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Title	Temporal skewness of pollination success in the spring ephemeral Trillium camschatcense
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1	Short	commu	nication
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- 2 Temporal skewness of pollination success in the spring ephemeral *Trillium*
- 3 camschatcense
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12 Abstract

13 Phenological overlap with pollinators is crucial for reproductive success in insectpollinated plants. In this study, we examined whether pollinator visitation successfully 14 15 occurred during an entire flowering season in two populations of the insect-pollinated spring ephemeral Trillium camschatcense in the Tokachi region of Hokkaido, northern 16 17 Japan. We bagged flowers and excluded pollinator visitation during either the first or the last half of the entire flowering season to compare pollination success between the two 18 19 periods. The two populations have experienced differing levels of climate warming in 20 the last 60 years, which impacted pollinator visitation. In the population experiencing 21 temperature rise more rapidly, fertilization rate and seed set decreased sharply when 22 bagged during the first half period, indicating that pollinator visitation is skewed to the 23 early part of the flowering season. The temporal skewness of pollination success would 24 be an early warning signal of the impacts of climate warming on the reproductive 25 success of T. camschatcense.

26

27 Keywords

climate warming, fertilization, phenological mismatch, bagging, seed set

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30 Introduction

47

31 Climate warming is altering the flowering phenology of many plant species (Root et al. 32 2003; Walther et al. 2002), particularly in early blooming spring ephemerals that sprout and flower directly after snowmelt (Fitter and Fitter 2002). Because the phenological 33 response to rising temperature differs between taxa and species (Parmesan 2007; Post 34 35 2019), pollinator activities does not necessarily shift in synchrony with flowering, potentially resulting in a temporal mismatch and reduced pollinator visitation (Kudo and 36 Cooper 2019). It is crucial to evaluate the degree of phenological overlap with 37 pollinators for insect-pollinated spring ephemeral plants as temperatures continue to 38 39 rise. Trillium camschatcense is an insect-pollinated, long-lived spring ephemeral 40 41 that sprouts in mid to late April and flowers in May in temperate deciduous forests. 42 Although most populations of *T. camschatcense* in Japan are self-compatible, those in 43 the Tokachi region of Hokkaido, northern Japan, are self-incompatible and owe seed production completely to insect pollinators (Ohara et al. 1996). Therefore, a 44 45 phenological mismatch with pollinators could be a serious threat to the reproductive 46 success of T. camschatcense.

3

In this study, we examined whether pollination successfully occurred during a

48	complete flowering season of Trillium camschatcense at two locations in the Tokachi
49	region, where long-term temperature data was available. We hypothesized that
50	pollination was more temporally limited due to a phenological mismatch at the location
51	experiencing more rapid warming.
52	
53	Materials and Methods
54	Study species
55	Trillium camschatcense is an understory herb whose primary pollinators are Coleoptera
56	and Diptera (Tomimatsu and Ohara, 2003). T. camschatcense does not produce
57	inflorescences made up of multiple flowers. Flowering individuals usually have one
58	flowering stem (sometimes two stems), with one flower and three leaves per stem. <i>T</i> .
59	camschatcense does not compensate for reproductive failure by producing additional
60	flowers later in the flowering season: the number of flowers per individual is pre-
61	determined before flowering onset. Most flowering stems synchronously sprout in
62	April, start anthesis in early May, and finish flowing around late May to early June.
63	After the flowering season, fruits ripen in July (Ohara and Kawano 2005; Ohara et al.
64	2006).

65

66 Study Sit	tes
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67	The present study was conducted in two populations located in: Obihiro (inland,
68	42.802° N, 143.103° E) and Hiroo (seacoast, 42.316° N, 143.324° E) (Fig. 1a). The two
69	populations were approximately 57 kilometers apart. Obihiro is a remnant forest
70	surrounded by agricultural lands. Hiroo is a part of a windbreak forest that stretches
71	along the coastline. The two populations were relatively large for the region in terms of
72	population density and habitat area (Fig. S1, S2).
73	
74	Temperature
75	We obtained the daily temperature of Obihiro and Hiroo from 1958 to 2019 from the
76	website of the Japan Meteorological Agency (JMA,
77	https://www.jma.go.jp/jma/index.html). We extracted the data for April and May, the
78	growing and flowering months of Trillium camschatcense. For each site, we carried out
79	a linear regression against the mean daily temperature per year, using year as an
80	explanatory variable.
81	
82	Flowering phenology and precipitation
83	In early April 2021, we randomly established two 3 m \times 3 m quadrats in each

84	population. Before flowering onset, we labeled all flowering stems within the quadrats
85	in late April (Fig. S3a). Flowering stems that sprouted later were labeled right after we
86	noticed their emergence. We monitored the labeled flowers at an interval of 2 to 11
87	days. We defined the first and the last day of the anthesis of each flower as the date on
88	which we first confirmed that the flower became open/withered. Along with
89	phenological surveys, daily precipitation during the flowering season in the study year
90	(2021), as well as that in the past years (from 1958 to 2019), was obtained from the
91	website of the JMA.

93 **Bagging experiment**

94	We divided the entire flowering period of Trillium camschatcense into two parts, the
95	first half and last half, and implemented three treatments: (1) the control, in which
96	flowers were left intact throughout the flowering period; (2) the first half, in which
97	flowers were left intact during the first half, but then bagged with cellophane bags
98	during the last half, (3) and the last half, in which flowers were bagged prior to anthesis
99	and then opened during the last half (Fig. S3b). The cellophane bags excluded
100	pollinators from flowers. Therefore, comparing seed production among the three
101	treatments could reveal the relative frequency of pollinator visitation during an

102 unbagged period.

103	Before flowering onset, we randomly selected 30 individuals for the first half
104	and the last half treatments around each 3 m \times 3 m quadrat, as well as 30 individuals
105	within the quadrat for control. Approximately 40 flowers were selected for each
106	treatment in each quadrat (Table S1). Flowers for the last half treatment were bagged at
107	that time. Because Trillium camschatcense in the Tokachi region usually flowers in May
108	and early June, we opened flowers for the last half treatment but bagged those for the
109	first half treatment within one week of the midpoint of May (May 16): on May 17 and
110	22 in Obihiro and Hiroo, respectively (Fig. S3c). We measured the length and width of a
111	leaf of each flowering stem to account for the confounding effects of plant size on
112	reproduction.
113	In early July, we collected fruits and counted the number of unfertilized ovules,
114	fertilized ovules that did not develop to seeds due to abortion, and seeds (Fig. S3d). We
115	recorded the presence/absence of stem injury caused by trampling and herbivory on
116	seeds by caterpillars, both of which took place before collecting fruits and might have
117	overridden the effects of bagging treatments by decreasing the number of seeds. We
118	defined "fertilization rate" and "seed set" as follows:
119	fertilization rate = $\frac{\text{sum of fertilized ovules and seeds}}{\frac{1}{1}$

total sum of ovules and seeds

120	seed set = $\frac{\text{sum of seeds}}{\text{total sum of ovules and seeds}}$
121	We constructed a generalized linear mixed model (GLMM) with binomial error and
122	logit link for fertilization rate and seed set, using the function "glmer" in the R package
123	"lme4" (Bates et al. 2015). Models were separately constructed for Obihiro and Hiroo.
124	For each response variable, we incorporated the treatment, size (= leaf length \times leaf
125	width), presence/absence of herbivory and stem injury as fixed effects, and individual
126	and quadrat as random effects. We carried out a multiple comparison test for treatment
127	by a Tukey method with Benjamini-Hochberg correction, using the R package
128	"multcomp" (Hothorn et al. 2008).
129	
130	Results
131	Temperature
132	The mean daily temperature of April and May in Obihiro significantly increased from
133	1958 to 2019 at a rate of 0.021 °C per year ($P = 0.005$, Fig. 1b). Although the
134	temperature in Hiroo was also rising, the rate of 0.012 °C per year was not statistically
135	significant ($P = 0.106$), indicating that Obihiro was experiencing more rapid warming
136	compared to Hiroo.

138 Flowering phenology and precipitation

139	In the four quadrats used for phenological survey (two in each population), all flowers
140	continued anthesis across the first and the last half period, depicting unimodal
141	trajectories (Fig. S4a). The timing of the onset, the peak, and the end of flowering was
142	earlier overall in Obihiro than in Hiroo. Daily precipitation of the study year (2021), as
143	well as the average daily precipitation over the past 60 years (1958-2019), mostly
144	remained low throughout the flowering period in both sites (Fig. S4b).
145	
146	Bagging experiment
147	The fertilization rate of the last half treatment was lower than that of control and first
148	half treatment in Obihiro (Table S2, Fig. 2a). On the other hand, there were no
149	significant differences among the three treatments in Hiroo (Table S2, Fig. 2b). The
150	seed set followed the same pattern as the fertilization rate: the last half treatment was
151	significantly lower than the others in Obihiro (Table S3, Fig. 2c), while no significant
152	differences were detected in Hiroo (Table S3, Fig. 2d).
153	Discussion
154	In Obihiro, the last half treatment decreased both fertilization rate and seed set, while
155	the first half treatment was comparable to the control (Fig. 2a, 2c), suggesting that

156	pollinators mostly visited flowers during the first half period, and that pollinator
157	visitation was infrequent during the last half. On the other hand, in Hiroo, neither
158	bagging treatment caused reproductive failure compared to the control (Fig. 2b, 2d),
159	indicating that pollinators actively visited flowers during the entire flowering period.
160	Pollinator scarcity during the last half period in Obihiro may be a result of
161	climate warming. Temperature rise was more pronounced in Obihiro than in Hiroo (Fig.
162	1b). Because phenological advance in response to warming is generally slower in plants
163	than in other taxa (Parmesan 2007; Post 2019), T. camschatcense may not have caught
164	up with the accelerating phenology of pollinators during the 60 years of rapid
165	temperature rise. Another possibility is pollinator-mediated plant-plant competition
166	(Rathcke 1983), in which the flowering onset of other plant species that had formerly
167	flowered after <i>T. camschatcense</i> advanced and overlapped with the last half period. We
168	think it is less likely because the study sites were thoroughly dominated by T .
169	camschatcense during its flowering period, and no other species appeared to compete
170	evenly (Fig. S2). Considering that precipitation remained low throughout the flowering
171	season (Fig. S4b), it was also unlikely that precipitation specifically inhibited pollinator
172	visitation during the last half period in Obihiro.
173	There are some remaining issues to be solved in future studies. Firstly, the seed

174	set was lower than the fertilization rate (Fig. 2c, 2d), indicating not all fertilized ovules
175	acquired enough resources to become seeds (i.e., resource limitation). A pollen
176	supplementation experiment is necessary to examine if the lowered reproductive success
177	during the last half period in Obihiro could be entirely attributed to reduced pollination
178	or could be partly caused by resource limitation as well. Secondly, the pollinating fauna
179	of Obihiro is not completely the same as that of Hiroo at a species level (Tomimatsu and
180	Ohara 2003). Considering the species-specific phenological shifts under climate
181	warming (Parmesan 2007; Post 2019), not only do the trends of temperature rise but the
182	pollinators' response might be essentially different between the two populations. Direct
183	observation of pollinating insects will help reassess the temporal skewness of visitation
184	with special attention to pollinator species composition.
185	This study reported the possibility of a phenological mismatch in Trillium
186	camschatcense with their pollinators. While habitat fragmentation has been considered
187	the primary threat to reproductive success in T. camschatcense in the Tokachi region
188	(Tomimatsu and Ohara 2002), phenological mismatches with pollinators have not been
189	explored. The impacts of the temporal skewness of pollination success on reproductive
190	success should be further examined in future studies.
191	

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201	
202	Data availability
203	The datasets generated during and/or analyzed during the current study are available
204	from the corresponding author upon reasonable request.
205	
206	Code availability
207	R scripts used in this study are available from the corresponding author upon reasonable
208	request.

210 Authors contribution

211 Y	T conceived the idea,	designed the	e study, and	analyzed th	e data. M	O and Y	T wrote the
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212 manuscript.

213

- 214 **Declarations**
- 215 Ethics approval NA
- 216 Consent to participate NA
- 217 Consent for publication NA
- 218 **Conflict of interest** The authors declare no competing interests.
- 219

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258 Figure captions





Fig. 1 (a) The location of the Tokachi region (gray) and the two studied populations:
Obihiro and Hiroo in the Tokachi region ofHokkaido. (b) Mean daily temperature in
April and May from 1958 to 2019 at Obihiro city (dark) and Hiroo town (light).
Regression lines and their 95 % confidence intervals are shown. Solid and dotted lines
indicate significant and non-significant effects of year, respectively



Fig. 2 Fertilization rate (a and b) and seed set (c and d) of the three treatments in
Obihiro and Hiroo: control (unbagged), first half (bagged during the last half of the
flowering period), and last half (bagged during the first half). Significant difference was
detected for pairs marked with different letters in each panel (significance level = 0.05).
No letters are assigned when there were no significantly different pairs