

1 **Floral visitors and reproductive success in two sequentially flowering**  
2 ***Lindera* shrubs (Lauraceae) of central Japan**

3 Yuji Tokumoto<sup>1</sup>, Michinari Matsushita<sup>2,3</sup>, Keiko Kishimoto-Yamada<sup>4</sup>, Aoi  
4 Nikkeshi<sup>5</sup>, Tomohiro Isogimi<sup>1</sup>, Michiko Nakagawa<sup>1</sup>

5

6 *1 Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601,*  
7 *Japan;*

8 *2 Faculty of Bioresource Sciences, Akita Prefectural University, Akita, 010-0195,*  
9 *Japan;*

10 *3 Forest Tree Breeding Center, Forestry and Forest Products Research Institute,*  
11 *Ibaragi 319-1301, Japan;*

12 *4 Center for Toki and Ecological Restoration, Niigata University, Niigata 950-2181,*  
13 *Japan;*

14 *5 Faculty of Life and Environmental Science, University of Tsukuba, Ibaragi 305-8572,*  
15 *Japan.*

16 Correspondence: Yuji Tokumoto, Graduate School of Bioagricultural Sciences, Nagoya  
17 University, Nagoya 464-8601, Japan; e-mail: tokumoto.ug@gmail.com, Tel. and Fax:  
18 +81 (0)52 789 4051.

19

20 **Floral visitors and reproductive success in two sequentially flowering**  
21 ***Lindera* shrubs (Lauraceae) of central Japan**

22 Abstract: Two *Lindera* species (*Lindera praecox* and *Lindera triloba*) are  
23 distributed sympatrically along the Pacific Ocean side of the main island of  
24 Japan. They are similar, but not identical, in habitat preference and flowering  
25 season. Slight differences in these traits have been proposed, but a comparative  
26 study on the reproductive ecology of these two species has not yet been  
27 conducted. To reveal the interspecies differences between these two *Lindera* spp.  
28 and characterize the life historical characteristics related to their reproduction, we  
29 assessed differences in floral visitors and reproductive ecology (fruit set, seed and  
30 pulp weights, and pollen limitation) between species and sexes over a period of 3  
31 years. A total of 39 species (458 individuals) of floral visitors, approximately  
32 70% of which comprised three Coleoptera species, were collected from plants of  
33 both species and sexes. The number of insect species that visited the late-  
34 flowering species, *L. triloba*, was significantly higher than the number that  
35 visited the early-flowering species, *L. praecox*. However, the numbers of visitors  
36 were not significantly different between species. The fruit set was higher in *L.*  
37 *triloba* than in *L. praecox*, but *L. praecox* pulps were heavier than those of *L.*  
38 *triloba*. Our study revealed interspecies differences in floral visitors and  
39 reproductive success between two species and indicate the possible differences of  
40 reproductive strategy of these two *Lindera* spp.

41 Keywords: flowering phenology, fruit set, *Lindera praecox*, *Lindera triloba*,

42

43

44 **Introduction**

45 Closely related species tend to have similar traits (Pagel 1999). However, slight  
46 differences in life history traits can offer evidence of significant historical events and  
47 selective pressures. A comparative study of species characteristics is an effective way to  
48 understand differences between closely related species and the historical events that  
49 have driven their evolution. Reproductive success and pollinator assessments are  
50 especially critical measures of fitness in plants, which are unable to move independently  
51 (Bradshw 2006). A comparative study of reproductive ecology in related plant species  
52 will clarify the characteristics of their life histories (Sans et al. 2004).

53 *Lindera* is a genus in the Lauraceae family that is distributed widely from  
54 tropical to temperate zones. There are over 100 *Lindera* species worldwide, seven of  
55 which are native to Japan. These dioecious shrubs bloom sequentially from February to  
56 May, with slightly overlapping flowering phenologies (Dupont and Kato 1999a). In  
57 particular, two *Lindera* species, *L. praecox* and *L. triloba*, inhabit the same regions and  
58 are in the same section Praeeces. They are often distributed sympatrically over wide  
59 ranges of Japan, but may prefer slightly different habitats (Nakagawa et al. 2015). They  
60 bloom at around the same time in April to May; the flowering period of each species  
61 lasts approximately 2 weeks, with *L. praecox* blooming approximately 2 weeks earlier  
62 than *L. triloba* (Dupont and Kato 1999a). The floral visitors of male plants of both  
63 species are mainly coleopteran and dipteran insects (Dupont and Kato 1999a). However,  
64 quantitative surveys of floral visitor numbers of both sexes have not been conducted for  
65 these two *Lindera* species and their pollinators remain unknown. In temperate regions,  
66 drastic environmental changes in the spring. Including increasing temperatures and  
67 strong thermal fluctuations (Kudo et al. 2008), mean that plants and animals

68 occasionally experience temperatures that are inadequate for growth or activity  
69 (Schemske et al. 1978; Kudo and Ida 2013); slight differences in flowering phenology  
70 are thus crucial for the reproductive success of the plants in these regions (Heinrich  
71 1976; Kudo et al. 2008; Kudo and Ida 2013). In dioecious plants and self-incompatible  
72 plant species, this season is the crucial time during which pollen must be transferred by  
73 pollinators from male to female plants. The pollination success of dioecious plants may  
74 be affected by floral visitor fauna and their behavior. Thus, interspecies differences in  
75 flowering phenology might lead to different floral visitor fauna and pollinators and  
76 result in differences in reproductive success. Comparing floral visitors and reproductive  
77 success between two sequentially flowering *Lindera* species will shed light on the  
78 reproductive strategies of the two species.

79 In this study, we aimed to compare the floral visitor fauna between both plant  
80 species and sexes, the behaviors of the main floral visitors, and the plants' reproductive  
81 success, examining species and sex differences in reproductive strategy in the two  
82 *Lindera* species.

83

## 84 **Materials and methods**

### 85 ***Study site***

86 Our study was conducted in Inabu Field at the Field Science Center of the Graduate  
87 School of Bioagricultural Sciences, Nagoya University, in Toyota, Aichi Prefecture,  
88 Japan (35°13'N, 137°34'E, 920–1230 m a.s.l.). The monthly mean temperature at this  
89 site ranges from –2.3°C in February to 21.1°C in July, and the annual mean temperature  
90 is 9.3°C (Nagoya University Forest 2004). The mean annual precipitation is 2,232.5  
91 mm, and the snow depth rarely exceeds 30 cm. *Cryptomeria japonica* (Thunb. ex L. f.)

92 D. Don (Cupressaceae), *Chamaecyparis obtusa* Sieb. & Zucc. (Cupressaceae), and  
93 *Larix kaempferi* (Lamb.) Carrière (Pinaceae) have been planted in Inabu field; however,  
94 the site also contains a natural central temperate zone deciduous broadleaf forest  
95 (Nagoya University Forest 2004).

96

### 97 ***Study species***

98 *Lindera praecox* (Siebold et Zuccarini) Blume and *Lindera triloba* (Siebold et  
99 Zuccarini) Blume are widely distributed in forest understories along the Pacific Ocean  
100 side of the Japanese islands of Honshu, Shikoku, and Kyushu (Horikawa 1972). Both  
101 species are deciduous dioecious shrubs and are dominant in forest understories. The two  
102 shrubs are in the same section of Praecoces, and are found in valleys and ridges within  
103 our study site, respectively (Nakagawa et al. 2015). Flower buds are produced in the fall  
104 of the previous year and bloom sequentially in the spring from April to May (Isogimi et  
105 al. 2014). The inflorescences and flower characteristics of the two species are similar  
106 (Dupont and Kato 1999b); both sexes produce three to five reproductive organs with  
107 short stalks within a single umbel. The flower is a yellow painted-bowl-shaped structure  
108 with six petals. The male flower has nine stamens with two anther locules, and the  
109 female flower has one pistil with a single ovule and nine staminodes. The corolla width  
110 of *L. triloba* in our study site was larger than that of *L. praecox* and the width of the  
111 male was greater than that of the female: *L. praecox* male,  $6.3 \pm 0.1$  mm (mean  $\pm$   
112 standard error [S.E.]; N = 86); *L. praecox* female,  $4.8 \pm 0.1$  mm (N = 46); *L. triloba*  
113 male,  $8.0 \pm 0.1$  mm (N = 98); *L. triloba* female,  $5.6 \pm 0.1$  mm (N = 62) (Isogimi et al.  
114 unpublished data). These trends were consistent with those observed at another study  
115 site (Dupont and Kato 1999b). Although Coleoptera and Diptera dominated the visitors  
116 of male flowers of both species, visitors to female plants remain unexamined (Dupont

117 and Kato 1999a).

118

119 ***Flowering phenology observation***

120 To investigate differences in phenology by species and sex, we randomly selected  
121 individual plants within the field where flowering plants of both species and sexes with  
122 nearly the same size were distributed sympatrically. In total, we selected 53 *L. praecox*  
123 males, 36 *L. praecox* females, 63 *L. triloba* males, and 37 *L. triloba* females. We  
124 recorded the total number of flowers on selected individuals and classified the flowering  
125 stages in 10% increments, such that 0%: all reproductive organs of female plants were  
126 flower buds or closed flowers (immature fruits) and those of male plants were abscised  
127 flowers; and 50%: roughly half of the reproductive organs were blooming, and the  
128 remaining reproductive organs were flower buds or immature fruits for females and  
129 abscised flowers for males. These flowering phenology classifications were applied to  
130 the same plants throughout the 3 consecutive years of the study (2011–2013).

131

132 ***Floral visitor collection***

133 We monitored 10–200 flowers in the observed plants for 10 minutes in the morning and  
134 afternoon and counted the floral visitors on these flowers. We conducted observations  
135 on a total of 27 days throughout the study period: 20 days for *L. praecox* males, 22 days  
136 for *L. praecox* females, 20 days for *L. triloba* males, and 15 days for *L. triloba* females  
137 (Table 1). We observed each floral visitor and classified their behaviors into four  
138 categories: staying (remaining only on reproductive organs), foraging (foraging pollen  
139 or nectar), moving (moving without foraging), and mating (two homogeneous floral  
140 visitors mating). After monitoring their behaviors, we caught floral visitors using

141 aspirators. We excluded the following arthropod groups from the data set used for  
142 further analyses: 1) Araneae (N = 17, Table 1), which scarcely moved and were  
143 generally predators, presumably waiting for prey; 2) Hemiptera (N = 93), which also  
144 stayed on the peduncles and rarely moved, suggesting that they were merely herbivores  
145 of *Lindera* flowers; and 3) Neuroptera (N = 1), which fed on pollen and were potentially  
146 pollinators of other plant species (Krenn et al. 2008) (however, we found only one  
147 individual moving on *Lindera* flowers (Table 1)). These three arthropod orders may be  
148 foragers or herbivores of *Lindera* species, rather than potential pollinators. We defined  
149 three insect orders (Coleoptera, Diptera, and Hymenoptera) as floral visitor species and  
150 used data on these taxa for the following analyses (see Statistical analysis). All samples  
151 were pinned and dried at 60°C for 72 hours. Sampled individuals of the order  
152 Coleoptera were identified to the species level (Table 1), and those of other taxa were  
153 identified to the family level. Specimens were stored at the Inabu Field Office.

154         We identified and quantified pollen on the bodies of the three dominant  
155 Coleoptera insect species (see Results) collected in 2012. Samples were dipped in 100  
156  $\mu$ l of 0.4 mol/l sucrose solution in tubes and shaken for 1 minute (Nikkeshi et al. 2016).  
157 Pollen species were identified and classified into three categories; *L. praecox*, *L. triloba*,  
158 and other species. The pollen grains in each 10  $\mu$ l solution were counted using a  
159 microscope. These counts were replicated five times and the scores were averaged as  
160 100- $\mu$ l densities.

161

## 162 ***Reproductive successes***

163 To investigate the plant reproductive success, we monitored the reproductive organs  
164 from flower to seed. We selected 4–11 individual female plants that were growing

165 sympatrically in the study area during each of our four study periods. In April, female  
166 flowers of both species were marked and the flowers in each inflorescence were counted  
167 (Table 2). In September of the fruit maturation year, we counted the fruits and collected  
168 those that were mature. Fruits were dried at 60°C for 72 hours and dried fruits, seeds,  
169 and pulp were weighed to the nearest 0.1 mg using an electronic analytical scale (New  
170 Classic MS, Mettler Toledo, OH).

171 In 2016, pollen limitation was determined in both species by performing a  
172 pollination treatment. Inflorescences not included in the observations described above  
173 were marked and pollen was attached to the stigma using ink brushes. We counted fruits  
174 of both species in September of the same year and calculated the fruit set of each  
175 inflorescence.

176

### 177 *Statistical analysis*

178 To confirm the comparability of sampling effort for each plant species and sex, we  
179 estimated the number of floral visitor species for each plant species and sex with 95%  
180 confidence intervals using the EstimateS software (ver. 9.1.0) (Colwell 2013) and drew  
181 sample-based rarefaction curves for floral visitor species number and cumulative study  
182 days.

183 To assess whether floral visitor composition differed between plant species and  
184 sexes, we calculated the Bray–Curtis similarity index of all plant species and sex  
185 combinations in the normalized floral visitor data. We then assessed the effects of plant  
186 species, sex, and their interaction (species  $\times$  sex) using permutational multivariate  
187 analysis of variance (PERMANOVA) with 20,000 permutations.



188           We calculated the percentages of each floral visitor behavior. There were  
189 insufficient Diptera and Hymenoptera visitors to include them in the species and sex  
190 comparisons (1–10 visiting individuals per flower per day, Table 1). To determine  
191 differences in the percentages of each behavior for only Coleoptera insects among the  
192 two plant species and sexes, we conducted Fisher’s exact test using the Benjamini and  
193 Hochberg *p*-adjustment method for multiple comparisons (Benjamini and Hochberg  
194 1995).

195           To assess between-species and between-sex differences in visiting individuals  
196 per flower per day among all floral visitors, the two orders (Coleoptera and Diptera) and  
197 the three dominant insect species (see Results), we constructed six generalized linear  
198 mixed models (GLMMs) with a Poisson distribution and log link. The response  
199 variables were the number of visiting individuals per flower per day among all floral  
200 visitors, each order, and the three dominant insect species for each plant species and  
201 sex. The fixed effects were species, sex, and the interaction of species and sex. We set  
202 year as a random effect, and the number of observed flowers used for counting floral  
203 visitors was included as the offset term to correct for sampling effort in the six GLMMs.

204           The between-species differences in measures of reproductive success (fruit set,  
205 fruit weight, seed weight, and pulp weight) were analyzed using GLMMs with species  
206 as a fixed effect and year and plant as random effects. For the pollen limitation analysis,  
207 we compared fruit set under natural conditions and following pollen treatment using a  
208 GLMM with a binomial distribution, with treatment as a fixed effect and plant  
209 individual terms as random effects.

210           Statistical analyses, except for the rarefaction curve, were performed using R  
211 software (ver. 3.1.2) (R Core Team 2014).

212

## 213 **Results**

### 214 *Flowering phenology*

215 The 3-year cumulative phenological trends of *L. praecox* at the study site showed that  
216 flowering began in the middle of April and lasted approximately 2 weeks (Figure 1).  
217 The phenology of *L. triloba* was delayed by approximately 2 weeks compared to that of  
218 *L. praecox*, with the flowering peak occurring in early May. Although between-species  
219 differences in flowering phenology were distinct, phenology was nearly synchronous  
220 between sexes of the same species.

221

### 222 *Floral visitor fauna and number of individuals, and behaviors of Coleoptera*

223 The total number of floral visitors to both species and sexes was 458, which we  
224 identified into 39 species within three orders (Table 1). Although the total numbers of  
225 census days for each species were 15–25 days (52–119 hours) over 3 consecutive years,  
226 the numbers of floral visitors for both species and sexes were very low: 1–3 individuals  
227 per 100 flowers per individual plant per day (Figure 2).

228 The rarefaction curves of both species and sexes were not saturated; however,  
229 we were able to detect general differences in species numbers (Figure 3). The number of  
230 floral visitor species found on *L. triloba* males (number of species: 25, 95% confidence  
231 interval: 18.5–35.5) was greater than that on *L. triloba* females (16 species, 11.6–20.4)  
232 and greater than that on both sexes of *L. praecox* (male: 14 species, 11.1–16.9; female:  
233 9 species, 4.4–13.6). Although there was no significant difference between *L. triloba*  
234 females and *L. praecox* males, the differences between females of the two species was  
235 significant (Table 1). The numbers of singletons on *L. triloba* males (13) and females  
236 (8) were greater than those on *L. praecox* (male: 2, female: 1) (Table 1). Although a

237 diversity of floral visitor species was observed on both *Lindera* species and sexes, three  
238 coleopteran species comprised approximately 70% of all floral visitor individuals:  
239 *Aphthona perminuta* (Chrysomelidae), *Meligethes flavicollis* (Nitidulidae), and *Anaspis*  
240 *marseuli* (Scraptiidae) (Figure 4). Floral visitor compositions did not differ between  
241 species and sexes (Figure 4) (PERMANOVA; plant species: pseudo- $F = 1.10$ ,  $p = 0.35$ ;  
242 sex: pseudo- $F = 1.00$ ,  $p = 0.43$ ; plant species  $\times$  sex: pseudo- $F = 0.62$ ,  $p = 0.386$ ).

243 The main behaviors of all coleopteran species observed on both *Lindera* species  
244 were foraging (46.6%) and staying (36.5%), although several individuals were  
245 categorized as mating (9.4%) and moving (7.6%;  $N = 373$ , Figure 5, Table 3). Staying  
246 was frequently observed on female plants, especially on *L. triloba* ( $p < 0.05$ ) (Figure 5,  
247 Table 3). Mating was the dominant behavior on *L. praecox* in both sexes ( $p < 0.05$ ), and  
248 foraging was frequently observed on male plants ( $p < 0.05$ ). Moving did not differ  
249 between plant species or between sexes ( $p > 0.05$ ).

250 The numbers of floral visitor individuals did not differ between plant species  
251 (species:  $\chi^2 = 0.06$ ,  $p = 0.81$ , Table 4, Figure 2); however, more floral visitors were  
252 observed on males than on females (sex:  $\chi^2 = 28.63$ ,  $p < 0.01$ ). This between-sex  
253 difference was commonly observed in both plant species (plant species  $\times$  sex:  $\chi^2 = 2.90$ ,  
254  $p = 0.09$ ). The trends of Coleoptera individuals were same as those of all floral visitor  
255 individuals; however, Diptera visited *L. triloba* more than *L. praecox* (species:  $\chi^2 =$   
256  $8.35$ ,  $p < 0.01$ ) (Table 4, Figure 2). Three dominant species showed different visiting  
257 trends among species: *A. perminuta* visited both plant species and sexes equally, more  
258 *M. flavicollis* were observed on males than on females, and more *A. marseuli* were  
259 observed on *L. praecox* than on *L. triloba*, as well as on male plants compared to  
260 females (Table 4, Figure 2). *Aphthona perminuta* and *A. marseuli* visited *L. praecox*  
261 males most among the plant categories in terms of mean individuals, whereas *M.*

262 *flavicollis* visited *L. praecox* of both sexes and *L. triloba* males equally (Table 4, Figure  
263 2).

264 According to the Coleoptera pollen identification results, pollen of both plant  
265 species was found on all three Coleoptera species, even when the beetles were collected  
266 on alternative plant species (Table 5).

267

### 268 ***Reproductive success and pollen limitation of the two Lindera species***

269 The fruit set of both species fluctuated among years and, except for 2012, the average  
270 scores of *L. triloba* were higher than those of *L. praecox* (Table 2). The 4-year average  
271 fruit set of *L. triloba* was  $11.9 \pm 0.9\%$ ; this score was significantly higher than that of *L.*  
272 *praecox* ( $9.2 \pm 1.0\%$ ;  $\chi^2 = 4.53$ ,  $p = 0.03$ , Table 2). In contrast to our fruit set results, the  
273 dry weight of *L. praecox* fruit and pulp was approximately 1.2 times higher than that of  
274 *L. triloba* (respectively,  $\chi^2 = 27.84$ ,  $p < 0.01$ ;  $\chi^2 = 63.25$ ,  $p < 0.01$ , Table 2). The  
275 between-species differences in seed weight was not detected ( $\chi^2 = 4.00$ ,  $p = 0.06$ ).

276 Pollination treatment did not change the fruit set results from those observed  
277 with natural pollination in either species ( $p > 0.05$ , Table 2). This result may indicate an  
278 absence of pollen limitation.

279

## 280 **Discussion**

### 281 ***Possible pollinators of two Lindera spp. and between-species and -sex*** 282 ***differences in floral visitor fauna***

283 We observed a total of 39 species in three insect orders as floral visitors; however,  
284 Coleoptera dominated in both species number and individuals, and the top three

285 Coleoptera species accounted for approximately 70% of all observed individuals  
286 (Figure 2). Three dominant Coleoptera species including closely related species have  
287 often been reported to visit other flowering plant species, indicating their function as a  
288 pollinator of these plants (Nadel and Peña 1994; Ishida 1996; Araki et al. 2005; Ohara  
289 et al. 2006; Kato et al. 2009; Matsuyama et al. 2009). Although *L. triloba* and *L.*  
290 *praecox* possess morphologically generalist-pollinated floral traits, including a painted-  
291 bowl shape and large number of flowers per individual (Bernhardt 2000), and although  
292 these species appear to be generalist-pollinated plants based on the floral visitor fauna  
293 on male plants (Dupont and Kato 1999a), the dominant floral visit trends and pollen  
294 identified from three Coleoptera species suggest that the two *Lindera* species are highly  
295 dependent on these insect species for pollination success, especially *A. perminuta* and  
296 *M. flavicollis*. *Anaspis marseuli* might transfer *L. praecox* pollen to individual female  
297 plants but this insect species was not observed on *L. triloba* females during the 3-year  
298 survey (Table 1). Thus, for *L. triloba*, the possibility of this insect as a pollinators are  
299 lower than other two species.

300 Fewer species visited *L. praecox* than *L. triloba*, and the number of singleton  
301 species visiting *L. triloba* was less than that visiting *L. praecox*; some Diptera and  
302 Hymenoptera species were observed only on *L. triloba* (Table 1). These differences  
303 may have been caused by the differing flowering phenology between the two species  
304 (Figure 1). As the spring progresses, greater numbers of insects, including Hymenoptera  
305 and Diptera, are observed (Kudo 1993; Dupont and Kato 1999a) because the foraging  
306 activities of Hymenoptera or Diptera are affected by temperature (Heinrich 1975). *L.*  
307 *praecox* blooms approximately 2 weeks earlier than *L. triloba*, and the 3-year average  
308 temperature during the flowering period of *L. praecox* (10.4°C, Julian days from 104  
309 [13 April] to 116 [26 April]) was about 3°C higher than that for *L. triloba* (13.1°C,

310 Julian days from 116 to 128 [7 May]) (Japan Meteorological Agency 2015). This slight  
311 difference in flowering timing in the spring might cause between-species differences in  
312 floral visitor species numbers.

313 In contrast to the differences in species number, the numbers of visiting  
314 individuals per flower per day of all floral visitor species, coleopterans, and the three  
315 dominant species visiting *L. praecox* were greater than those on *L. triloba* (Figure 1,  
316 Table 1). Additionally, the primary behavior practiced by coleopterans on *L. praecox*  
317 was mating and that on *L. triloba* was foraging (Figure 3). Generally, coleopterans visit  
318 flowers to find places to mate, food rewards, and heat sources (Bernhardt 2000).  
319 Because the dominant Coleoptera species, *A. perminuta* and *A. marseuli*, often visit  
320 plants that bloom during the same season or slightly later than *L. triloba* (Ishida 1996;  
321 Ohara et al. 2006; Ohsawa 2007; Matsuyama et al. 2009; Kato et al. 2009). These insect  
322 species may forage on other flowers while *L. triloba* is flowering. Thus, these  
323 Coleoptera might utilize *L. praecox* for mating and switch foraging sites from *L.*  
324 *praecox* to *L. triloba* and/or other flowering plants after *L. praecox* flowers. Such  
325 behavior would explain the differences we observed in visiting individuals between the  
326 two plant species.

327 For both *L. praecox* and *L. triloba*, insects visited male flowers more frequently  
328 than female flowers (Table 1, Figure 1). This male bias in visitation frequency has been  
329 observed in many dioecious plants and is often caused by differences in floral rewards,  
330 such as pollen and nectar (Bawa 1980; Farwig et al. 2004; Glaettli and Barrett 2008;  
331 Matsuyama et al. 2009; Vlasáková and Jarau 2011). Male plants do not invest in  
332 reproductive organs to the same extent as females and can thus allocate more resources  
333 to flower size, flower number, and floral rewards (Buckley and Avila-Sakar 2013). In  
334 *Lindera* species, nectar is secreted from flowers of both sexes, with a sugar

335 concentration of less than 10% (Dupont and Kato 1999a). The male has stamens with  
336 sticky pollen, whereas the female has staminodes (Rohwer 1993, Dupont and Kato  
337 1999a). Thus, the female flowers attract floral visitors by nectar or mimicry of male  
338 flowers without pollen and male-biased visitation might be caused by these differences.  
339

340 ***Between-species differences in reproductive success of two Lindera spp. and***  
341 ***implications for reproductive strategy***

342 Neither plant species showed any change in fruit set with additional pollen treatment.  
343 Our surveys to test pollen limitation seemed to demonstrate the absence of such a  
344 limitation. Pollen limitation functions in the early growing stages of reproductive  
345 organs, especially during periods of pollination (Knight et al. 2005). In our survey, plant  
346 reproductive organs were observed during the flowering (April and May) and fruiting  
347 periods (September of the same year); the survey did not consider the differences  
348 among individual of micro-scale climate, resource availability, or herbivores on  
349 reproductive organs. If these factors affected the inflorescence of both species equally  
350 during the study period at Inabu Field, the artificial pollen treatment results would likely  
351 have suggested an absence of pollen limitation in both species. Another *Lindera*  
352 species, *L. benzoin*, which has been shown to have approximately one attached pollen  
353 grain per flower, can produce mature fruit (Niesenbaum 1993). Thus, pollination by  
354 infrequent pollinators might be sufficient for fruit setting in both *L. praecox* and *L.*  
355 *triloba*.

356 The fruit set in both *Lindera* species was around 10% throughout the study  
357 period, which appears to be consistent with that in other *Lindera* species (Niesenbaum  
358 1993; Devall et al. 2001). The average fruit set was significantly higher in *L. triloba*  
359 than in *L. praecox* (Table 2), whereas the trend observed in fruit and pulp weights was

360 the opposite. (Table 3). Seed size did not show the same trend, being essentially the  
361 same in both species. Recent studies have shown that fruit size has been evolved under  
362 a mixture of selective pressures from dispersers' size and seed size (Herrera 2002;  
363 Alcántara & Rey 2003). The lack of significant differences in seed size between the two  
364 species may indicate that the effect of dispersers drives between-species differences in  
365 the weight of fruit and pulp. Birds have been considered as possible dispersers of  
366 *Lindera* spp., as the intact pulp of *Lindera* spp. often prevents seed germination,  
367 whereas seeds that pass through a bird gut are able to germinate (Sri-ngernyuang et al.  
368 2003; Smith et al. 2004; Aleric and Kirkman 2005; Hawkins et al. 2011). Although we  
369 did not determine the dispersers of these two *Lindera* spp., species-level differences in  
370 pulp weight may lead to different seed dispersal and germination strategies.

371         Some reproductive organs in *L. praecox* were aborted due to frost, leading to  
372 low fruit set. One proximate explanation for *L. praecox*'s heavier fruit may be that this  
373 species increased the size of its remaining reproductive organs, and especially of the  
374 amount of pulp, in response to this loss. Another explanation may be related to the  
375 findings of previous studies that a bright light environment positively affects fruit set in  
376 *Lindera* species (Niesenbaum 1993). According to a study of branch architecture and  
377 distribution in two *Lindera* species, *L. triloba* was distributed in dry sites such as ridges  
378 and *L. praecox* in the moist sites, such as valleys (Nakagawa et al. 2015). Although both  
379 species were distributed in the forest understory, where access to sunlight was disrupted  
380 by canopy cover, the light environment of *L. triloba* was brighter than that of *L.*  
381 *praecox*, which is distributed on ridges (Nakagawa et al. 2015). Thus, light environment  
382 may lead to differences in fruit set between these *Lindera* species.

383         The current study showed between-species differences in floral visitor fauna and  
384 reproductive success of two *Lindera* species. Previous studies of these plant species



385 demonstrated between-species differences in plant growth and architectural traits  
386 (Isogimi et al. 2014; Nakagawa et al. 2015). *Lindera praecox* has higher ramet survival  
387 and growth; however, *L. triloba* has higher ramet production (Isogimi et al. 2014). The  
388 number of branches and shoots of the main ramet, leaf area, and dry weight of *L. triloba*  
389 were greater than those of *L. praecox* (Nakagawa et al. 2015). Additionally, we assessed  
390 between-species differences in female reproductive success; however, an assessment of  
391 male reproductive success, such as pollen deposition and germination of each male  
392 individual via pollinators, would require a genetic analysis, which has not yet been  
393 conducted yet. Further studies of between-species differences in male reproductive  
394 success, seed germination, seedling survival, and resource allocation to the reproductive  
395 organs might clarify differences in reproductive strategies within the life cycles of these  
396 *Lindera* species.

397

398 Geolocation information

399 35°13'N, 137°34'E

400

401 Acknowledgements

402 We are grateful to Professor Nobuhiro Tomaru, Professor Naoki Hijii, Dr. Hisashi Kajimura,  
403 Mr. Yasuji Imaizumi, Mr. Hiroshi Ando, Mr. Norio Yamaguchi, and Mr. Naoki Takabe for the  
404 permission to conduct this research and their kind support at the Inabu Field, Field Science  
405 Center of the Graduate School of Bioagricultural Sciences, Nagoya University. We also thank  
406 Dr. Toshio Kishimoto for identifying the Byturidae, Cryptophagidae, Kateretidae, Melyridae,  
407 and Phalacridae (Coleoptera), Dr. Sadatomo Hisamatsu for identifying the Nitidulidae  
408 (Coleoptera), Dr. Wataru Suzuki for identifying the Eilateridae (Coleoptera), Dr. Hikaru  
409 Yoshitake for identifying the Curculionidae and Attelabidae (Coleoptera), and Mr. Taiki Oguri  
410 for identifying the Araneae. Financial support was provided from Research grant 2016 by the  
411 Ecological Society of Japan, Chubu branch to YT.

412

413 Declaration of interest statement  
414 The authors declare that there is not any conflict of interest on this study.  
415

416 References:

417 Alcántara JM, Rey PJ. 2003. Conflicting selection pressures on seed size: evolutionary  
418 ecology of fruit size in a bird-dispersed tree, *Olea europaea*. *J Evol Biol.* 16:  
419 1168–1176.

420 Aleric KM, Kirkman LK. 2005. Seed germination observations of the federally listed  
421 *Lindera melissifolia*. *Castanea.* 70(2): 157–160.

422 Araki K, Yamada E, Ohara M. 2005. Breeding system and floral visitors of *Convallaria*  
423 *keiskei*. *Plant Species Biol.* 20(2): 149–153.

424 Bawa KS. 1980. Evolution of dioecy in flowering plants. *Annu Rev Ecol Syst.* 11: 15–  
425 39.

426 Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and  
427 powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol.* 57:  
428 289–300.

429 Bernhardt P. 2000. Convergent evolution and adaptive radiation of beetle-pollinated  
430 angiosperms. *Plant Syst Evol.* 222: 293–320.

431 Bradshw AD. 2006. Unravelling phenotypic plasticity – why should we bother? *New*  
432 *Phytologist.* 170: 644-648.

433 Buckley NE, Avila-Sakar G. 2013. Reproduction, growth, and defense trade-offs vary  
434 with gender and reproductive allocation in *Ilex glabra* (Aquifoliaceae). *Am J*  
435 *Bot.* 100(2): 357–364.

436 Colwell RK. 2013. EstimateS: Statistical estimation of species richness and shared  
437 species from samples. Version 9. User's Guide and application published at:  
438 <http://purl.oclc.org/estimates> [accessed 1 November 2014]

439 Corbet SA, Fussell M, Ake R, Fraser A, Gunson C, Savage A, Smith K. 1993.  
440 Temperature and the pollinating activity of social bees. *Ecol Entomol.* 18(1):  
441 17–30.

442 Danieli-Silva A, Varassin IG. 2013. Breeding system and thrips (Thysanoptera)  
443 pollination in the endangered tree *Ocotea porosa* (Lauraceae): implications for  
444 conservation. *Plant Species Biol.* 28: 31–40.

- 445 Devall M, Schiff N, Boyette D. 2001. Ecology and reproductive biology of the  
446 endangered Pondberry, *Lindera melissifolia* (Walt) Blume. *Natural Areas J.* 21,  
447 250–258.
- 448 Dupont YL, Kato M. 1999a. Phenology and flower-visiting entomofauna of six species  
449 of *Lindera* (Lauraceae) in Japan. *Nord J Bot.* 19(6): 707-718.
- 450 Dupont YL, Kato M. 1999b. Sex ratio variation in dioecious plant species: A  
451 comparative ecological study of six species of *Lindera* (Lauraceae). *Nord J Bot.*  
452 19(5): 529-540.
- 453 Farwig N, Randrianirina EF, Voigt FA, Kraemer M, Böhning-Gaese K. 2004.  
454 Pollination ecology of the dioecious tree *Commiphora guillauminii* in  
455 Madagascar. *J Trop Ecol.* 20(3): 307–316.
- 456 Glaetli M, Barrett SCH. 2008. Pollinator responses to variation in floral display and  
457 flower size in dioecious *Sagittaria latifolia* (Alismataceae). *New Phytol.* 179(4):  
458 1193–1201.
- 459 Hawkins TS, Walck JL, Hidayati SN. 2011. Seed ecology of *Lindera melissifolia*  
460 (Lauraceae) as it relates to rarity of the species. *J Torrey Bot Soc.* 138(3): 298–  
461 307.
- 462 Heinrich B. 1975. Energetics of pollination. *Annu Rev Ecol Syst.* 6: 139–170.
- 463 Heinrich B. 1976. Flowering phenologies: bog, woodland, and disturbed habitats.  
464 *Ecology.* 57(5): 890–899.
- 465 Herrera CM. 2002. Correlated evolution of fruit and leaf size in bird-dispersed plants:  
466 species-level variance in fruit traits explained a bit further? *Oikos.* 97(3): 426–  
467 432.
- 468 Horikawa Y. 1972. Atlas of the Japanese Flora, an introduction to plant sociology of  
469 east Asia. Gakken, Tokyo, Japan. Japanese.
- 470 Hothorn T, Bretz F, Westfall P. 2008. Simultaneous inference in general parametric  
471 models. *Biom J.* 50(3): 346–363.
- 472 House SM. 1989. Pollen movement to flowering canopies of pistillate individuals of  
473 three rain forest tree species in tropical Aus *J Ecol.* 14: 77–94.
- 474 Ish-Am G, Barrientos-Priego F, Castañeda-Vildozola A, Gazit S. 1999. Avocado  
475 (*Persea americana* Mill.) pollinators in its region of origin. *Rev Chapingo Ser*  
476 *Hortic.* 5: 137–143.
- 477 Ishida K. 1996. Beetle pollination of *Magnolia praecocissima* var. *borealis*. *Plant*  
478 *Species Biol.* 11: 199–206.

- 479 Isogimi T, Matsushita M, Nakagawa M. 2014. Species-specific sprouting pattern in two  
480 dioecious *Lindera* shrubs: The role of physiological integration. *Flora*. 209(2):  
481 718–724.
- 482 Japan Meteorological Agency. 2016. Climate data from  
483 <http://www.data.jma.go.jp/obd/stats/etrn/index.php> [accessed 25 April 2015]
- 484 Kato Y, Araki K, Ohara M. 2009. Breeding system and floral visitors of *Veratrum*  
485 *album* subsp. *oxysepalum* (Melanthiaceae). *Plant Species Biol.* 24(1): 42–46.
- 486 Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR,  
487 Johnston MO, Mitchell RJ, Ashman TL. 2005. Pollen limitation of plant  
488 reproduction: pattern and process. *Ann Rev Ecol Evol.* 36: 467–497.
- 489 Krenn HW, Gereben-Krenn BA, Steinwender BM, Popov A. 2008. Flower visiting  
490 Neuroptera: Mouthparts and feeding behaviour of *Nemoptera sinuata*  
491 (Nemopteridae). *Eur J Entomol.* 105(2): 267–277.
- 492 Kudo G. 1993. Relationship between flowering time and fruit set of the entomophilous  
493 alpine shrub, *Rhododendron aureum* (Ericaceae), inhabiting snow patches. *Am J*  
494 *Bot.* 80(11): 1300–1304.
- 495 Kudo G, Nishikawa Y, Kasagi T, Kosuge S. 2004. Does seed production of spring  
496 ephemerals decrease when spring comes early? *Ecol Res.* 19(2): 255–259.
- 497 Kudo G, Ida TY, Tani T. 2008. Linkages between phenology, pollination,  
498 photosynthesis, and reproduction in deciduous forest understory plants. *Ecology.*  
499 89(2): 321–331.
- 500 Kudo G, Ida TY. 2013. Early onset of spring increases the phenological mismatches  
501 between plants and pollinators. *Ecology.* 94(10): 2311–2320.
- 502 Lafer GSH. 1999. Contributions to the knowledge of Coleoptera fauna (insecta) of  
503 Kunashir, Kuril islands. *Far Eastern Entomol.* 77: 1–16.
- 504 Matsushita M, Nakagawa M, Tomaru N. 2011. Sexual differences in year-to-year  
505 flowering trends in the dioecious multi-stemmed shrub *Lindera triloba*: effects  
506 of light and clonal integration. *J Ecol.* 99(6): 1520–1530.
- 507 Matsuyama S, Osawa N, Sakimoto M. 2009. Generalist pollinators in the dioecious  
508 shrub *Rhus trichocarpa* Miq. (Anacardiaceae) and their role in reproductive  
509 success. *Plant Species Biol.* 24(3): 215–224.
- 510 Nadel H, Peña JE. 1994. Identity, behaviour, and efficacy of *Nitidulid* beetles  
511 (Coleoptera: Nitidulidae) pollinating commercial *Annona* species in Florida.  
512 *Environ Entomol.* 23(4): 878–886.

513 Nagoya University Forest. 2004. The 5th management plan of the Nagoya University  
514 Forest. Aichi: Nagoya University Forest, Japan. Japanese.

515 Nakagawa M, Isogimi T, Inanaga M, Abe K, Okada T, Yoichi W, Kobayakawa K,  
516 Toyama C, Ito K, Kawashima N, et al. 2015. Inter-specific and sexual  
517 differences in architectural traits of two dioecious *Lindera* species (Lauraceae).  
518 Plant Ecol. 216(1): 99–109.

519 Niemirski R, Zych M. 2011. Fly pollination of dichogamous *Angelica sylvestris*  
520 (Apiaceae): how (functionally) specialized can a (morphologically) generalized  
521 plant be? Plant Syst Evol. 294: 147–158.

522 Niesenbaum RA. 1993. Light or pollen –seasonal limitations on female reproductive  
523 success in the understory shrub *Lindera benzoin*. J Ecol. 81(2): 315–323.

524 Nikkeshi A, Hiraiwa KM, Ushimaru A, Hoshizaki K, Makita A, Inoue M. 2016.  
525 Established method of deposited pollen grains on the surface of pollinated insect  
526 body. Jpn J Palynol. 62, 1–5.

527 Ohara M, Araki K, Yamada E, Kawano S. 2006. Life-history monographs of Japanese  
528 plants. 6: *Convallaria keiskei* Miq. (Convallariaceae). Plant Species Biol. 21(2):  
529 119–126.

530 Ohsawa M. 2007. The role of isolated old oak trees in maintaining beetle diversity  
531 within larch plantations in the central mountainous region of Japan. For Ecol  
532 Manag. 250(3): 215–226.

533 Ohsawa M, Nagaike T. 2006. Influence of forest types and effects of forestry activities  
534 on species richness and composition of Chrysomelidae in the central  
535 mountainous region of Japan. For Biodivers Conserv. 15: 1179–1191.

536 Pagel M. 1999. Inferring the historical patterns of biological evolution. Nature. 401:  
537 877–884.

538 Pyke GH. 1991. What does it cost a plant to produce floral nectar? Nature. 350: 58–59.

539 R Core Team. 2014. R: A language and environment for statistical computing. R  
540 Foundation for Statistical Computing, Vienna, Austria. URL [http://www.R-](http://www.R-project.org/)  
541 [project.org/](http://www.R-project.org/) [accessed 1 November 2014]

542 Rohwer JG. 1993. Lauraceae. In The families and genera of vascular plants. Edited by  
543 K. Kubitzki. Springer Verlag, Berlin, German, pp. 366–390.

544 Sans FX, Garcia-Serrano H, Aflan I. 2004. Life-history traits of alien and native senecio  
545 species in the Mediterranean region. Acta Oecologica 26: 167–178.

- 546 Schemske DW, Willson MF, Melampy MN, Miller LJ, Verner L, Schemske KM, Best  
547 LB. 1978. Flowering ecology of some spring woodland herbs. *Ecology*. 59(2):  
548 351–366.
- 549 Smith CGIII, Hamel PB, Devall MS, Schiff NM. 2004. Hermit thrush is the first  
550 observed dispersal agent for pondberry (*Lindera melissifolia*). *Castanea*.  
551 69(1):1–8.
- 552 Sri-ngernyuang K, Chai-Udom K, Kanzaki M, Ohkubo T, Yamakura T. 2003. Survival  
553 and germination of an experimental seed bank population of two species of  
554 Lauraceae in a tropical montane forest in Thailand. *J For Res*. 8: 311–316.
- 555 Vlasáková B, Jarau S. 2011. Dioecious *Clusia nemorosa* achieves pollination by  
556 combining specialized and generalized floral rewards. *Plant Ecol*. 212(8): 1327–  
557 1337.
- 558

560 Table 1. List of arthropod species visiting both sexes of *Lindera praecox* and *Lindera*  
561 *triloba*.

Arthropod species				Plant species and sex				Total number of visiting individuals	
Order	Family	Genus	Species	<i>L. praecox</i>		<i>L. triloba</i>			
				Male	Female	Male	Female		
Araneae	Araneidae	<i>Eriophora</i>	<i>sachalinensis</i>	0	0	1	0	1	
			<i>minutus</i>	1	0	0	0	1	
	Tetragnathidae	<i>Tetragnatha</i>	<i>yesoensis</i>	0	4	0	2	6	
			<i>foliata</i>	0	0	1	0	1	
	Theridiidae	<i>Chrysso</i>	<i>fujisawai</i>	2	1	3	0	6	
			<i>subdola</i>	0	0	1	0	1	
	Thomisidae	<i>Synaema</i>	<i>chikunii</i>	0	0	0	1	1	
			<i>unicolor</i>	0	0	1	0	1	
	Coleoptera	Attelabidae	<i>Deporaus</i>	<i>unicolor</i>	0	0	1	0	1
		Chrysomelidae	<i>Aphthona</i>	<i>perminuta</i>	75	10	55	19	159
<i>Luperomorpha</i>				<i>collaris</i>	4	0	0	0	4
<i>Nonarthra</i>				<i>cyanea</i>	4	0	14	0	18
Cryptophagidae		<i>Cryptophagus</i>	<i>sp.</i>	0	0	0	1	1	
Curculionidae		<i>Imachra</i>	<i>nipponica</i>	0	0	0	2	2	
Elateridae		<i>Ectinus</i>	<i>sericeus</i>	0	0	1	1	2	
Kateretidae		<i>Heterhelus</i>	<i>japonicus</i>	0	0	1	0	1	
Nitidulidae		<i>Epuraea</i>	<i>japonica</i>	9	1	19	2	31	
			<i>Meligethes</i>	<i>flavicollis</i>	47	10	38	2	97
Scolytidae			<i>sp.</i>	0	0	0	1	1	
Scraptiidae		<i>Anaspis</i>	<i>marseuli</i>	42	6	18	0	66	
Staphylinidae		<i>Eusphalerum</i>	<i>sp.</i>	2	0	7	1	10	
Diptera		Bibionidae		<i>sp.1</i>	0	0	1	0	1
				<i>sp.2</i>	0	0	1	0	1
		Chironomidae		<i>sp.1</i>	1	0	0	0	1
				<i>sp.2</i>	0	0	0	2	2
				<i>sp.3</i>	0	0	0	1	1
		Dolichopodidae		<i>sp.1</i>	1	0	4	0	5
	<i>sp.2</i>			0	0	0	1	1	
	Drosophilidae		<i>sp.</i>	2	0	8	3	13	
	Helomyzidae		<i>sp.</i>	1	1	0	0	2	
	Mycetophilidae		<i>sp.1</i>	0	0	2	2	4	
			<i>sp.2</i>	0	0	0	1	1	
	Scathophagidae		<i>sp.</i>	0	0	1	0	1	
	Sciariidae		<i>sp.1</i>	5	2	0	0	7	
			<i>sp.2</i>	0	1	1	0	2	
			<i>sp.3</i>	0	0	2	0	2	
			<i>sp.4</i>	0	1	0	0	1	
	Simuliidae		<i>sp.</i>	0	0	0	1	1	
	Syrphidae		<i>sp.1</i>	0	0	1	0	1	
			<i>sp.2</i>	1	0	4	0	5	
	Tabanidae		<i>sp.</i>	0	0	2	0	2	
	Trichoceridae		<i>sp.</i>	0	0	1	0	1	
	Hemiptera	Aphididae		<i>sp.1</i>	28	27	13	15	83
				<i>sp.2</i>	1	0	2	2	5
				<i>sp.3</i>	0	0	1	0	1
		Carsidaridae		<i>sp.</i>	0	0	1	0	1
		Lygaeidae		<i>sp.</i>	1	0	0	0	1
		Unknown		<i>sp.</i>	0	0	2	0	2
Hymenoptera			Formicidae	<i>sp.</i>	0	0	1	0	1
	Halictidae		<i>sp.</i>	3	1	0	1	5	
	Ichneumonidae		<i>sp.</i>	0	0	1	0	1	
	Tenthredinidae		<i>sp.1</i>	0	0	1	0	1	
			<i>sp.2</i>	0	0	2	0	2	
Neuroptera		<i>sp.</i>	0	0	1	0	1		
Total number of arthropods				230	65	214	61	569	
Total number of floral visitors (Coleoptera, Diptera, and Hymenoptera)				197	33	187	41	458	
Total research days				20	22	20	15	27	
Total number of floral visitor species				14	9	25	16	39	
95% confidence intervals of floral visitor species number				11.1-16.9	4.4-13.6	18.5-35.5	11.6-20.4		
Number of singleton floral visitors				2	1	13	8		

563

564 **Table 2.** Sample numbers for reproductive success assessment during the 4-year study period with the results of generalized linear mixed models  
 565 (GLMMs) analyzing interspecies differences in reproductive successes.

566

Species	Year	Treatment	No. individuals	No. inflorescences	No. flowers	Fruit set (%) *		Fruit weight (mg) *			Seed weight (mg)		Pulp weight (mg) *		
						(Mean ± S.E.)	(Mean ± S.E.)	(N)	(Mean ± S.E.)	(N)	(Mean ± S.E.)	(N)	(Mean ± S.E.)	(N)	
<i>L. praecox</i>	Sum total	Natural pollination	17	1390	4692	9.21 ± 1.03	327.3 ± 4.6	261	200.6 ± 4.1	127	118.3 ± 1.9	127			
	2011	Natural pollination	4	307	1179	2.88 ± 0.76	319.7 ± 6.7	78	193.3 ± 4.5	78	126.4 ± 2.4	78			
	2012	Natural pollination	5	270	1021	22.33 ± 2.83	286.3 ± 4.1	74	NA		NA				
	2013	Natural pollination	5	447	1345	7.40 ± 1.76	397.1 ± 11.5	59	NA		NA				
	2016	Natural pollination		11	366	1147	6.99 ± 1.31	317.5 ± 9.0	50	212.3 ± 7.6	49	105.4 ± 2.2	49		
		Additional pollination			115	400	4.35 ± 1.37	322.6 ± 11.3	17	223.8 ± 7.2	19	97.9 ± 4.2	17		
<i>L. triloba</i>	Sum total	Natural pollination	16	1188	5283	11.95 ± 0.89	251.9 ± 2.5	378	174.0 ± 2.2	269	71.5 ± 0.8	268			
	2011	Natural pollination	5	330	1560	15.52 ± 1.42	236.2 ± 2.7	188	164.8 ± 2.0	188	71.5 ± 1.0	188			
	2012	Natural pollination	5	281	1333	9.13 ± 1.17	239.9 ± 4.4	72	NA		NA				
	2013	Natural pollination	4	417	1730	9.75 ± 1.27	325.0 ± 7.9	34	NA		NA				
	2016	Natural pollination		8	160	660	13.56 ± 3.36	267.8 ± 5.4	84	195.6 ± 4.7	81	71.6 ± 1.7	80		
		Additional pollination			128	543	10.14 ± 2.09	304.2 ± 7.6	54	223.6 ± 5.5	57	80.9 ± 3.3	54		

567 \*: Interspecies differences in reproductive successes of natural pollination were detected from the results of GLMM analyses.

568



569

570 **Table 3.** Percentages of four categories of coleopteran behavior on both sexes of *Lindera praecox* and *Lindera triloba*.

571

Species	No. of individuals	Percentage of each behavior			
		Staying *	Mating *	Foraging *	Moving *
<i>L. praecox</i> Female	27	48.1 ab	29.6 a	14.8 b	7.4 a
Male	163	32.2 b	13.6 ab	48.6 a	5.6 a
<i>L. triloba</i> Female	29	62.1 a	0.0 c	31.0 ab	6.9 a
Male	154	34.4 b	2.6 b	53.0 a	9.9 a
Total	373	36.5	9.4	46.6	7.6

572 \* Different alphabet indicates different group from Fisher's exact test within same behaviour

573

574

575

576

577 **Table 4.** Results of generalized linear mixed models (GLMMs) analyzing individuals of six taxa (total number of floral visitors, Coleoptera,  
 578 Diptera, *Aphthona perminuta*, *Meligethes flavicollis*, and *Anaspis marseuli*) visiting flowers of *Lindera praecox* and *Lindera triloba* of both  
 579 sexes at the Field Science Center of Nagoya University, Japan.

Explanatory variable	Total no. of floral visitors		Coleoptera		Diptera		<i>Aphthona perminuta</i>		<i>Meligethes flavicollis</i>		<i>Anaspis marseuli</i>	
	$\chi^2$	p	$\chi^2$	p	$\chi^2$	p	$\chi^2$	p	$\chi^2$	p	$\chi^2$	p
Plant species	0.06	0.81	3.09	0.08	8.35	<0.01	1.09	0.30	3.41	0.07	12.92	<0.01
Sex	28.63	<0.01	12.06	<0.01	0.47	0.49	3.35	0.07	5.56	0.02	9.74	<0.01
Plant species × Sex	2.90	0.09	1.22	0.27	0.00	0.96	6.28	0.01	2.68	0.10	3.03	0.08

580

581

582 **Table 5.** Average number of attached pollen grains on three dominant insect species (*Aphthona perminuta*, *Meligethes flavicollis*, and *Anaspis*  
 583 *marseuli* [Coleoptera]) on flowers of *L. praecox* and *L. triloba* of both sexes.

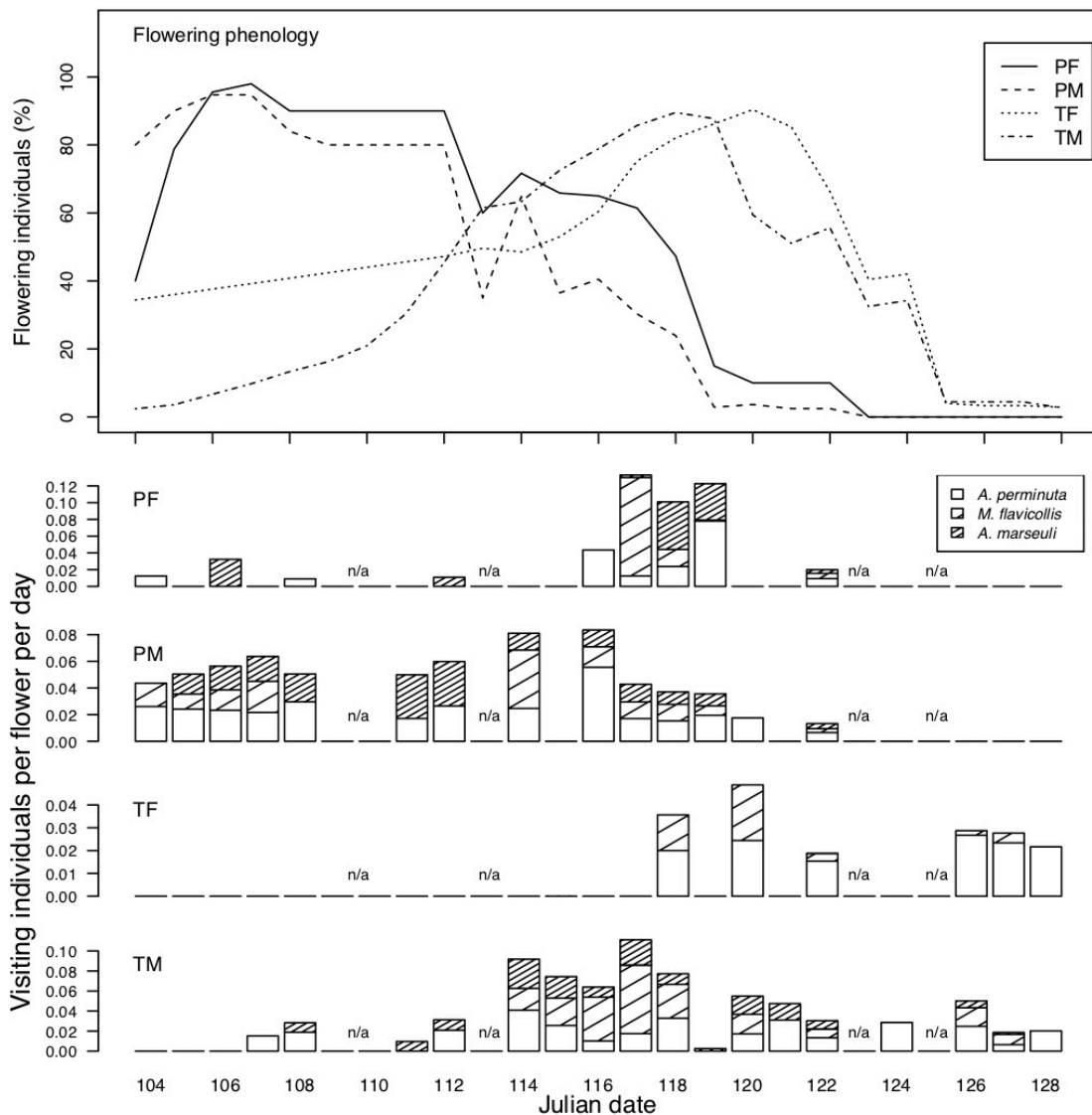
Insect species	Plant species and sex		Average attached pollen number of			No. individuals
			<i>L.praecox</i>	<i>L.triloba</i>	Other spp. <sup>*, †</sup>	
<i>A. perminuta</i>	<i>L.praecox</i>	Female	40.0	16.0	2.0 (1)	2
		Male	90.4	35.3	55.7 (8)	19
	<i>L.triloba</i>	Female	14.0	2.0	16.0 (2)	5
		Male	30.7	17.5	22.0 (7)	17
<i>M. flavicollis</i>	<i>L.praecox</i>	Female	11.0	11.0	20.0 (1)	4
		Male	33.0	46.8	27.1 (8)	22
	<i>L.triloba</i>	Female	90.0	78.0	12.0 (4)	4
		Male	27.4	41.7	43.3 (10)	48
<i>A. marseuli</i>	<i>L.praecox</i>	Female	-	-	-	0
		Male	59.4	94.4	65.9 (8)	33
	<i>L.triloba</i>	Female	-	-	-	0
		Male	10.0	43.5	20.0 (4)	9

\*: Other spp. include *Cerasus leveilleana* (Rosaceae), *Rubus palmatus var. coptophyllus* (Rosaceae), Other Rosaceae sp., *Petasites japonicus* (Asteraceae), Other Asteraceae sp., *Stachyurus praecox* (Stachyuraceae), *Heloniopsis orientalis* (Melanthiaceae), *Lonicera japonica* (Caprifoliaceae), *Rhododendron* sp. (Ericaceae), Salicaceae spp. 1, 2 and 3, and Unknown spp. 1 and 2.

†: the number in parentheses were number of other species

584

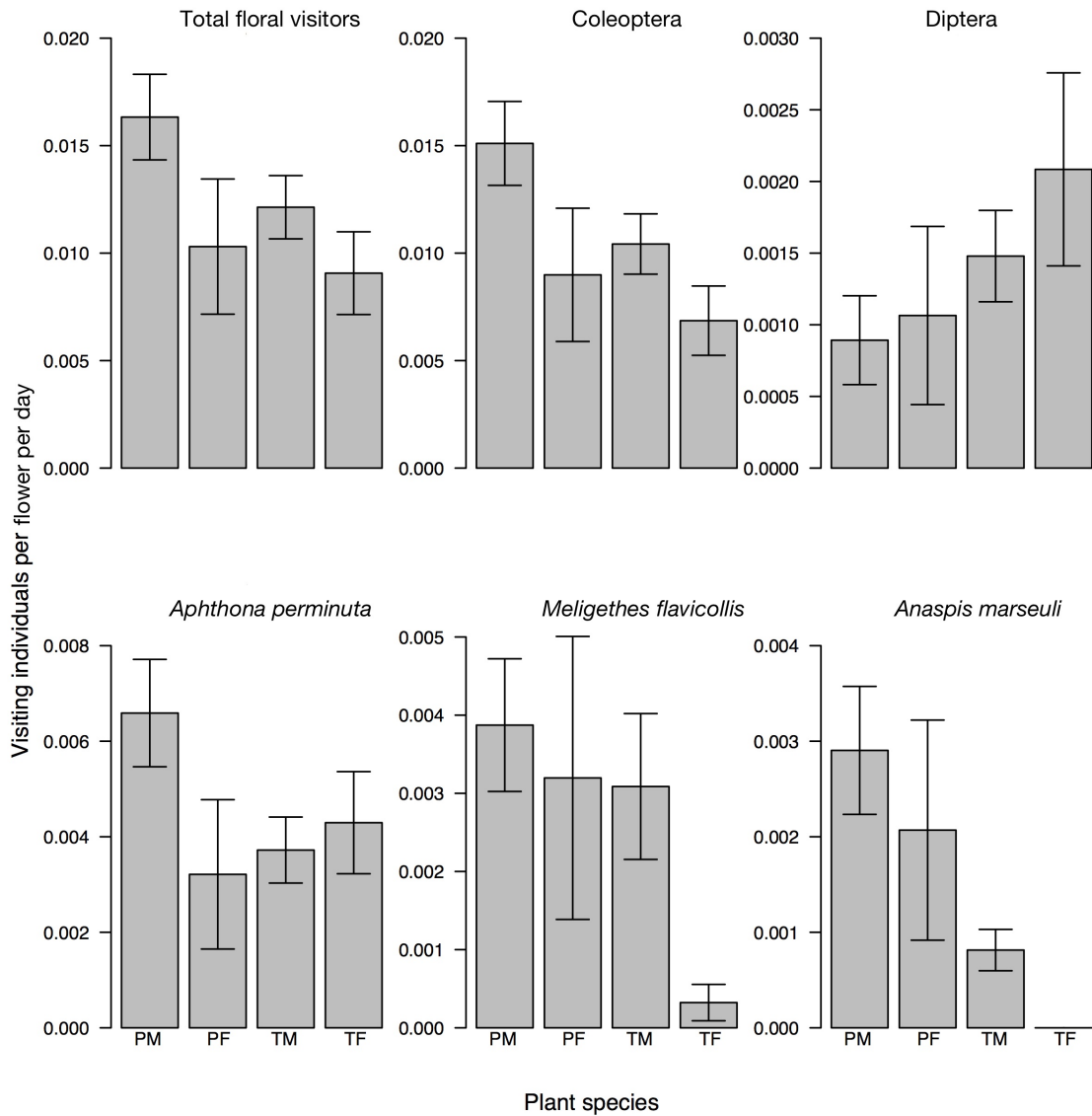
585



586

587 **Figure 1.** Flowering phenology fluctuation (3-year average) and visiting individuals of  
 588 three insect species on both sexes of *Lindera praecox* and *Lindera triloba*. In the upper  
 589 box, lines show the fluctuation of average percentages of flowering individuals; each  
 590 line type and abbreviation indicates a plant species/sex combination as follows: dashed  
 591 line, PM (*L. praecox* male); solid line, PF (*L. praecox* female); long-dashed-dotted line,  
 592 TM (*L. triloba* male); dotted line, TF (*L. triloba* female). In the bottom four boxes, bar  
 593 plots show the time series of visiting individuals per flower per day for three insect  
 594 species (*Aphthona perminuta*, *Meligethes flavicollis*, and *Anaspis marseuli*). The x-axis  
 595 represents Julian days 104 (13 April) to 128 (7 May).

596

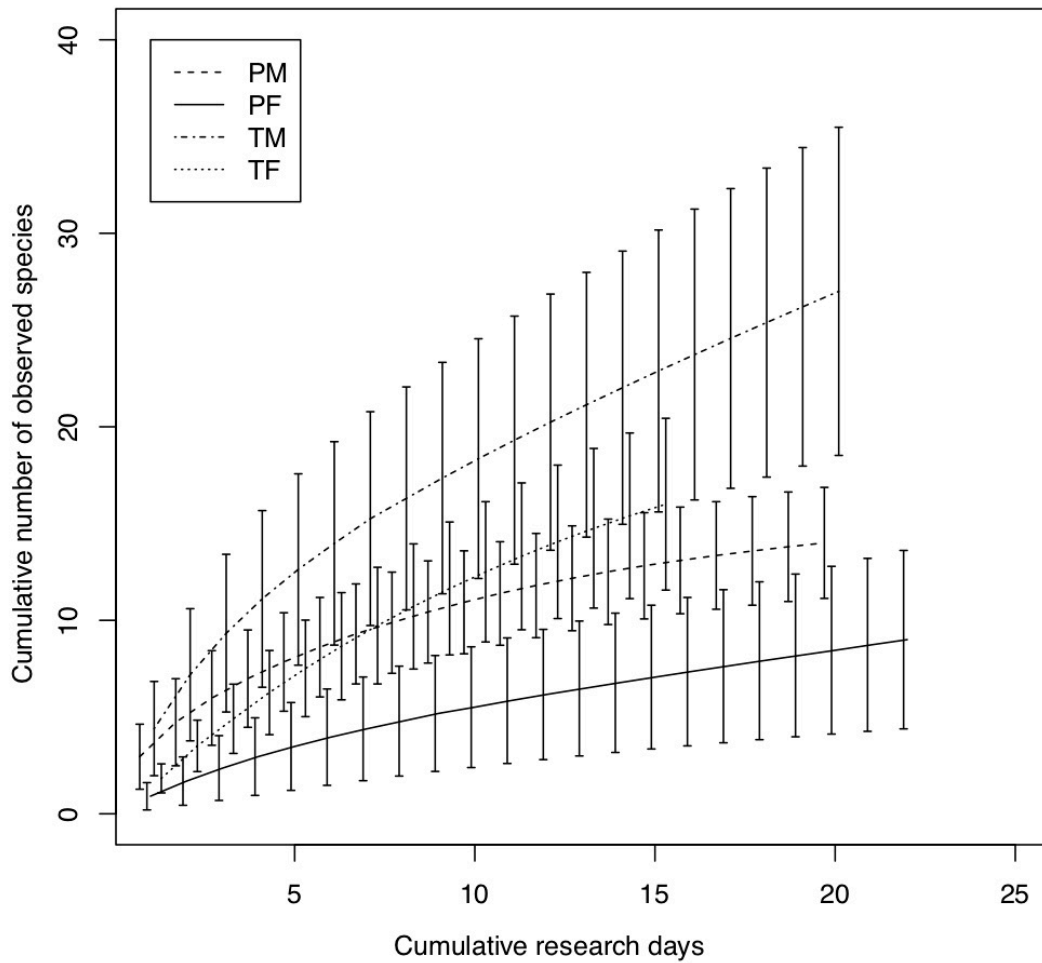


597

598 **Figure 2.** Visiting individuals per flower per day on two *Lindera* species among total  
 599 floral visitors, two insect orders (Coleoptera and Diptera), and three insect species  
 600 (*Aphthona perminuta*, *Meligethes flavicollis*, and *Anaspis marseuli*). For the  
 601 abbreviations of each plant species and sex on the x-axis, refer to the legend of Figure 1.  
 602 Bar indicates standard error (S.E).

603

604



605

606 **Figure 3.** Rarefaction curves between species number and cumulative research days for  
 607 both sexes of *Lindera praecox* and *Lindera triloba*. For the key to line types, refer to the  
 608 legend of Figure 1.

609

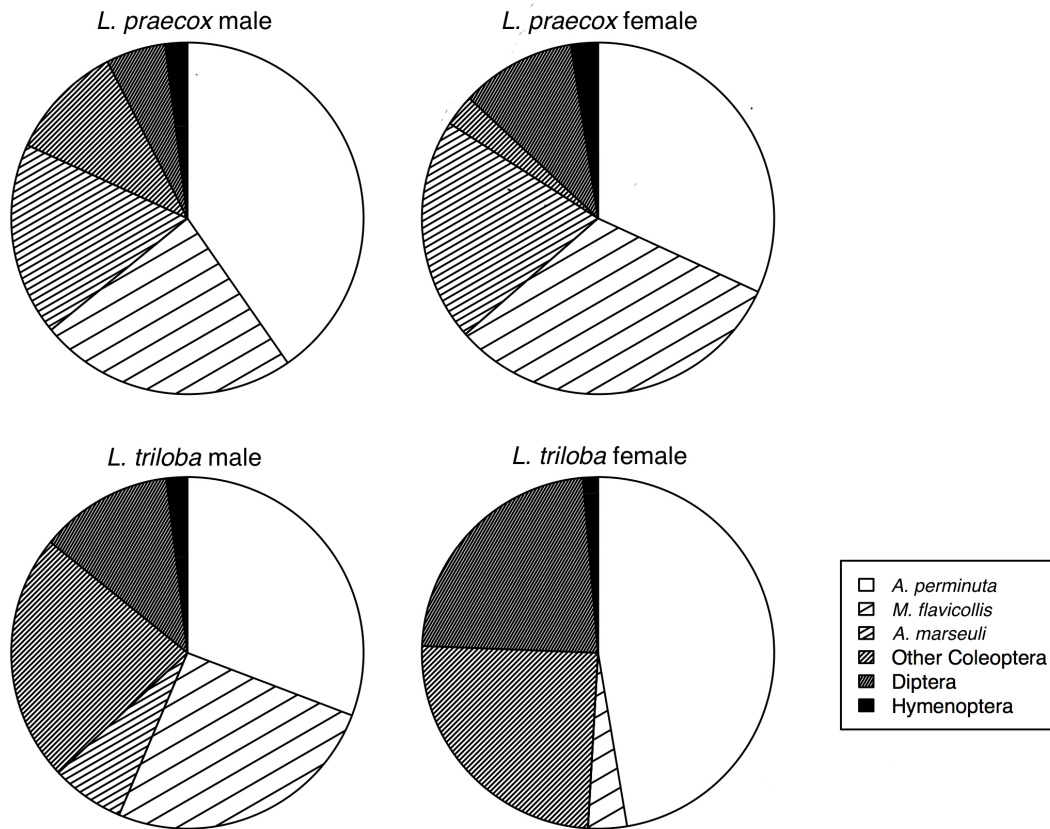
610

611

612

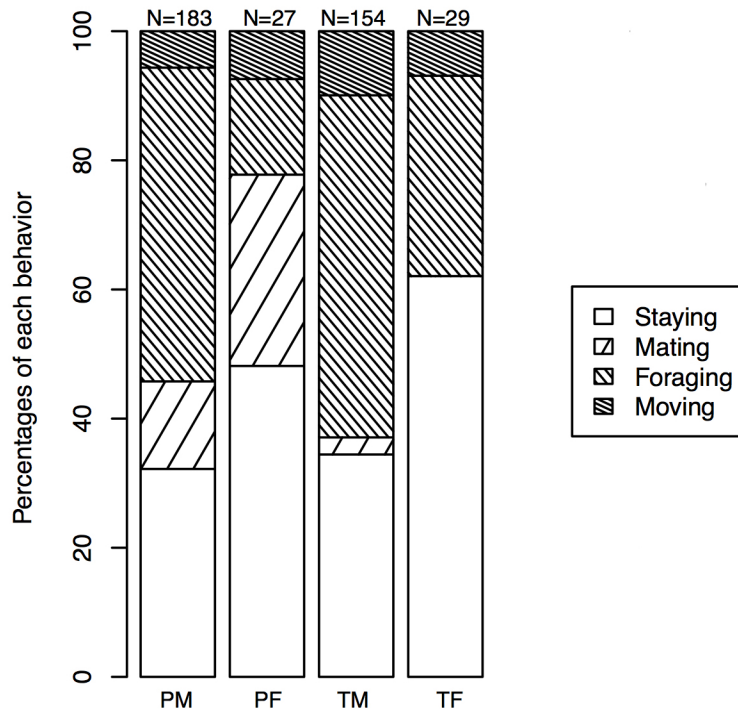
613

614



615  
 616 **Figure 4.** Percentages of each floral visitor faunal group: three dominant Coleoptera  
 617 species (*Aphthona perminuta*, *Meligethes flavicollis*, and *Anaspis marseuli*), other  
 618 Coleoptera, Diptera, and Hymenoptera. Results are expressed as individual frequencies  
 619 per plant individual per day.

620  
 621  
 622  
 623  
 624  
 625  
 626  
 627



628

629 **Figure 5.** Coleoptera behavior partitioned by plant species and sex. For the  
 630 abbreviations of each plant species and sex on the *x*-axis, refer to the legend of Figure 1.

631

632