Covering and shading by neighbouring plants diminish pollinator visits to and reproductive success of a forest edge specialist dwarf species

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1 **Original Article**

2 **Covering and shading by neighbouring plants diminish pollinator visits to and reproductive**

3 **success of a forest edge-specialist dwarf species**

4

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13 Running head: Covering and shading effects on pollination

14

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Abstract

1

2 • The pollination and reproductive success of flowering plants can be negatively

3 influenced by neighbouring heterospecific plants in various ways, such as resource

4 competition and reproductive interference. We hypothesised that covering, along with

5 shading, by neighbouring plants may reduce pollinator visits to and reproductive success

6 of plants by reducing floral attractiveness and pollinator activity and by interrupting

7 flower access, respectively.

8 • To test this hypothesis, we examined whether shaded and covered flowers suffered from

9 pollinator limitation and low reproductive success in a population of the dwarf herb

10 *Lithospermum zollingeri*, which co-exists with woody and herbaceous plants in

11 anthropogenically maintained forest-edge meadows. Here, shaded and covered flowers

12 were defined as those under the shade of the woods and those whose front portion was

13 covered by any vegetative part of neighbouring plants, respectively.

14 • The shaded and covered flowers were visited by significantly fewer pollinators than

15 sunlit and open flowers in the field. However, three major pollinator species responded

16 differently to shading and covering. Significant pollen limitation reduced seed set in

17 covered flowers, and shaded flowers produced fewer seeds. Pollen removal from the

18 anthers was not influenced by shading or covering.

19 • Our study demonstrates the negative effects of covering on pollinator visits and seed

20 production. It also elucidates the negative effects of shading on reproductive success in

21 *L. zollingeri*, which depends on managed semi-natural conditions. Management

22 abandonment, which has increased shaded and covered conditions in artificial forest-

1 edge meadows and open forest floors, might promote a rapid reduction in the population

2 of such dwarf plants.

3 **Keywords**: *Bombylius*; *Eucera*; *Oligoneura*; plant-pollinator interaction; pollen receipt;

4 pollinator functional complementarity; stoneseed; tubular flower

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6

7 **Introduction**

8 The pollination and reproductive success of flowering plants can be both positively and

9 negatively influenced by neighbouring heterospecific plants in various ways, such as

10 pollination facilitation, resource competition, and reproductive interference (Ghazoul,

11 2006; Burdfield-Steel and Shuker, 2011; Achehoug *et al*., 2016). For example, co-

12 flowering heterospecific individuals are known to mitigate negative Allee effects on

13 pollination success in rare or isolated plants (Ghazoul, 2006). In contrast, within a

14 community, the presence of neighbouring plants usually reduces the availability of light,

15 water, and nutrient resources and consequently decreases the biomass and reproductive

16 performance (the number of flowers, fruits, and seeds) of a given plant (Achehoug *et al*.,

17 2016). Furthermore, reproductive interference by flowering neighbours often reduces

18 pollinator visits and conspecific pollen grains delivered to a given plant, which may then

19 suffer from pollen limitation during sexual reproduction (reviewed by Morales and

20 Traveset, 2008; Mitchell *et al*., 2009).

21 Neighbouring plants without flowers may also have negative effects on the

22 pollination success of insect-pollinated flowering plants. Shading of whole plants, flowers,

3

1 or inflorescences (hereinafter referred to as shading) by the aboveground vegetative organs

2 (leaves, stems, and trunks) of neighbouring plants can decrease pollinator visits to the

3 shaded plant by reducing floral attractiveness (brightness) and pollinator activities, due to

4 the dark and low temperature conditions (Fig. 1; Kilkenny and Galloway, 2007; McKinney

5 and Goodell, 2010; Eckerter *et al*., 2019). In particular, the activities of various bee species

6 are known to decrease under low light and temperature conditions (Herrera, 1995*a, b*).

7 Reduced pollinator activities under shaded conditions often cause pollen-limited seed

8 production (Kilkenny and Galloway, 2007; Eckerter *et al*., 2019), although this

9 phenomenon has been examined only in limited plant species.

10   Here, we hypothesised that the covering of flowers (hereinafter referred to as

11 covering) by the vegetative organs of neighbouring plants can also decrease pollinator visits

12 by interrupting and limiting the access routes to and landing sites on the flowers and by

13 hiding attractive tepals (Fig. 1). Especially in tubular or bilaterally symmetrical flowers,

14 specialised pollinators tend to be forced to orient constantly on each flower, leading to a

15 higher pollination success (Ushimaru and Hyodo, 2005; Armbruster *et al*., 2009; Nikkeshi

16 *et al*., 2015; Armbruster and Muchhala, 2020). These constant behaviours of pollinators are

17 thought to be induced by the complex structure and lateral orientation of these flowers

18 (Neal *et al*., 1998; Ushimaru and Hyodo, 2005; Armbruster and Muchhala, 2020). When

19 the leaves or stems of neighbouring plants cover the front portion of such flowers,

20 pollinators cannot access the flowers. Thus, covering by neighbouring plants can also

21 reduce pollination and reproductive success in a given plant, although this possibility has

22 not been tested.

4

1   In this study, we examined the effects of both shading and covering by

2   neighbouring woods or herbs on pollinator visits, pollination, and reproductive success in

3   *Lithospermum zollingeri*. This dwarf herb species is predominantly distributed in forest-

4   edge meadows (Fig. 1A,B). Forest-edge specialist species are ideal subjects for this

5   research topic because they often experience different shading conditions depending on the

6   species identity, size of neighbouring woody plants and the geographical aspect of the edge

7   (e.g. south- and north-facing edges) (Fig. 1C; Ohara and Ushimaru, 2015). Moreover, the

8   flowers of dwarf species are likely to be covered by the leaves and stems of neighbouring

9   herbs and shrubs in meadows (Fig. 1D). *Lithospermum zollingeri* produces laterally

10   oriented long-tubed flowers, which are usually approached by long-tongued bee and fly

11   pollinators from the front (Fig. 1E,F, see *Study species*). By examining natural variations in

12   shading and covering conditions of *L. zollingeri*, we addressed the following questions: (1)

13   Do shaded and covered flowers receive fewer visits by bee and fly pollinators? (2) Do the

14   effects of shading and covering differ amongst the observed pollinators? and (3) whether

15   shading and covering decrease pollen receipt and removal, as well as seed production by

16   reducing pollinator visits?

17

18   **Materials and methods**

19   Study site, plots, and species

20   We studied a population of *L. zollingeri* A. DC. (Boraginaceae) in meadows between

21   terraced paddy fields and secondary forests in Yamada Area, Kobe, Hyogo Prefecture,

22   Japan (34°69, 135°20, Fig. 1A,B). The species is a spring-blooming, stoloniferous

5

1 perennial herb, which is distributed in East Asia and usually grows along forest edges and

2 on open forest floors within traditional agroforestry (*satoyama*) landscapes in Japan. In the

3 study site, *L. zollingeri* is mostly found along the forest-edge meadows, which are managed

4 by periodical mowing (Fig. 1A,B). We set six 1×3 m^2 study plots (A-F), where flowering

5 patches were observed; each plot was distributed approximately 3–15 m from the nearest

6 plot.

7   A flowering ramet of *L. zollingeri* produces 5.2 nondichogamous perfect flowers

8 on average (2–9 flowers, n = 58), from mid-April to early May in the study site; 1–3

9 flowers simultaneously open in each ramet. The horizontally or obliquely oriented tubular

10 flowers were predominantly visited by the following three insect species with long

11 proboscises (Fig. 1E,F): a long-horned bee species (*Eucera nipponensis*), the large bee-fly

12 (*Bombylius major*), and a hunch-back fly species (*Oligoneura nigroaenea*). We

13 preliminarily collected a few individuals for each pollinator species and identified species

14 based on their morphologies. The flower is purple immediately after opening and changes

15 to blue within approximately 1–2 days (Fig. 1E,F). Individual flowers open for 7–10 days

16 (RI, 'personal observation'). According to our preliminary observations, the pollinators did

17 not discriminate flowers with different colours. The stigma and anthers are placed within

18 the corolla tube and four ovules are produced in the ovary at the base of the flower. A

19 preliminary bagging experiment revealed strong pollinator dependence for fruiting in *L.*

20 *zollingeri*; only one out of 35 bagged flowers on nine ramets produced a fruit.

21

22 Effects of shading and covering on pollinator visits

1   To examine the effects of shading and covering on the behaviours of pollinators, we

2   observed the visits of pollinators to flowers in the six plots for 5 days (20, 22, 27, 29 Apr,

3   and 4 May) in 2018 (Tables S1 and S2). We observed pollinator visits in the morning

4   (0700–1200 h) because they were less frequent in the afternoon during our preliminary

5   observation. For pollinator observation, we classified all flowers within the target plot into

6   four types depending on 2×2 factorial categories (sunlit/shaded and open/covered).

7   'Shaded' and 'sunlit' flowers were defined as those under the shade of trees (and/or shrubs)

8   and those under direct sunlight, respectively, during a given observation trial (Fig. 1). We

9   defined 'covered' and 'open' flowers as those whose front portion (within approximately 5

10   cm hemisphere from the corolla tube mouth) was covered by any vegetative part of

11   heterospecific and conspecific plants and those uncovered, respectively (Fig. 1).

12   In each observation trial, we first counted the number of flowers of each type, and

13   then counted the total number of visits by each pollinator species to each flower type in the

14   plot. Only visits in which the pollinators inserted their proboscises into the corolla tubes

15   were counted. We conducted each trial for 20 min, during which the shading status of each

16   flower was consistent, although the status of some flowers changed depending on the

17   position of the sun. In a single observation day, each study plot was observed 1–2 times

18   (three times for plot D on 20 April and 4 May). We conducted 50 observation trials (1000

19   min). In each trial, at least two flower types were simultaneously observed: 15, 13, and 22

20   trials in which two, three, and four types were observed, respectively. In all trials, the

21   cumulative number of sunlit and open, sunlit and covered, shaded and open, and shaded

22   and covered flowers was 366, 147, 1160, and 573, respectively. Owing to their longevity,

7

1   some flowers could be observed for more than 1 d. The maximum number of flowers per

2   plot varied from 36 to 109 and was 60.5 on an average for the six plots (Table S2). We

3   treated data obtained from 10 trials under cloudy conditions on 27 April and 4 May as

4   shaded flower data (Table S1). It should be noted that the results were similar even when

5   we excluded data obtained under cloudy conditions.

6

7   Effects of shading and covering on pollination success

8   We examined how shading and covering influence female and male reproductive success in

9   *L. zollingeri*. As an index of shading (light) conditions for a whole day for a given flower,

10 potential direct sunlight duration (DSD; min) to a flower on 27 April (the middle day of the

11 study period) was calculated from a hemispherical photograph using CanopOn 2 (takenaka-

12 akio.org/etc./canopon2/index.html). A shorter DSD indicates that the flower was more

13 shaded by the neighbouring trees and shrubs. The hemispherical photograph was taken

14 from 1 m above the flower using the Fisheye Lens under cloudy conditions to avoid

15 halation, on 27 April (COOLPIX 990, fisheye converter FC-E8; Nikon, Tokyo, JP). When

16 several target flowers (ramets) were distributed nearby (within the 30-cm radius), a single

17 photograph of these flowers (ramets) was captured. We used the same criteria of pollinator

18 observation for categorising open/covered flowers in this analysis.

19   To examine female reproductive success, we examined pollen receipt on the

20 stigmas and seed set of the study flowers. We arbitrarily selected one or two flowers from

21 each of the 12 open and 8 covered ramets (19 open and 14 covered flowers) and collected

22 the stigma after each flower closed and stored it in a microtube. In the laboratory, we

1   counted the number of pollen grains on each stigma under a light microscope (×100,

2   Olympus CH30, Tokyo, Japan). Furthermore, we marked 306 flowers on 60 ramets (165

3   flowers on 30 open ramets and 141 flowers on 30 covered ramets) and assessed seed set

4   (seed number/ovule number) of each flower approximately 1 month after flowering.

5   To examine pollen removal from a given study flower as an index of male

6   reproductive success (Ushimaru *et al.*, 2014), we arbitrarily selected 30 closed flowers on

7   30 ramets (15 uncovered and 15 covered flowers) that were exposed to pollinators and

8   collected and stored their anthers with corolla tubes. We also collected 12 flower buds. We

9   stored each flower and bud sample separately in 200 µL 99.9% ethanol. The samples were

10   mashed with a stick and shaken to detach pollen grains from the anthers in the solution. We

11   then estimated the number of pollen grains per flower by counting the number of grains in

12   three 10-µL aliquots of each sample under a light microscope (×100). For each flower, we

13   estimated pollen removal as follows: (the mean estimated number of pollen grains in the

14   flower buds) - (the estimated number of pollen grains remaining in the closed flower) as an

15   integer.

16

17   Data analyses

18   *Pollinator visits*: Generalised linear mixed models (GLMMs) with Poisson error and

19   logarithmic link were used to analyse the effects of shading and covering on pollinator

20   visits. A zero-inflated Poisson GLMM was applied to analyse large bee-fly and hunch-back

21   fly visit data with excess zeros. In each GLMM, shading (sunlit/shaded, 0/1) and covering

22   (open/covered, 0/1) conditions were incorporated as explanatory variables, and the total

1   number of visits of all pollinators or each major pollinator species (*E. nipponensis*, *B.*

2   *major,* or *O. Nigroaenea*) to each flower type was used as the response variable. We

3   excluded the interaction between shading and covering as the explanatory variable in all

4   models, except for *B. major* because our preliminary analyses revealed no significant

5   effects of the interaction in these models. In the models, we also included the DSD total number

6   of flowers per plot and observation time (time since 0700 h) as covariates to control for

7   their potential effects on pollinator visits in the analyses. We further incorporated the

8   number of flowers of each type as an offset term to examine the visit frequency per flower.

9   The observation date and plot identities were incorporated as random terms.

10

11   *Female and male reproductive success*: We examined the effects of shading and covering

12   on pollen receipt and removal using GLMMs with negative binomial errors and logarithmic

13   link. In the model, the response variable was the number of pollen grains on the stigma or

14   pollen removal from each study flower. To examine the effects of shading and covering on

15   seed set, we used a GLMM (binomial errors and logit link) in which seed set per flower

16   (the seed number/ovule number of four) was the response variable. We also conducted a

17   GLMM analysis in which the response variable was seed set per ramet (the total seed

18   number/total ovule number of the ramet) because some open ramets produced higher

19   numbers of flowers (mean±SE, range; 5.5±0.3, 3–9) than covered ramets (4.7±0.2, 2–6).

20   We should note that there was no significant difference in the mean flower number between

21   the open and covered ramets (Table S3). In all GLMMs, we included the DSD and covering

status (open/covered, 0/1) as the explanatory variables and nested plot and ramet identities

as random effects.

Results

Pollinator visits

We observed 380 visits by 162 pollinators to the study flowers. Long-horned bees were the

most dominant (54% of total visits), followed by bee-flies (27%) and hunch-back flies

(16%). Most *Eucera* individuals were males that sought nectar, similar to the two fly

species, and only a few females were observed to visit flowers. We found that only two

Xylocopa appendiculata circumpolar and four butterfly individuals visited *L. zollingeri*

flowers.

Shading and covering significantly decreased the total visits per flower, and the

shaded and covered flowers received the fewest visits among all flower types (Fig. 2, Table

1). The responses to shading and covering differed among the major pollinator species.

Visits by long-horned bees were significantly decreased by shading and covering (Fig. 2,

Table 1). Shading and the interaction between shading and covering had significantly

negative effects on bee-fly visits, whereas covering did not influence their visits under

sunlit conditions (Fig. 2, Table 1). Furthermore, hunch-back fly visits significantly

decreased with covering but not with shading (Fig. 2, Table 1).

Visits by long-horned bees and hunch-back flies significantly decreased and

increased with the total number of flowers per plot, respectively, whereas visits by bee-flies

were not influenced by this variable (Table 1). Visits by long-horned bees and hunch-back

1   flies significantly increased with time in the morning, whereas those by bee-flies

2   significantly decreased with this variable (Table 1). The total visit frequency was not

3   influenced by total flower number per plot or observation time (Table 1).

4

5   Female and male reproductive success

6   The DSD varied from 0 to 235 min for the study flowers, indicating that all study flowers

7   experienced shade for at least several hours a day in the study site. Pollen receipt on the

8   stigma was significantly fewer in covered flowers than in open flowers, but it did not

9   change with the DSD (Fig. 3, Table 2). The seed set of each individual flower significantly

10   and marginally decreased with the decrease in the DSD and with covering, respectively;

11   whereas that of each ramet significantly decreased with both the decrease in the DSD and

12   covering (Fig. 3, Table 2). Pollen removal was not influenced by DSD or covering (Fig. 3,

13   Table 2).

14

15   **Discussion**

16   The findings of our research with the forest-edge dwarf herb species *L. zollingeri* support

17   our hypothesis regarding the effects of covering and are similar to the results of previous

18   studies on the negative effects of shading.

19   Although the total number of pollinator visits decreased with coverage, there were

20   interspecific differences among the pollinators in the visits to covered flowers. Long-

21   horned bees and hunch-back flies avoided the covered flowers. The bees seemed to avoid

22   flight into the bushes of stems and leaves around the flowers. The hunch-back flies were

12

1   observed handling covered flowers for longer than on open flowers owing to the hindrance

2   to insert their proboscises into the tubes by the vegetative parts; although we did not record

3   handling times (AU and RI, 'personal observation'). The longer handling time on covered

4   flowers might subsequently lead to avoidance behaviour in the flies. Furthermore, bee-flies

5   most frequently visited covered flowers under sunlit conditions. Their high hovering ability

6   possibly enables them to fly through a bush of stems and leaves during their search for

7   flowers. Their number of visits to covered flowers substantially decreased under shaded

8   conditions, suggesting that this species avoids darker conditions.

9   Lower pollen receipt and seed set in covered flowers suggested that reduced

10   pollinator visits caused pollen limitation in female reproductive success in these flowers. In

11   contrast, pollen removal was not influenced by the cover. A possible explanation for this

12   discrepancy between male and female reproductive success is that pollen grains are carried

13   more in quantity or accidentally lost by infrequent pollinators that stay on covered flowers

14   for a relatively long duration. Harder (1990) reported that the amount of pollen removal

15   increased with increasing visit duration. However, we could not examine this possibility

16   with our dataset, and this needs to be investigated in the future. Pollen removal has often

17   been considered a poor proxy of male success, especially when pollinators actively collect

18   pollen on flowers. In our case, all the dominant pollinators were nectar seekers. Pollen- and

19   nectar-collecting bees removed the same amount of pollen in *Pedicularis bracteosa*

20   (Harder, 1990), but the generality of the finding is still unknown. To further examine the

21   covering effect on male siring success, more detailed pollinator observation and paternity

22   analysis using molecular markers is necessary.

1   Shading negatively influenced the behaviour of not only the long-horned bees, but

2   also that of the large bee-flies, which is consistent with the findings of previous studies on

3   bee behaviour (Herrera, 1997; Kilkenny and Galloway, 2007; McKinney and Goodell,

4   2010; Eckerter *et al*., 2019). In early spring with cool ambient conditions, shading further

5   lowered the temperature, and this possibly discouraged these bees and flies from seeking

6   flowers (Herrera, 1995*a*; Inouye *et al*., 2015). Conversely, the hunch-back flies preferred

7   flowers under shade, although the pattern was not significant. It should be noted that the

8   hunch-back flies were the only pollinators found under cloudy conditions at the study site.

9   The shade and cloudy condition-tolerant foraging behaviour of *O. nigroaenea* could be due

10   to its dark body, which enables it to easily reach and maintain body temperatures conducive

11   to flight (Willmer and Unwin, 1981). Since we observed pollinator visits mainly under

12   sunny conditions, the visit frequency of hunch-back flies might be underestimated.

13   The interspecific differences in the response to shading conditions might provide

14   pollination function complementarity (Fründ *et al*., 2013). Despite the significantly low

15   number of pollinator visits to the shaded flowers, pollen receipt and removal were not

16   affected by the DSD in *L. zollingeri*. Pollination by hunck-back flies might compensate for

17   the low levels of pollination by long-horned bees and bee-flies in relatively more shaded

18   flowers. Additionally, sunlight for a short duration during a day could be sufficient for the

19   pollination of *L. zollingeri* flowers by the bee and bee-fly species.

20   In contrast, seed production was reduced in the more shaded flowers (especially

21   those on ramets that received direct sunlight for less than 1 h a day), suggesting that

22   shading prevented fruit and seed maturation after fertilisation. This is because pollen

1   receipt was not influenced by the degree of DSD. Shading by woody plants often directly

2   reduces reproductive success by limiting resource allocation to reproduction in forest-floor

3   herbs (Kilkenny and Galloway, 2007; Kudo *et al*., 2008). It should be noted that the

4   number of flowers per ramet did not change with the DSD (Table S3), indicating that

5   flower production per ramet was not influenced by shading, unlike that in European

6   bilberry (Eckerter *et al*., 2019). Our results suggest that shading for almost the whole day

7   might cause severe resource limitation during fruit and seed development, even in forest-

8   edge specialist plants. Alternatively, low flower temperature caused by shading might

9   diminish pollen tube growth and seed development, as often found in spring blooming

10   herbs (Kudo, 1995). These ideas should be further examined using hand-pollination and

11   temperature control experiments in future research.

12   The relationship between the frequency of visits and the number of flowers per

13   plot, and patterns of temporal change in foraging activity in the morning differed among the

14   pollinator species. With an increase in the total number of flowers, the number of visits by

15   the long-horned bees significantly decreased, whereas those by the hunch-back flies

16   significantly increased. Unlike previously reported positive responses to floral density

17   (Campbell and Motten, 1985; Thompson, 2001), visits by bee-flies did not increase with

18   increasing flower number per plot. These findings suggest that only the hunch-back flies

19   were attracted by an increase in the number of flowers per unit area. Both long-horned bees

20   and hunch-back flies were more active around noon, whereas the bee-flies decreased their

21   activities towards noon (Table 1). Daily activity patterns often vary largely between bees

22   and flies and among species within groups depending on their thermoregulatory ability,

1   energy requirements and their interactions with habitat environments (Herrera, 1990; Rader

2   et al., 2013). The interspecific behavioural differences are likely to mitigate the effects of

3   these explanatory variables on total pollinator visits in our study (Table 1). Together with

4   the differences in the responses to covering and shading conditions, the behavioural

5   diversity among the three major pollinators may provide functional complementarity in the

6   pollination process of *L. zollingeri* (Blüthgen and Klein, 2011; Fründ *et al*., 2013). Since

7   we did not observe direct interferences between any pollinator pairs, their behavioural

8   differences might be due to differences in physiological features or indirect resource

9   competition (Fründ *et al*., 2013). Thus, visitations by diverse pollinator species may

10   maintain stable pollination success even in specialised floral features.

11   Traditionally, agroforestry management practices with mowing and logging

12   reduced covered and shaded habitat conditions (Ushimaru *et al*., 2008; Ohara and

13   Ushimaru, 2015), which may be preferable for the successful pollination of dwarf herb

14   species on forest edges and floors. However, the recent prevalence of agricultural land and

15   secondary forest abandonment has promoted cessation of these management practices

16   throughout the country, increasing the shaded and covered conditions in forest-edge

17   meadows and forest floors (Uematsu *et al*., 2010; Ministry of the Environment of Japan,

18   2012). Under expanding land abandonment, *L. zollingeri* is currently listed as more than

19   near-threatened in the Red List of the 19 prefectures (ca. 40% of all prefectures' list).

20   Several grassland and forest-floor herbs, which depend on relatively open semi-natural

21   habitats, have also rapidly decreased owing to land abandonment in the country (Ministry

22   of the Environment of Japan, 2012; Ushimaru *et al*., 2018, 2020), similar to those in other

16

1 Palaearctic regions (Dengler *et al*., 2020). Since we examined only one species as a case

2 study, the negative effects of shading and covering after land abandonment on pollination

3 and reproductive success should be examined for more endangered herb species worldwide.

4 This can aid in testing the generality of the effects and contribute to the understanding of

5 the causes of ongoing local species extinction events.

6

7 Conclusion

8 Our findings demonstrated that covering significantly reduced female reproductive success

9 by causing pollinator limitations in *L. zollingeri*. Meanwhile, shading diminished seed

10 production of the species, likely due to resultant resource or temperature limitation. Thus,

11 neighbouring heterospecific woody and herbaceous species may have negative effects on

12 the pollination and reproductive success of dwarf species at artificially managed forest

13 edges and floors in various ways, although visits by diverse pollinators could slightly

14 mitigate these effects. The recent global prevalence of management abandonment could

15 enhance negative shading and covering effects, thereby acting as a potential threat to dwarf

16 herb species living in semi-natural grasslands and forests.

17

18 Supporting information

19 Table S1. Weather conditions during the study period in 2018.

20 Table S2. Temporal changes in the maximum flower number in the six study plots on the

21 days of pollinator observation.

22 Table S3. GLMM result for the number of flowers per ramet in *Lithospermum zollinger*.

1

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1   **Figure legends**

2   Fig. 1. Forest edge habitats (A and B), sunlit and shaded (C) and covered flowers (D), and

3   pollinator species (E, *Eucera nipponensis* visiting shaded flowers; F, *Oligoneura*

4   *nigroaenea* on an open flower) on open flowers in *Lithospermum zollingeri*. In photos A

5   and B, the arrows indicate study flower-patch places of *L. zollingeri*.

6   Fig. 2. Visit frequency per flower during a 20-min observation of all pollinators and the

7   three major pollinator species to each flower type. Mean and S.E. are shown. See Table

8   1 for analytical results and sampling numbers.
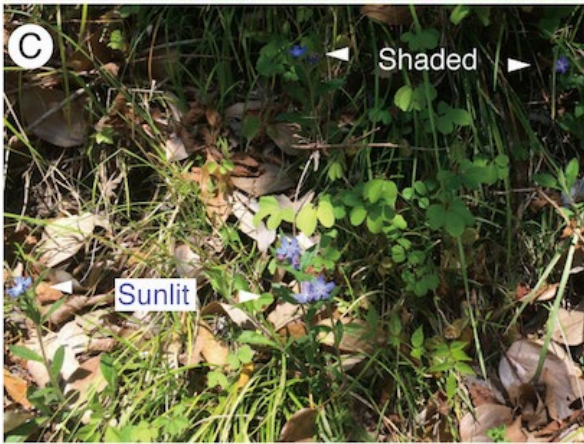
9   Fig. 3. Relationships between shading and covering conditions and female (pollen receipt

10   and seed set per flower) and male reproductive success (pollen removal per flower).

11   Shorter direct sunlight duration indicates more shaded conditions. *$P < 0.05$; $^{+}P < 0.10$.

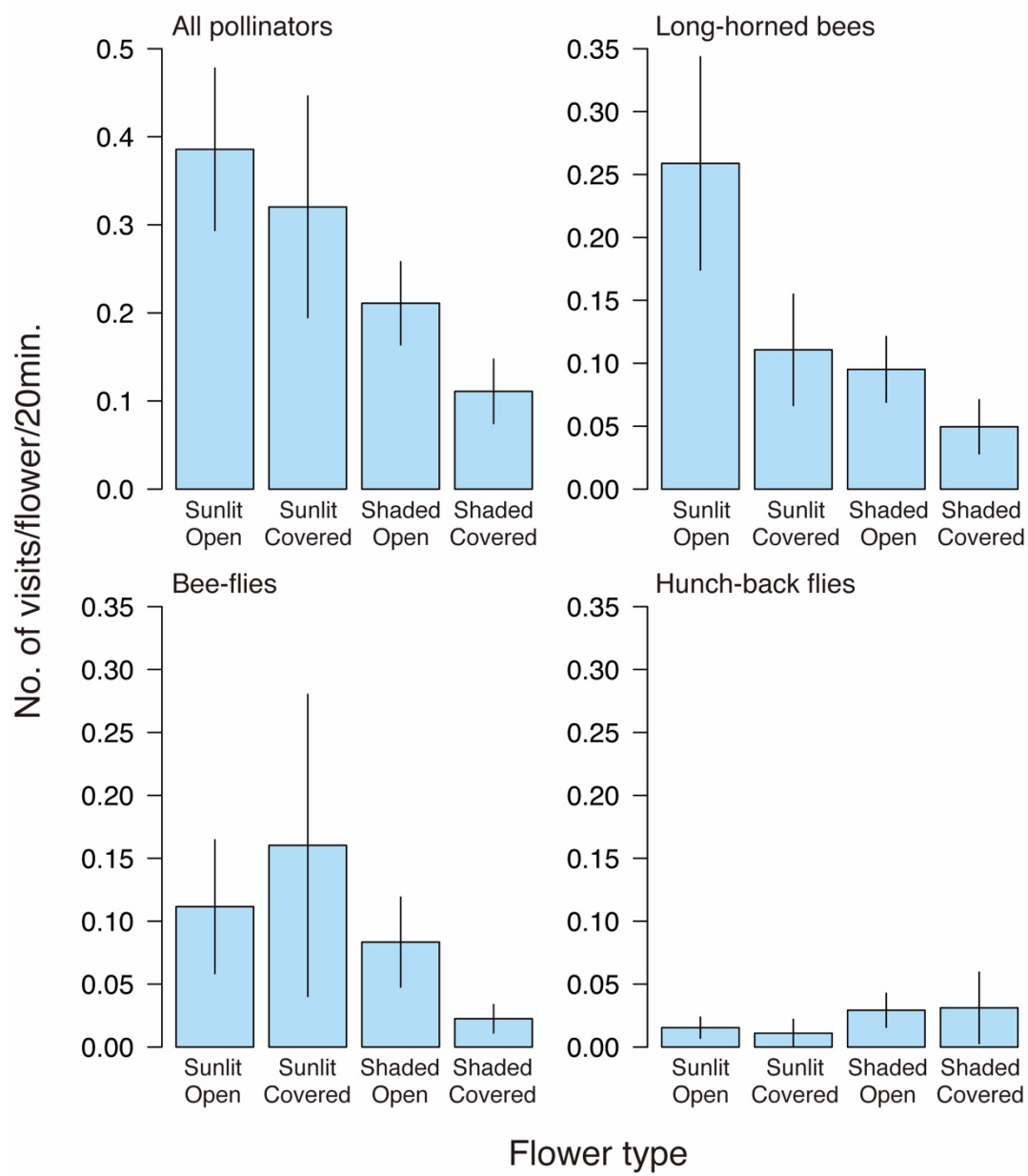12   See Table 2 for analytical results and sampling numbers.

13

Table 1. GLMM results of pollinator visit analyses. Pollinator species (individual number and total visit number), estimated coefficient, and standard error (SE) for each variable are shown. Variables with significant P values are shown in bold.

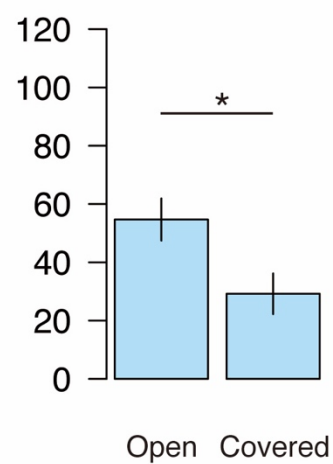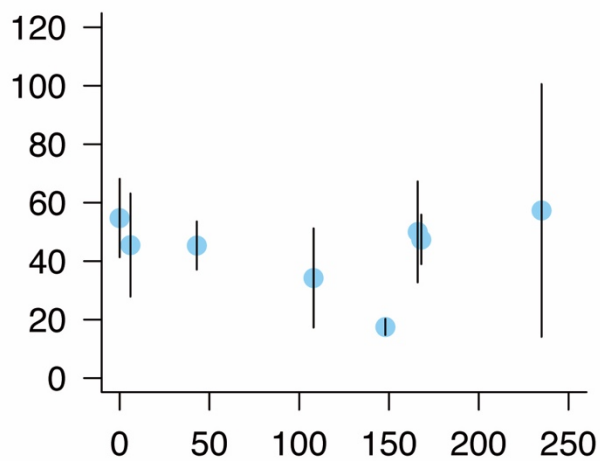| Species	Explanatory variable	Coefficient ± SE	z	P
Long-horned bees (88, 206)				
	Shading	-0.554 ± 0.153	-3.61	**<0.001**
	Covering	-0.579 ± 0.211	-2.75	**0.006**
	Flower number per plot	-0.077 ± 0.011	-6.85	**<0.001**
	Observation time	0.006 ± 0.002	3.29	**<0.001**
	Intercept	-2.134 ± 1.726	-1.24	0.216
Bee-flies (39, 103)				
	Shading	-0.828 ± 0.273	-3.03	**0.002**
	Covering	-0.362 ± 0.398	0.91	0.365
	Shading × covering	-1.264 ± 0.574	-2.20	**0.028**
	Flower number per plot	-0.002 ± 0.018	0.14	0.891
	Observation time	-0.015 ± 0.004	-3.59	**<0.001**
	Intercept	-11.842 ± 5.417	-2.19	**0.029**
Hunch-back flies (29, 61)				
	Shading	0.215 ± 0.523	0.41	0.681
	Covering	-0.847 ± 0.378	-2.24	**0.025**
	Flower number per plot	0.048 ± 0.020	2.37	**0.018**
	Observation time	0.008 ± 0.003	2.48	**0.013**
	Intercept	-8.690 ± 1.872	-4.64	**<0.001**
All pollinators (162, 380)				
	Shading	-0.443 ± 0.116	-3.83	**<0.001**
	Covering	-0.605 ± 0.142	-4.28	**<0.001**
	Flower number per plot	-0.0004 ± 0.004	-0.09	0.926
	Observation time	0.001 ± 0.001	0.52	0.606
	Intercept	-1.827 ± 0.515	-3.55	**<0.001**

1

Table 2. GLMM results of female and male reproductive success in *Lithospermum zollingeri*. Estimated coefficient and standard error (SE) are shown for each variable. Variables with significant and marginally significant *P* values are shown in bold and are underlined, respectively. Sampled flower and ramet numbers (open, covered) are shown in the parenthesis.

Response variable	Explanatory variable	Coefficient±SE	*z*	*P*
Pollen receipt per flower (19, 14)				
	Direct sunshine duration	-0.001±0.002	-0.79	0.428
	Covering	-0.694±0.283	-2.46	**0.014**
	Intercept	4.178±0.288	14.53	**<0.001**
Seed set per flower (165, 141)				
	Direct sunshine duration	0.005±0.002	2.32	**0.021**
	<u>Covering</u>	-0.508±0.279	-1.82	<u>0.068</u>
	Intercept	-3.037±0.328	-9.26	**<0.001**
Seed set per ramet (30, 30)				
	Direct sunshine duration	0.005±0.002	2.33	**0.020**
	Covering	-0.518±0.252	-2.06	**0.040**
	Intercept	-2.951±0.305	-9.69	**<0.001**
Pollen removal per flower (15, 15)				
	Direct sunshine duration	-0.508±0.279	-0.26	0.79
	Covering	0.172±0.131	1.32	0.19
	Intercept	7.641±0.092	83.1	**<0.001**

1

No. of visits/flower/20min.

All pollinators

Sunlit Open | Sunlit Covered | Shaded Open | Shaded Covered

Long-horned bees

Sunlit Open | Sunlit Covered | Shaded Open | Shaded Covered

Bee-flies

Sunlit Open | Sunlit Covered | Shaded Open | Shaded Covered

Hunch-back flies

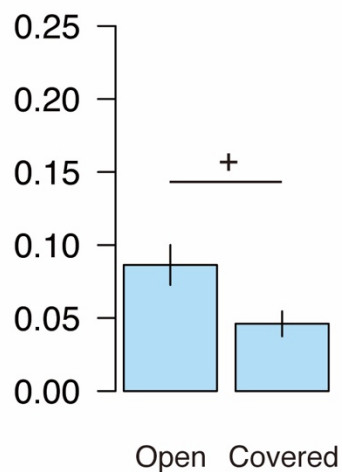Sunlit Open | Sunlit Covered | Shaded Open | Shaded Covered
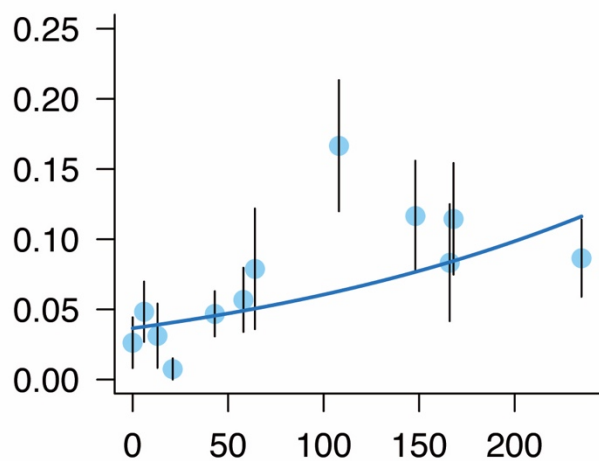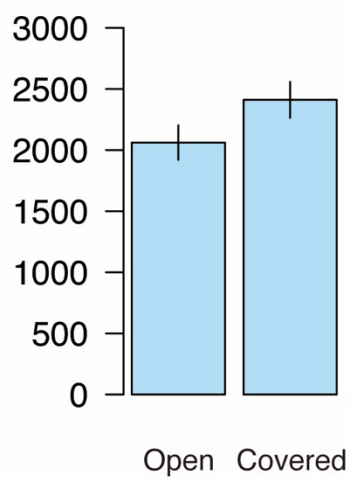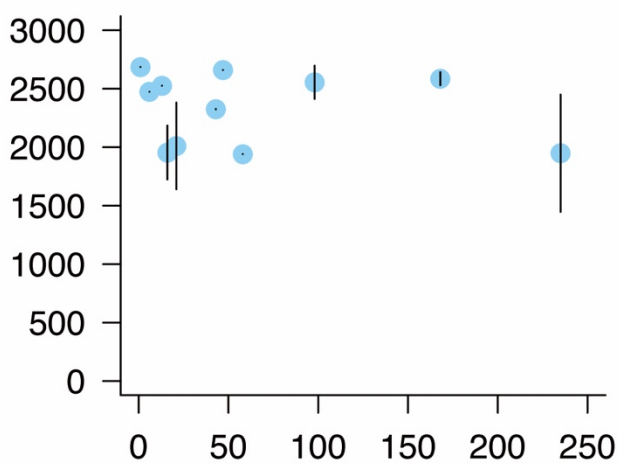
Flower type

Pollen receipt per stigma

Seed set per flower

Pollen removal per flower

Direct sunlight duration (min.)

Flower type

Open   Covered

1

Table S1. Weather conditions during the study period in 2018. Data were recorded at a nearby (34°41.8' N, 135°12.7' E) automated meteorological data acquisition point of the Japan Meteorological Agency. Pollinators were observed on the dates indicated in bold.

Month	Date	Precipitation	Air temperature			Sunshine Duration	Weather in the daytime
			Mean	Max.	Min.		
April	**20**	**0**	**19.3**	**23.9**	**14.3**	**12.2**	**Sunny**
	21	0	19.7	24.2	15.0	12.3	Sunny
	22	**0**	**19.8**	**23.1**	**16.3**	**11.7**	**Sunny & cloudy**
	23	0	20.2	24.0	16.8	10.0	Cloudy & sunny
	24	46	19.2	21.1	18.0	0.0	Rainy
	25	40.5	17.2	19.7	13.9	0.8	Cloudy
	26	0	16.4	20.6	13.0	9.2	Slightly cloudy
	27	**0**	**17.9**	**20.9**	**15.4**	**3.0**	**Cloudy**
	28	0	17.6	20.9	13.8	12.6	Sunny
	29	**0**	**19.4**	**23.7**	**14.2**	**12.6**	**Sunny**
	30	0	19.6	22.4	17.6	4.6	Cloudy & sunny
May	1	0	20.5	26.9	16.2	11.7	Sunny & slightly cloudy
	2	21.5	20.2	21.8	19.0	0.0	Rainy & cloudy
	3	0.5	18.9	21.1	15.9	7.2	Slightly cloudy & sunny
	4	**0**	**15.9**	**19.1**	**12.0**	**10.5**	**Sunny**

2

1

Table S2. Temporal changes in the maximum flower number in the six study plots on the days of pollinator observation.

Month	Date	Maximum number of flowers per plot					
		A	B	C	D	E	f
April	20	47	14	27	53	28	12
	22	35	29	48	68	37	24
	27	26	32	71	108	28	36
	29	23	40	78	109	53	28
May	3	0	14	28	88	16	12

2

3

Table S3. GLMM result for the number of flowers per ramet in *Lithospermum zollingeri*. Effects of shading and covering were examined. Estimated coefficient and standard error (SE) are shown for each variable. Significant *P* values are shown in bold.

Explanatory variable	Coefficient ± SE	*z*	*P*
Direct sunshine duration	0.000464 ± 0.000831	0.56	0.58
Covering	-0.181 ± 0.118	-1.53	0.13
Intercept	1.697 ± 0.108	15.75	**<0.001**