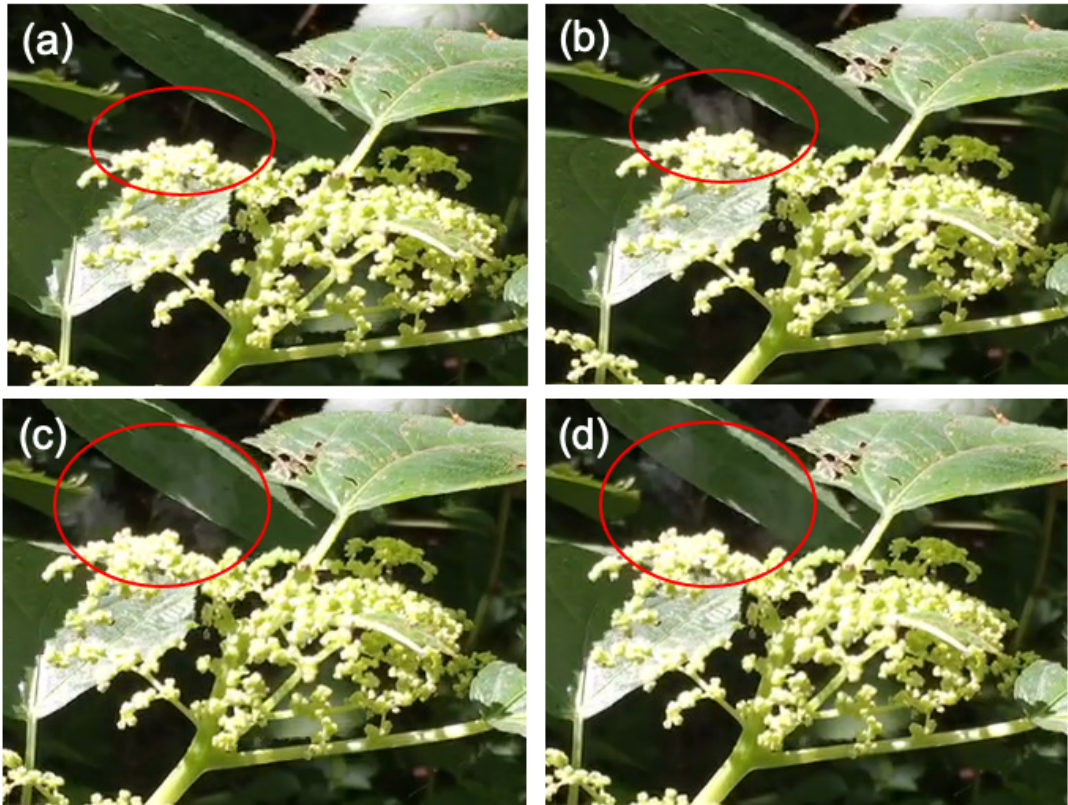
201

202

203

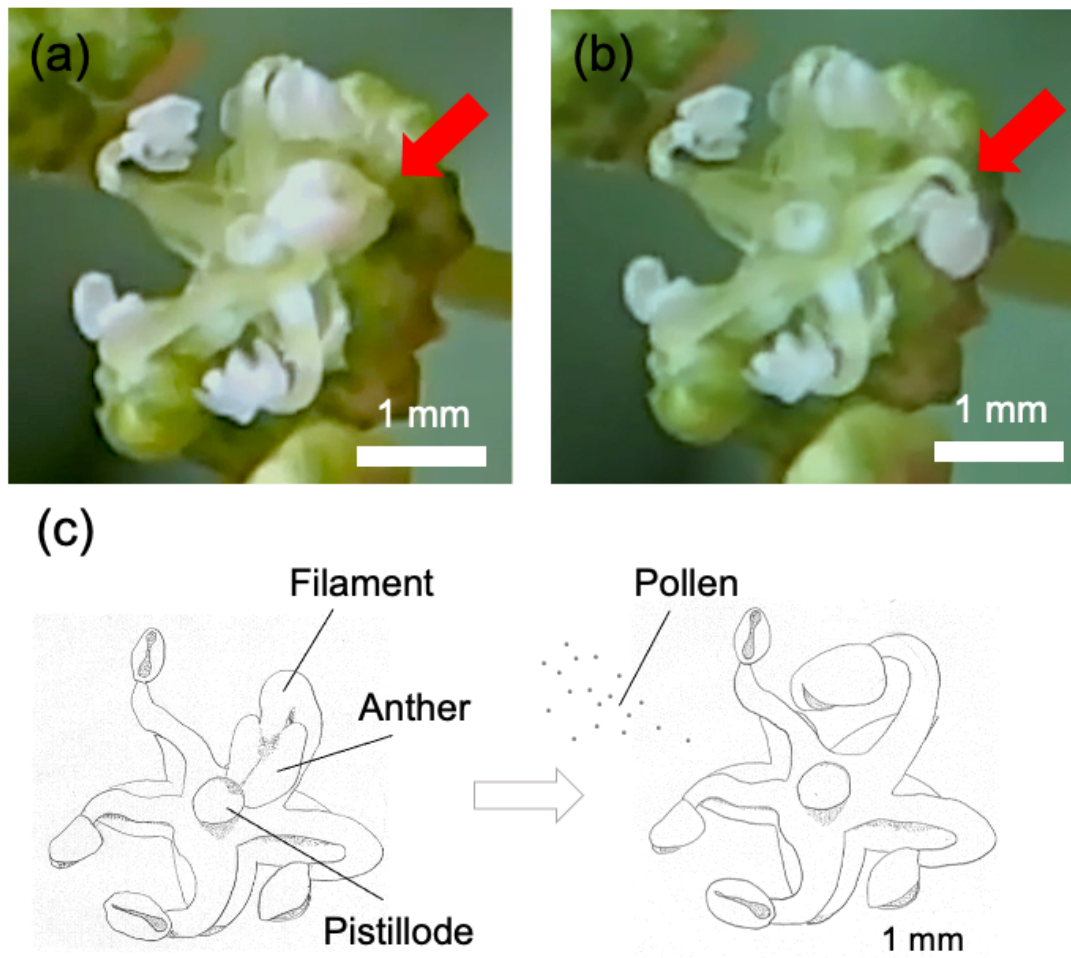
204

205



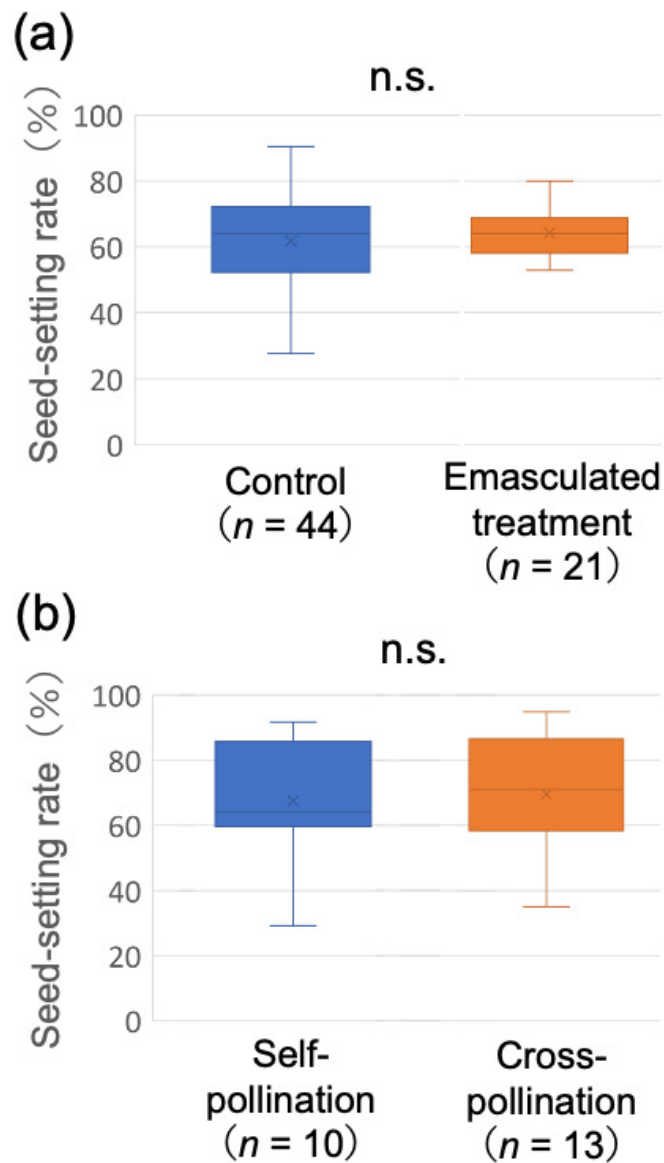
206 Figure 2: High-speed consecutive photographs of pollen dispersal from male flowers.
207 Photos taken by a digital camera: OLYMPUS STYLUS TG-2 Tough (Olympus
208 Corporation). Photos shown at (a) 0.00 second, (b) 0.04 s, (c) 0.09 s, (d) 0.10 s. The
209 red color ellipses show the diffusion states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

210
211
212
213
214
215
216
217



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.

221
 222
 223
 224
 225
 226



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