**Short communication**

# Temporal skewness of pollination success in the spring ephemeral *Trillium camschatcense*

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**Abstract**

Phenological overlap with pollinators is crucial for reproductive success in insect-pollinated plants. In this study, we examined whether pollinator visitation successfully occurred during an entire flowering season in two populations of the insect-pollinated spring ephemeral *Trillium camschatcense* in the Tokachi region of Hokkaido, northern 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 periods. The two populations have experienced differing levels of climate warming in the last 60 years, which impacted pollinator visitation. In the population experiencing temperature rise more rapidly, fertilization rate and seed set decreased sharply when bagged during the first half period, indicating that pollinator visitation is skewed to the early part of the flowering season. The temporal skewness of pollination success would be an early warning signal of the impacts of climate warming on the reproductive success of *T. camschatcense*.

**Keywords**

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

## Introduction

Climate warming is altering the flowering phenology of many plant species (Root et al. 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 response to rising temperature differs between taxa and species (Parmesan 2007; Post 2019), pollinator activities does not necessarily shift in synchrony with flowering, potentially resulting in a temporal mismatch and reduced pollinator visitation (Kudo and Cooper 2019). It is crucial to evaluate the degree of phenological overlap with pollinators for insect-pollinated spring ephemeral plants as temperatures continue to rise.

*Trillium camschatcense* is an insect-pollinated, long-lived spring ephemeral that sprouts in mid to late April and flowers in May in temperate deciduous forests. Although most populations of *T. camschatcense* in Japan are self-compatible, those in the Tokachi region of Hokkaido, northern Japan, are self-incompatible and owe seed production completely to insect pollinators (Ohara et al. 1996). Therefore, a phenological mismatch with pollinators could be a serious threat to the reproductive success of *T. camschatcense*.

In this study, we examined whether pollination successfully occurred during a complete flowering season of *Trillium camschatcense* at two locations in the Tokachi region, where long-term temperature data was available. We hypothesized that pollination was more temporally limited due to a phenological mismatch at the location experiencing more rapid warming.

## Materials and Methods

### Study species

*Trillium camschatcense* is an understory herb whose primary pollinators are Coleoptera and Diptera (Tomimatsu and Ohara, 2003). *T. camschatcense* does not produce inflorescences made up of multiple flowers. Flowering individuals usually have one flowering stem (sometimes two stems), with one flower and three leaves per stem. *T. camschatcense* does not compensate for reproductive failure by producing additional flowers later in the flowering season: the number of flowers per individual is pre-determined before flowering onset. Most flowering stems synchronously sprout in April, start anthesis in early May, and finish flowing around late May to early June. After the flowering season, fruits ripen in July (Ohara and Kawano 2005; Ohara et al. 2006).

**Study Sites**

The present study was conducted in two populations located in: Obihiro (inland, 42.802° N, 143.103° E) and Hiroo (seacoast, 42.316° N, 143.324° E) (Fig. 1a). The two populations were approximately 57 kilometers apart. Obihiro is a remnant forest surrounded by agricultural lands. Hiroo is a part of a windbreak forest that stretches along the coastline. The two populations were relatively large for the region in terms of population density and habitat area (Fig. S1, S2).

**Temperature**

We obtained the daily temperature of Obihiro and Hiroo from 1958 to 2019 from the website of the Japan Meteorological Agency (JMA, https://www.jma.go.jp/jma/index.html). We extracted the data for April and May, the growing and flowering months of *Trillium camschatcense*. For each site, we carried out a linear regression against the mean daily temperature per year, using year as an explanatory variable.

**Flowering phenology and precipitation**

In early April 2021, we randomly established two 3 m × 3 m quadrats in each population. Before flowering onset, we labeled all flowering stems within the quadrats in late April (Fig. S3a). Flowering stems that sprouted later were labeled right after we noticed their emergence. We monitored the labeled flowers at an interval of 2 to 11 days. We defined the first and the last day of the anthesis of each flower as the date on which we first confirmed that the flower became open/withered. Along with phenological surveys, daily precipitation during the flowering season in the study year (2021), as well as that in the past years (from 1958 to 2019), was obtained from the website of the JMA.

**Bagging experiment**

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

Before flowering onset, we randomly selected 30 individuals for the first half and the last half treatments around each 3 m × 3 m quadrat, as well as 30 individuals within the quadrat for control. Approximately 40 flowers were selected for each treatment in each quadrat (Table S1). Flowers for the last half treatment were bagged at that time. Because *Trillium camschatcense* in the Tokachi region usually flowers in May and early June, we opened flowers for the last half treatment but bagged those for the first half treatment within one week of the midpoint of May (May 16): on May 17 and 22 in Obihiro and Hiroo, respectively (Fig. S3c). We measured the length and width of a leaf of each flowering stem to account for the confounding effects of plant size on reproduction.

In early July, we collected fruits and counted the number of unfertilized ovules, fertilized ovules that did not develop to seeds due to abortion, and seeds (Fig. S3d). We recorded the presence/absence of stem injury caused by trampling and herbivory on seeds by caterpillars, both of which took place before collecting fruits and might have overridden the effects of bagging treatments by decreasing the number of seeds. We defined “fertilization rate” and “seed set” as follows:

$$\text{fertilization rate} = \frac{\text{sum of fertilized ovules and seeds}}{\text{total sum of ovules and seeds}}$$

$$\text{seed set} = \frac{\text{sum of seeds}}{\text{total sum of ovules and seeds}}$$

We constructed a generalized linear mixed model (GLMM) with binomial error and logit link for fertilization rate and seed set, using the function “glmer” in the R package “lme4” (Bates et al. 2015). Models were separately constructed for Obihiro and Hiroo. For each response variable, we incorporated the treatment, size (= leaf length × leaf width), presence/absence of herbivory and stem injury as fixed effects, and individual and quadrat as random effects. We carried out a multiple comparison test for treatment by a Tukey method with Benjamini-Hochberg correction, using the R package “multcomp” (Hothorn et al. 2008).

## Results

### Temperature

The mean daily temperature of April and May in Obihiro significantly increased from 1958 to 2019 at a rate of 0.021 °C per year (*P* = 0.005, Fig. 1b). Although the temperature in Hiroo was also rising, the rate of 0.012 °C per year was not statistically significant (*P* = 0.106), indicating that Obihiro was experiencing more rapid warming compared to Hiroo.

**Flowering phenology and precipitation**

In the four quadrats used for phenological survey (two in each population), all flowers continued anthesis across the first and the last half period, depicting unimodal trajectories (Fig. S4a). The timing of the onset, the peak, and the end of flowering was earlier overall in Obihiro than in Hiroo. Daily precipitation of the study year (2021), as well as the average daily precipitation over the past 60 years (1958-2019), mostly remained low throughout the flowering period in both sites (Fig. S4b).

**Bagging experiment**

The fertilization rate of the last half treatment was lower than that of control and first half treatment in Obihiro (Table S2, Fig. 2a). On the other hand, there were no significant differences among the three treatments in Hiroo (Table S2, Fig. 2b). The seed set followed the same pattern as the fertilization rate: the last half treatment was significantly lower than the others in Obihiro (Table S3, Fig. 2c), while no significant differences were detected in Hiroo (Table S3, Fig. 2d).

**Discussion**

In Obihiro, the last half treatment decreased both fertilization rate and seed set, while the first half treatment was comparable to the control (Fig. 2a, 2c), suggesting that pollinators mostly visited flowers during the first half period, and that pollinator visitation was infrequent during the last half. On the other hand, in Hiroo, neither bagging treatment caused reproductive failure compared to the control (Fig. 2b, 2d), indicating that pollinators actively visited flowers during the entire flowering period.

Pollinator scarcity during the last half period in Obihiro may be a result of climate warming. Temperature rise was more pronounced in Obihiro than in Hiroo (Fig. 1b). Because phenological advance in response to warming is generally slower in plants than in other taxa (Parmesan 2007; Post 2019), *T. camschatcense* may not have caught up with the accelerating phenology of pollinators during the 60 years of rapid temperature rise. Another possibility is pollinator-mediated plant-plant competition (Rathcke 1983), in which the flowering onset of other plant species that had formerly flowered after *T. camschatcense* advanced and overlapped with the last half period. We think it is less likely because the study sites were thoroughly dominated by *T. camschatcense* during its flowering period, and no other species appeared to compete evenly (Fig. S2). Considering that precipitation remained low throughout the flowering season (Fig. S4b), it was also unlikely that precipitation specifically inhibited pollinator visitation during the last half period in Obihiro.

There are some remaining issues to be solved in future studies. Firstly, the seed set was lower than the fertilization rate (Fig. 2c, 2d), indicating not all fertilized ovules acquired enough resources to become seeds (i.e., resource limitation). A pollen supplementation experiment is necessary to examine if the lowered reproductive success during the last half period in Obihiro could be entirely attributed to reduced pollination or could be partly caused by resource limitation as well. Secondly, the pollinating fauna of Obihiro is not completely the same as that of Hiroo at a species level (Tomimatsu and Ohara 2003). Considering the species-specific phenological shifts under climate warming (Parmesan 2007; Post 2019), not only do the trends of temperature rise but the pollinators' response might be essentially different between the two populations. Direct observation of pollinating insects will help reassess the temporal skewness of visitation with special attention to pollinator species composition.

This study reported the possibility of a phenological mismatch in *Trillium camschatcense* with their pollinators. While habitat fragmentation has been considered the primary threat to reproductive success in *T. camschatcense* in the Tokachi region (Tomimatsu and Ohara 2002), phenological mismatches with pollinators have not been explored. The impacts of the temporal skewness of pollination success on reproductive success should be further examined in future studies.

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**Data availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Code availability**

R scripts used in this study are available from the corresponding author upon reasonable request.

**Authors contribution**

YT conceived the idea, designed the study, and analyzed the data. MO and YT wrote the manuscript.

**Declarations**

**Ethics approval** NA

**Consent to participate** NA

**Consent for publication** NA

**Conflict of interest** The authors declare no competing interests.

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**Figure captions**

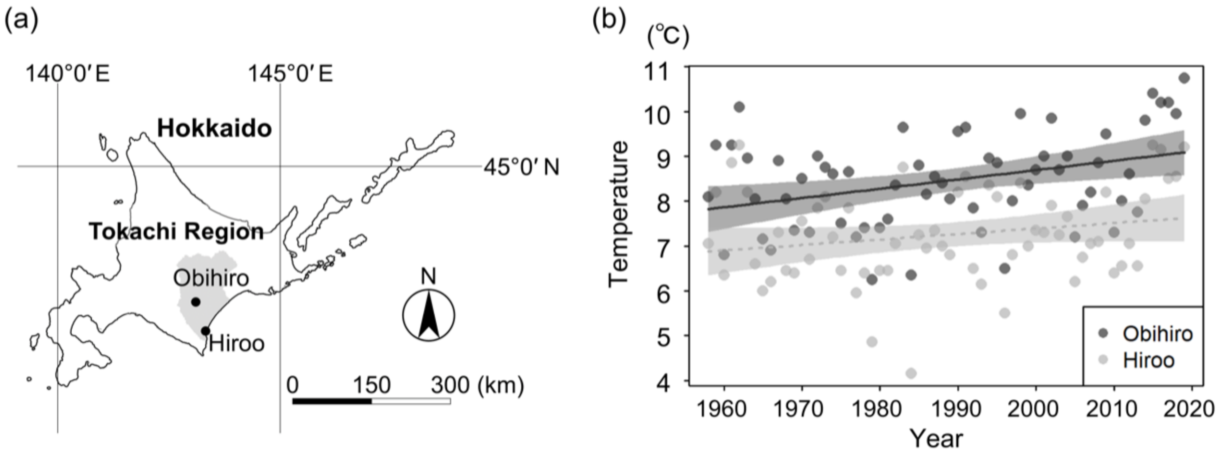


**Fig. 1** (a) The location of the Tokachi region (gray) and the two studied populations: Obihiro and Hiroo in the Tokachi region ofHokkaido. (b) Mean daily temperature in April and May from 1958 to 2019 at Obihiro city (dark) and Hiroo town (light). Regression lines and their 95 % confidence intervals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

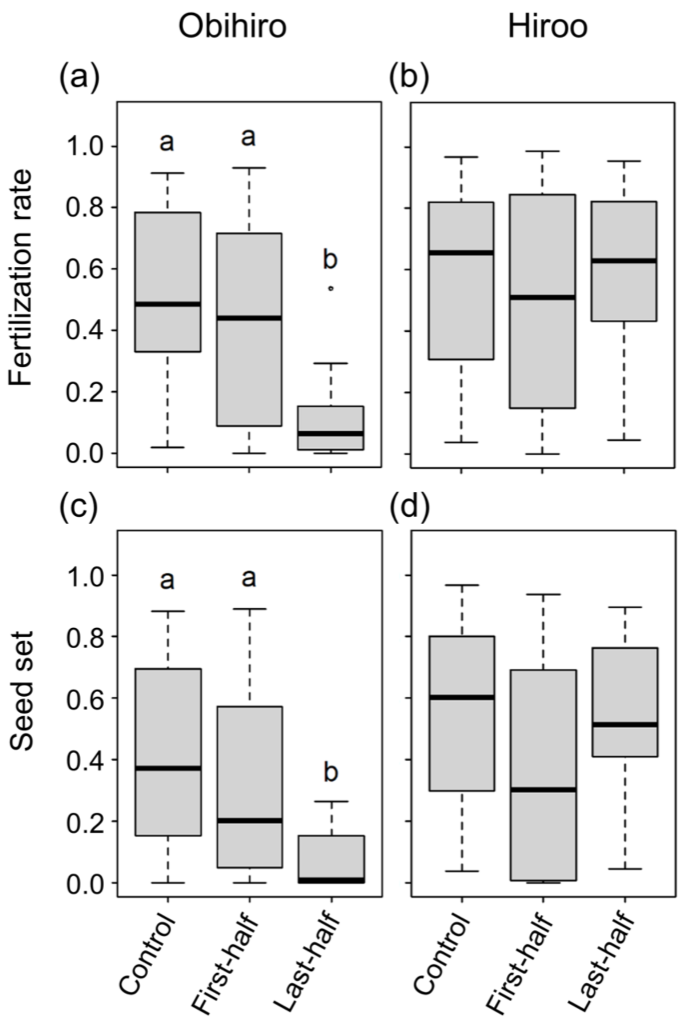


**Fig. 2** Fertilization rate (a and b) and seed set (c and d) of the three treatments in Obihiro and Hiroo: control (unbagged), first half (bagged during the last half of the flowering period), and last half (bagged during the first half). Significant difference was detected for pairs marked with different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