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The selective advantage of a mast-flowering behavior in *Veratrum album* subsp. *oxysepalum*: implications of the predator satiation hypothesis

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Short title: **Ecological significance of mast flowering in *Veratrum***

Abstract

Premise: The synchronous highly variable flower or seed production among years within a population— i.e., masting – has been reported in numerous perennial plants. Although ecological advantages of masting are recognized as the enhancement of pollination efficiency and/or escape from predator attack, little is known about the degree of these advantages and the variation in masting behavior among populations of conspecific plants.

Methods: We observed flowering ramet density and reproductive success (fruit-set success and herbivorous damage) of a perennial herb, *Veratrum album* subsp. *oxysepalum*, across six lowland and six alpine populations in northern Japan during 2–3 years. We then analyzed the relationship between floral density and reproductive success to clarify the ecological significance of mast flowering. Furthermore, flowering intervals of individual plants were estimated by counting annual scars on rhizomes.

Results: Mast flowering was observed in the most populations, but flowering intervals of individual plants were shorter in the alpine populations compared with the lowland populations. Floral damage by stem borers (dipteran larvae) and seed predation by lepidopteran larvae were intense in the lowland populations. The seed production of individual ramets increased with higher floral density owing to the effective avoidance of floral-stem damage and seed predation. Although stem borers were absent in alpine habitat, seed predation decreased with higher floral density also in the alpine populations. Pollination success was independent of floral density in both of the alpine and lowland populations.

Conclusions: These results strongly support the predator satiation hypothesis for the mast flowering behavior in this species.

KEYWORDS

elevation; herbivory; intraspecific variation; masting behavior; Melanthiaceae; predator satiation.

INTRODUCTION

Masting in plants is defined as a synchronous highly variable flower or seed production among years by a population of perennial plants (Janzen, 1976; Kelly, 1994; Kelly and Sork, 2002; Kelly et al., 2008; Pearse et al., 2016). Masting provides fitness advantages if an occasional big investment in reproduction simultaneously among plants results in higher reproductive success than a stable and moderate resource allocation for reproduction (i.e., the economy of scale hypothesis; Norton and Kelly, 1988). Generally, masting behavior has been considered as a life-history strategy of specific species and has been recognized in various plant taxa from many regions of the world (Herrera et al., 1998; Kelly and Sork, 2002; Koenig et al., 2003). The pattern of masting is formed by the periodicity and variability of the reproductive output of individual plants (Sork et al., 1993; Herrera et al., 1998; Henkel and Mayor, 2019; Bogdziewicz et al., 2020b) and the reproductive synchrony among plants within a population (Koenig and Knops, 2000; Liebhold et al., 2004; Haynes et al., 2013; Mooney et al., 2011; Bogdziewicz et al., 2020b). For a comprehensive understanding of masting behavior as a life-history strategy of plants, it is crucial to clarify the variation pattern of flowering behavior in masting plants and its selective force (Koenig et al., 2003).

As the ecological advantages of masting, the predator satiation hypothesis (Janzen, 1971; Silvertown, 1980; Kelly and Sullivan, 1997; Shibata et al., 1998; Bogdziewicz et al., 2020c; Zwolak et al., 2022) and the pollination efficiency hypothesis (Nilsson and Wařtłjung, 1987; Norton and Kelly 1988; Smith et al., 1990; Shibata et al., 1998; Kelly et al., 2001) have the most empirical support. The former predicts a selective advantage of lower predation damage by mast fruiting (Janzen, 1971), whereas the latter predicts higher pollination success by mast flowering (Kelly et al., 2001). These hypotheses are applicable not only for the life-history trait of specific species but also for that of individual populations of same species growing at various environmental conditions. Several studies reported the variation in masting behavior among populations of same species (Sullivan and Kelly, 2000; Satake et al., 2004; Lázaro et al., 2006; Crone et al., 2011; Allen et al., 2012). As ecological situations, such as species compositions and abundance of herbivores and effective pollinators, often vary among populations, different masting patterns may be generated among populations (Sullivan and Kelly, 2000; Kelly et al., 2001). Furthermore, a recent study reported that global warming has disturbed the pattern of masting in European beech, resulting in the less effective pollination and more seed predation damage (Bogdziewicz et al., 2020a). Although several studies suggest the importance of interspecific variation in selective pressures acting on masting behavior (Kelly and Sork, 2002, Koenig et al., 2003, Bogdziewicz et al., 2020c), few studies focus on intraspecific variation. Therefore, the comparisons of populations in different ecological situation are crucial to understanding the evolutionary process of masting behavior responding to local selective pressures.

Elevational change forms an important environmental gradient for plant growth and distribution, and there is variation in masting patterns between high- and low-elevation populations in some species (Sullivan and Kelly, 2000; Kelly et al., 2001; Allen et al., 2012). Various environmental conditions, such as temperature, radiation, precipitation, snow cover,

and UV intensity, change steeply along the elevation gradient (Körner, 2007). This change generates differences in phenology, reproductive performance, and biological interactions of local populations (Hodkinson, 2005; Rasmann et al., 2014; Sakaguchi et al., 2017). In species having a wide distribution range of elevations, for instance, the intensity of seed predation damage often decrease in the populations at higher elevations (Lord and Kelly, 1999; Sullivan and Kelly, 2000). In this case, selective pressures acting on masting behavior may decrease along the elevation gradient, resulting in obscure masting behaviors in high-elevation populations (i.e., less synchronized or continuous reproduction). Accordingly, comparisons of populations at different elevations are available to understand the intraspecific variation of masting behavior and the ecological significance of masting in terms of plant-herbivore interactions.

Veratrum album subsp. *oxysepalum* (Turcz.) Hultén (Melanthiaceae) is a perennial clonal herb growing in the understory of deciduous forests and moist grasslands from lowland to alpine regions in eastern Asia. Mast flowering behavior occurs in this species and other *Veratrum* species (e.g., Iler and Inouye, 2013). Interestingly, the intervals of mast flowering occurrence seem to vary among populations in this species: once every 5–6 years in the lowland populations and once every 2–3 years in the alpine populations (G. Kudo, unpublished data). The proximate factors of mast flowering in *Veratrum* species were analyzed in another species, *V. tenuipetalum*, in which a cool summer temperature seemed to be related to the floral-bud formation (Iler and Inouye, 2013). However, the ecological advantage of masting behavior in *Veratrum* species is unknown. So far, the masting phenomenon has mostly been studied in woody plants or graminoids, whereas studies on animal-pollinated herbaceous plants are limited, e.g., *Phormium*, *Celmisia* and *Astragalus* species (Schauber et al., 2002; Kelly et al., 2013; Crone, 2013).

This study aims to clarify the mast flowering behavior (i.e., yearly variation in floral density at population level and flowering intervals at plant level) and its ecological significance (i.e., pollination success and herbivorous damage) in *V. album* subsp. *oxysepalum* populations growing at different elevations. Herbivorous damages to floral stems and fruits are reported in the type species *V. album* L. in Europe (Schaffner et al., 2001). Because of large differences in the growing environments between lowland and alpine habitats, such as meteorological conditions and growing season length, distribution and activity of herbivorous insects may vary along the elevation gradient. In addition, if selective pressures acting on herbivorous and/or pollination processes vary between lowland and alpine populations, the masting behavior may also vary according to elevation. Thus, we tested the predator satiation hypothesis and the pollination efficiency hypothesis for the masting behavior in this species. Specifically, we address the following questions:

- (1) How extent does floral density vary between years within a population of lowland and alpine habitats?
- (2) Do flowering intervals of individual plants differ between lowland and alpine populations?

- (3) How extent does herbivorous damage to reproductive organs vary between populations and elevations?
- (4) Which selective advantage (predation avoidance or pollination efficiency) is more related to the masting behavior in this species?

MATERIALS AND METHODS

Study species and sites

Veratrum album subsp. *oxysepalum* (hereafter *V. album*) is a summer-green perennial herb growing in the moist forest floor and meadow habitats from lowland to alpine regions in central to northern Japan. Flowering of this species usually occurs synchronously within a population after consecutive non-flowering years at a local scale. Shoot growth initiates soon after snowmelt and height growth is completed within one month of the emergence. This species has a thick lateral rhizome on which annual growth scars are formed (Appendix S1; see the Supplementary Data with this article). The longevity of rhizomes is 5–30 years. When flowering occurs, the rhizome branches into 1–3 lateral parts from the apical growth point (Tani, 2005). Thus, flowering intervals are countable from the annual scar number on the rhizome segments between the branching points. Reproductive ramets produce a single floral stem on which 10–15 lateral inflorescences are arranged as a raceme. Flowering occurs about 50 days after the emergence, and fruits mature about one month after that. As most of the leaves on reproductive ramets start to senesce at the time of flowering, the length of the photosynthetic period is shorter than three months. This species is physiologically self-incompatible (Kato et al., 2009), and various insects visit flowers, including dipteran, coleopteran, and hymenopteran insects (Y. Ito and G. Kudo, personal observation). Several herbivorous insects attack the reproductive organs of *V. album* in western Europe (Schaffner et al., 2001), and one major seed predator is *Eupithecia veratraria* larvae (Lepidoptera: Geometridae; Hesse et al., 2008). Furthermore, the mechanical damage to floral stems by *Earomyia crystallophila* (Diptera: Lonchaeidae) larvae, a stem borer, has been reported (Ozerov and Krivosheina, 2017). Stem borers eat piths (parenchyma) of floral stems. As non-flowering stems of *V. album* are hollow without piths, herbivory damage by stem borers is absent in vegetative ramets (Y. Ito and G. Kudo, personal observation).

We conducted field surveys for 12 *V. album* populations in Hokkaido during 2019–2021: seven sites in 2019 and all of the 12 sites in 2020 and 2021 (Table 1, Figure 1). Although mast flowering usually occurs every few years in each population as mentioned before, we observed masting events in many populations either of these years (see Results). That opportunity enabled us to evaluate the ecological advantages of masting in this species. We classified these sites into lowland populations (5–178 m elevation) and alpine populations (1760–1990 m elevation). All of the lowland populations located in the understory of deciduous forests, where shoots emerged in late April soon after snowmelt, flowering occurred in early June, and fruits matured in mid-July. The alpine populations were in the snow-meadows of the Taisetsu Mountains, where plant growth progressed approximately two months later than that of the lowland populations due to cool weather and late snowmelt time

at higher elevations (i.e., growth started in late June). Here, flowering occurred in early August, and fruits mature in mid-September.

Data collection in the study plots

We established multiple 5 m × 1 m plots in each of the study sites, according to the whole population size (ranging from 8 to 30 plots; Table 1). We recorded the number of flowering ramets in each plot to estimate the abundance of flowering ramets in each population. We then randomly selected 10 flowering ramets in each plot (or all flowering ramets if the number of flowering ramets was less than 10) and recorded the numbers of flowers and fruits.

We investigated herbivorous damage to the reproductive organs of *V. album* during the flowering period (for floral-stem herbivory) and the fruiting period (for seed predation). For the floral-stem damage, we quantified the proportion of decayed parts (necrotic tissue) on the inflorescences of each ramet. Inflorescences of ramets infected by stem borers often suffer from necrosis before flowering. We visually quantified the proportion of necrosis in each lateral inflorescence and translated it into four scores as follows: 0 % necrosis = score 0, 1–49 % necrosis = score 1, 50–99 % necrosis = score 2, and 100 % necrosis = score 3 (Appendix S2). The scores of individual inflorescences were then averaged for the entire ramet as an intensity of the floral-stem damage in each ramet. In the preliminary survey, we confirmed a positive correlation between the average score of necrosis and the number of stem borers (dipteran larvae) in the floral stem ($r = 0.79$, $N = 44$; Appendix S2).

Regarding the degree of seed predation, we measured the proportion of fruits attacked by seed predators. The occurrence of seed predation was detectable due to the existence of small holes in fruits. To quantify the frequency of fruits damaged by seed predators, we counted the numbers of intact and attacked fruits every year. We excluded the data of plants, whose inflorescence was damaged by strong winds or herbivory of large mammals. When the total number of flowering ramets was less than 15 across the plots within a site, we added samples of flowering ramets outside of the plots to create a minimum sample size of 15 per population. All field surveys quantifying the number of flowering ramets, flower and fruit number and herbivorous damages were repeated in each site during two or three years (Table 1).

Species identification and distribution of herbivorous insects

To investigate the geographic distribution of herbivorous insects, we judged the presence or absence of herbivorous damage across the 34 populations for floral-stem damage and 14 populations for seed predation in Hokkaido (Appendix S3). We confirmed the presence of herbivorous insects when herbivorous damage was visible in either 2019 or 2020. Their absence was defined when there were no signs of herbivorous damage in both years.

Herbivorous insects were collected and preserved in 100 % ethanol for identification by DNA barcoding at a family level by the sequencing CO1 gene (Hebert et al., 2003). The DNA of sampled insects was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The PCR mixture contained 1.0 µl sample DNA, 0.5 µM each of primers, and

GoTaq DNA polymerase (Promega Corporation, Madison, WI, USA) in a total volume of 20 μ l. The thermal profile for PCR was followed by the procedure of Geller et al. (2013). We used a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) for terminator reaction. The sequencing was conducted using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA), and the sequence data were aligned by MEGAX (Kumar et al., 2018). We identified samples by comparing sequence data with the sequence obtained from the NCBI database (<https://www.ncbi.nlm.nih.gov>).

Measurement of flowering intervals using annual scars on rhizome

To examine flowering intervals from annual scars on rhizomes, we excavated the rhizomes of 15–20 individuals in the six lowland populations (AS, NP, TB, OB, EN, and HO) and three alpine populations (HS, KR, and HK) in 2021. For the sampling of this measurement, we selected flowering individuals as possible. Because the number of flowering plants was small in most of the lowland populations in this year (see Results), however, some non-flowering individuals were included. The flowering intervals of individual plants were estimated by counting the number of annual scars between the branching segments in which flowering occurred. For plants in which multiple flowering events were not detected on their rhizomes (due to decay of old tissues), we excluded them from the analysis.

Statistical analysis

For analyzing yearly variations of flowering ramets over the three years, we compared the density of flowering ramets per plot between the years in each population by the generalized linear model (GLM) postulating a negative binomial error distribution in which the density of flowering ramets per plot was a response variable and observation year was an explanatory variable.

Flowering intervals were compared among populations by generalized linear mixed models (GLMM) and Turkey's post-hoc test using the lme4 package (Bates et al., 2015). In the GLMM postulating a Poisson error distribution, flowering interval was a response variable, site ID was an explanatory variable, and plant ID was set as a random factor.

The effects of floral-stem abundance on herbivorous damage (floral-stem damage and seed predation) and pollination success (fruit-set rate) were analyzed by GLMMs using the glmmTMB package (Brooks et al., 2017). The floral-stem abundance in each population was calculated using the sum of flowering ramet numbers across the plots in a population and converted as a density per 1 m² (hereafter floral density). In the analysis, we excluded the data of years and populations in which the number of samples was < 15 due to insufficient statistical comparison. Floral density was an explanatory variable, and plot in each site was a random variable in every GLMM. In the analysis of floral-stem damage (i.e., scores by the proportion of decayed parts on the inflorescences), GLMM postulating a gamma error distribution was used. Since the data of seed predation and fruit-set success contained a lot of zero values, we used zero-inflated negative binomial GLMM (Brooks et al., 2017) to reduce

the overdispersion. The zero-inflated GLMM is composed of a conditional model and a zero-inflation model that analyzes a probability of an extra zero (Brooks et al., 2017). In the analysis of seed predation, damaged fruit number was a response variable and fruit number was an offset term after logarithmic transformation (Agresti, 1996). In the analysis of fruit-set success, fruit number was a response variable and flower number was an offset term after logarithmic transformation (Agresti, 1996). In all models, interactions between floral density and elevation type were considered. We conducted all analyses using the statistical software R, version 3.6.0 (R Core Team, 2019).

RESULTS

Flowering behavior

The density of flowering ramets varied significantly between years (2019–2021 or 2020–2021) in all populations, except for the EN population (Figure 2, Appendix S4). Among lowland populations, the HO, TB, and AS populations showed high floral densities in 2019, while low floral densities in 2020 and 2021. In contrast, the OB and NP populations showed high floral densities in 2020, while low floral densities in 2019 and 2021. Floral densities of the EN population was consistently low over the observation period, indicating no masting events during the three years. Among alpine populations, the HS population showed high floral densities in 2019 and 2021, while a low floral density in 2020. Other alpine populations showed high floral densities in 2021, while low floral densities in 2020. Thus, most of the populations showed large fluctuations in flower production between years.

Flowering intervals of individual plants were significantly shorter in the alpine populations than in the lowland populations (Figure 3). The average flowering intervals were longer than six years in the lowland populations (7.1 yr in HO, 6.5 yr in TB, 6.9 yr in AS, 6.2 yr in OB, 6.8 yr in NP, and 7.1 yr in EN), whereas they were five years or shorter in the alpine populations (4.3 yr in HS, 5.0 yr in KR, and 4.3 yr in HK).

Distribution of herbivorous insects

We found floral-stem borers in the pre-flowering period and seed predators in the fruiting period as the major herbivorous insects of *V. album*. In the CO1 sequence, the floral-stem borer was the dipteran larvae of Lonchaeinae sp., with 96 % sequence matching. The seed predator was the lepidopteran larvae of *Eupithecia veratraria* (Geometridae), with a 99 % sequence matching.

In the extensive observation of herbivorous damages over 34 populations, floral-stem damage by dipteran larvae occurred in all lowland populations (21 populations), but not in the alpine populations (13 populations; Appendix S3). Seed predation by lepidopteran larvae was detected in both of the lowland and alpine populations, but absent in some. Three of seven populations were free from seed predation in each lowland and alpine habitat (Appendix S3).

Ecological advantage of mast flowering

The effects of floral density on herbivorous damage and pollination efficiency by GLMMs are summarized in Table 2. Floral-stem damage by stem borers was observed only in the lowland populations, where the damage score significantly decreased with a higher floral density (Figure 4A).

For the analysis of seed predation, three populations in each of the lowland and alpine habitats were excluded because of the absence of seed predators. The number of damaged-fruits by seed predators significantly decreased with the floral density in both of the lowland and alpine populations, although the intensity of predation damage was higher in the lowland populations (conditional model; Table 2). The frequency of non-damaged fruits increased with floral density in both habitats but differently between the lowland and alpine populations (significant interaction between elevation type and floral density in zero-inflation model; Table 2). The intensity of predation damage was moderate in the alpine populations, where predation damage was absent at a lower level of floral densities in comparison with the lowland populations (Figure 4B).

Fruit-set rate was independent of floral density both of the lowland and alpine populations, while the extent of fruit-set rate was significantly higher in the alpine populations than in the lowland populations (conditional model; Table 2). The frequency of complete failure in fruit-set was independent of floral density, but a significant interaction was detected between elevation type and floral density (zero-inflation model; Table 2). This is because the proportion of non-fruiting ramets increased when the density of flowering ramets was low only in the lowland populations (Figure 4C). These results indicate that the contribution of floral density to pollination success is limited in this species.

DISCUSSION

The abundance of flowering ramets fluctuated greatly between years in most of the populations, indicating that mast flowering is a widespread behavior in *V. album*. Flowering intervals were shorter in the alpine populations compared with the lowland populations. Floral-stem herbivory was observed only in the lowland populations, while seed predation existed in both of the lowland and alpine populations. These herbivorous damages decreased significantly with higher density of flowering ramets. In contrast, pollination success was mostly independent of flowering abundance in both of the lowland and alpine populations. These results suggest that the selective advantage of mast flowering is related to the avoidance of herbivorous damages, supporting the predator satiation hypothesis (Janzen, 1971).

Herbivory intensity and floral density

The dominant herbivorous insects in *V. album* were stem borers (dipteran larvae of Lonchaeinae family) and seed predators (lepidopteran larvae of *Eupithecia veratraria*). Stem borers attack only flowering stems filled with pith (parenchyma). Because the damage by stem borers progresses during the pre-flowering period by which inflorescences subsequently decay before flowering onset (Appendix S2), not only seed production but also pollen

donation are intensively restricted by the stem herbivory. The present study revealed that the herbivorous damage by the stem borers was effectively decreased with the abundance of flowering ramets in the population (Figure 4A). While most previous studies on the predator saturation hypothesis focused on seed predation (Janzen, 1971; Kelly et al., 2008; Bogdziewicz et al., 2020c), this is a first study indicates that floral-stem herbivory before flowering could act as a selective pressure of mast flowering.

Although the herbivorous damage of floral stems was common in the lowland populations, stem herbivory was absent in the alpine populations (Appendix S3), indicating that the herbivory pressure by the stem borers is specific to the low elevation habitat. In contrast, seed predation by *E. veratraria* larvae occurred in both of the lowland and alpine populations (Appendix S3). This moth species was reported as a main seed predator of *Veratrum* species in western Europe, which greatly reduced seed production over the fruiting period (Hesse et al., 2008). Because the seed predation damage decreased with the abundance of flowering ramets in both of the lowland and alpine populations (Figure 4B), mast flowering is beneficial for avoiding seed predation. However, the intensity of seed predation was milder in the alpine populations, where moderate level of floral density seemed to be effective for a predator satiation compared with the lowland populations (Figure 4B). Plant-herbivore interactions (Lord and Kelly, 1999; Sullivan and Kelly, 2000) and the distribution patterns of herbivorous insects (Hodkinson, 2005) often vary at different elevations. Also in the present study, the selective pressure of herbivorous damage was smaller in the alpine populations than in the lowland populations.

Pollination success and floral density

Fruit-set success was weakly related to the floral density in both of the lowland and alpine populations (Figure 4C), suggesting that the selective advantage of mast flowering for pollination efficiency is limited in *V. album*. Mast flowering often provides a selective advantage for the pollination efficiency of wind-pollinated plants, but this is not common in animal-pollinated plants (Kelly and Sork, 2002). This is because the pollination success of animal-pollinated plants will decelerate when too much flower production satiates pollinator visits, while the saturation of pollination success is not common in wind-pollinated plants due to wide pollen dispersal (Kelly and Sork, 2002). However, fruit-set success was lower in the lowland populations, and significant interaction between elevation and flowering density was detected in the zero-inflation model (Table 2). *Veratrum album* flowers in lowland forests are visited by coleopteran and dipteran insects at low frequency, and they often suffer from low pollination success, especially under the condition of low floral density (Kato et al., 2009). In contrast, *V. album* flowers growing in alpine meadow is frequently visited by various insects, such as dipteran, coleopteran, and hymenopteran insects (Y. Ito and G. Kudo, personal observation), suggesting that pollen limitation might be severe in the lowland populations. Thus, flowering density might partially contribute to pollination success in the lowland populations, but the contribution of mast flowering to pollination efficiency as an ultimate factor is questionable.

However, careful considerations are needed to reject the pollination efficiency hypothesis as a driver of the mast-flowering behavior in this species because we evaluated pollination success on the basis of fruit-set success. First, the lack of relationship between floral density and female reproductive success might be influenced not only by pollinator visitation frequency but also by pollen quality (Wilcock and Neiland 2002). As *V. album* is self-incompatible and multiplies clonally (Kato et al., 2009; Kleijn and Steinger, 2002), individual patches are often composed of same clones. This might lead to frequent self-pollination within a clonal patch in mast-flowering years, resulting in a limited seed production. Second, high floral density might increase pollination success by means of pollen donation (i.e., male success), that has not been measured in this study. To conclude the possibility of the pollination efficiency hypothesis in this species, therefore, further studies are necessary.

Variation in flowering intervals between the lowland and alpine populations

The herb-chronological analysis of rhizomes indicated the significant difference in flowering intervals between the lowland and alpine populations (Figure 3). Long flowering intervals in the lowland populations might be related to the effectiveness of predation avoidance. The predator satiation hypothesis predicts that predators suffer from starvation in the years of low reproductive output of host plants, resulting in a reduction of predator density, and the subsequent masting event satiates herbivorous damage by which plants can escape from predator attack (Silvertown, 1980). If the spatial scale of reproductive synchrony of host plants is smaller than the flight distance of predators, however, predators would move to other populations producing more resources, that reduces the effectiveness of masting behavior as a strategy of predator starvation (Curran and Webb, 2000; Satake and Bjørnstad, 2004). Because the lowland populations commonly suffer from intense herbivorous damages by both of stem borers and seed predators, predator starvation during the non-flowering years should be crucial to satiate herbivorous damages in a mast year more than the alpine populations. As *V. album* populations are often distributed patchily at short distance, furthermore, herbivorous insects will easily move between local populations for oviposition on host plants if the flowering synchrony between adjacent populations is not perfect.

During the three years observation, mast flowering in the lowland populations occurred discontinuously in 2019 or 2020 even between adjacent populations, such as NP and TB. On the other hand, non-flowering year existed across the populations in 2021 (Figure 2). This suggests that the long flowering intervals in the lowland populations allow to occur non-flowering years across many populations even when flowering synchronization is not perfect among populations. Hence, longer intervals between masting events might contribute to starving herbivorous insects by a higher occurrence of synchronous non-flowering years at a regional scale. However, the possibility of this hypothesis is available only when herbivores highly depend on *V. album* as a host plant and the mobility of herbivores is not limited in a local population scale. Although previous studies reported that the floral-stem borers of Lonchaeinae family (dipteran larvae) and the seed predator *Eupithecia veratraria*

(lepidopteran larvae) highly depended on *Veratrum* species in western Europe (Schaffner et al., 2001; Hesse et al., 2008; Ozerov and Krivosheina, 2017), the feeding habits of stem-borer and seed-predator insects in Hokkaido are unknown. Therefore, it is crucial to reveal the life-histories of the herbivorous insects to clarify the ecological significance of intraspecific variation in the masting behavior of *V. album*.

CONCLUSIONS

The present study demonstrate that the masting behavior of *V. album* is advantageous for decreasing herbivorous damages by stem borers and seed predators, supporting the predator satiation hypothesis. The selective pressure acting on flowering synchrony varied among populations depending on the growing habitats, where the lowland populations were more susceptible to herbivorous damage than the alpine populations, resulting in longer flowering intervals. On the other hand, dependency of the herbivorous insects on *V. album* and their mobility between populations are not studied in this study. For further understanding of the evolutionary significance of the masting behavior in *V. album*, clarification of the life-histories of herbivorous insects, determinant mechanism of the flowering synchrony (proximate factor of masting), and genetic relationship between the lowland and alpine populations (evidence of local adaptation) are needed.

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AUTHOR CONTRIBUTIONS

Both YI and GK conceived the idea, conducted the field survey, analyzed the data, and wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data set used in this study is available in the Dryad Digital Repository:

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

Appendix S1. Photographs of rhizomes of *Veratrum album*. Left: lowland individual; right: alpine individual. *V. album* has a thick lateral rhizome on which annual growth scars are formed. When flowering occurs, the rhizome branches into 1–3 lateral parts from the apical growth point. Thus, flowering intervals are countable from the annual scar number of the rhizome segments between the branching points. On the photographs, flowering intervals of the plants are shown.

Appendix S2. (A) A picture of dipteran floral-stem borer, Lonchaeinae sp. larvae. (B) Examples of damage scores of inflorescence necrosis caused by floral-stem borers. (C) Relationship between the number of floral-stem borers and damage score. There was a significant positive correlation between the number of insects and damage score ($r = 0.790$, $N = 44$, $P < 0.001$).

Appendix S3. Distribution of herbivorous damages in *Veratrum album* in Hokkaido. Presence or absence of damages by floral-stem borers and seed predators in 34 populations are expressed by ○ or ×, respectively.

Appendix S4. Results of the GLMs for yearly variations in flowering ramet density over two or three years. Refer to Table 1 for the site cord.

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TABLE 1. Descriptions of the main study sites in Hokkaido. See Figure 1 for the location of each site.

Site name	Site cord	Elevation type	Elevation (m)	No. plots	Obs. year
Hoshioki	HO	Lowland	8	8	2019, 2020, 2021
Nopporo	NP	Lowland	65	10	2019, 2020, 2021
Eniwa	EN	Lowland	18	8	2019, 2020, 2021
Tobetsu	TB	Lowland	5	8	2019, 2020, 2021
Asahikawa	AS	Lowland	178	10	2019, 2020, 2021
Obihiro	OB	Lowland	51	8	2019, 2020, 2021
Hisago	HS	Alpine	1760	30	2019, 2020, 2021
Mt. Kaun	KN	Alpine	1850	8	2020, 2021
Mt. Goshiki	GS	Alpine	1865	8	2020, 2021
Mt. Hakuun	HK	Alpine	1990	8	2020, 2021
Mt. Kuro	KR	Alpine	1832	8	2020, 2021
Mt. Asahi	MA	Alpine	1785	8	2020, 2021

TABLE 2. Summary of GLMMs for herbivorous damage (floral-stem damage and seed predation) and fruit-set rate responding to flowering ramet density (FRD) and elevation type (alpine and lowland habitats). For the GLMM of floral-stem herbivory, data of alpine populations are excluded because of no stem borers in the alpine habitat. Both of conditional and zero-inflation models are shown in the GLMMs of damaged-fruits rate and fruit-set rate.

	Variable		Coefficient	SE	z value	P value
	Objective	Explanation				
Floral-stem herbivory (N = 420)	Intercept		0.677	0.112	6.05	< 0.001
	FRD		-1.134	0.081	-14.08	< 0.001
	Conditional model					
	Intercept		-1.501	0.449	-3.35	< 0.001
	FRD		-0.845	0.357	-2.37	0.018
	Elevation (low)		1.271	0.597	2.13	0.033
Damaged-fruits rate (N = 518)	Elevation (low) × FRD		-0.155	0.371	-0.42	0.675
	Zero-inflation model					
	Intercept		-0.557	0.346	-1.61	0.107
	FRD		2.023	0.361	5.61	< 0.001
	Elevation (low)		-2.167	0.679	-3.19	0.001
	Elevation (low) × FRD		-1.455	0.498	-2.92	0.003
	Conditional model					
	Intercept		-1.063	0.111	-9.61	< 0.001
	FRD		0.022	0.018	1.21	0.226
	Elevation (low)		-0.484	0.156	-3.11	0.002
Fruit-set rate (N = 908)	Elevation (low) × FRD		0.104	0.066	1.57	0.118
	Zero-inflation model					
	Intercept		-3.495	0.378	-9.241	< 0.001
	FRD		0.029	0.092	0.312	0.755
	Elevation (low)		2.087	0.458	4.558	< 0.001
	Elevation (low) × FRD		-1.637	0.448	-3.653	< 0.001

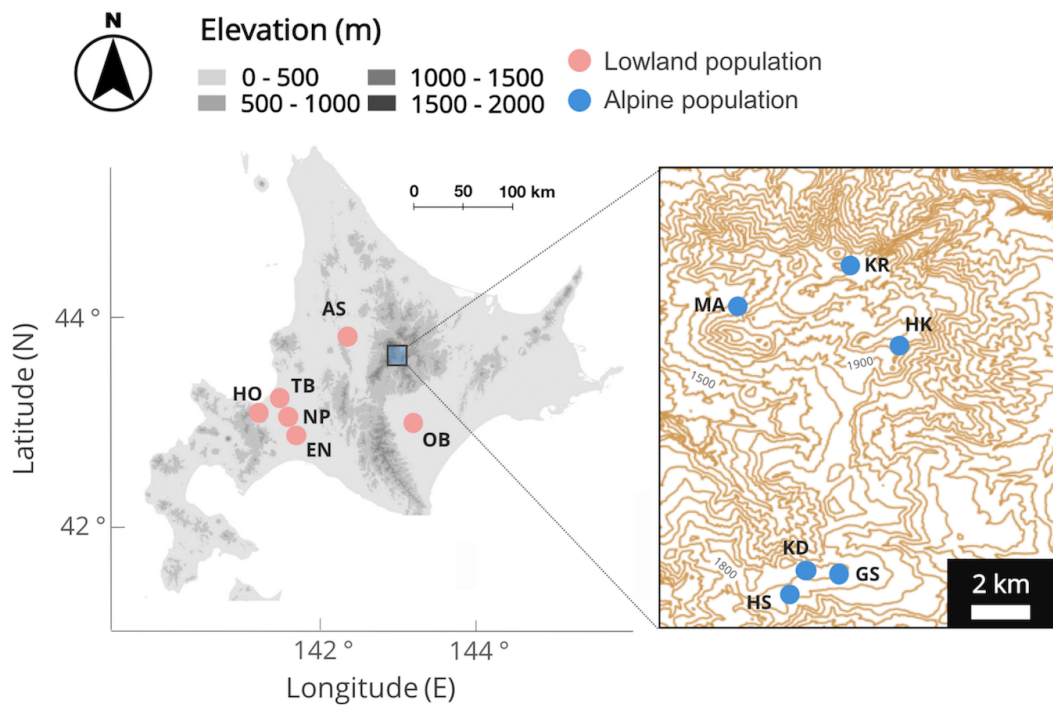


FIGURE 1. Locations of study sites in Hokkaido of northern Japan. The color difference indicates elevational habitat type. Red: lowland population, blue: alpine population. Refer to Table 1 for details of the sites.

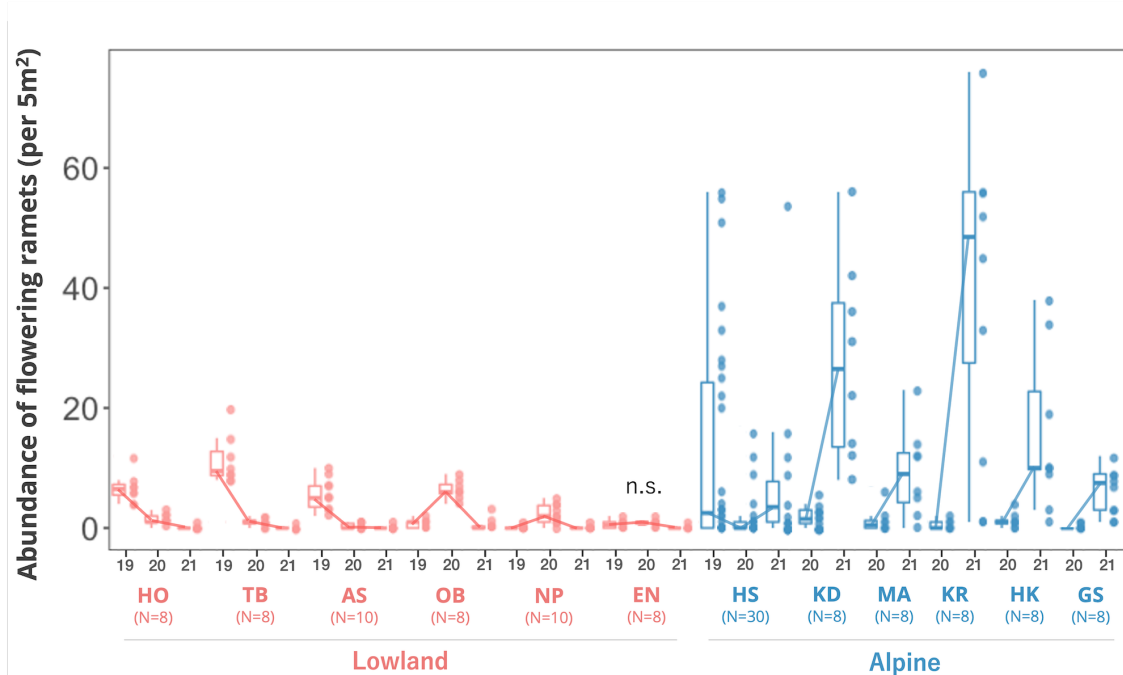


FIGURE 2. Yearly variation in the abundance of flowering ramets in each population (per 5-m × 1-m plot) during 2019–2021 (HO, TB, AS, OB, NP, EN, and HS) and 2020–2021 (KD, MA, KR, HK, and GS). The abundance of flowering ramets significantly varied between years in all populations ($P < 0.05$), except for EN (n.s.; $P > 0.05$). A box-and-whisker plot is shown in each year of the populations. Each point to be next to box-and-whisker plot indicates the value of each study plot within the population. Red: lowland population; blue: alpine population. Refer to Table 1 for the site cord.

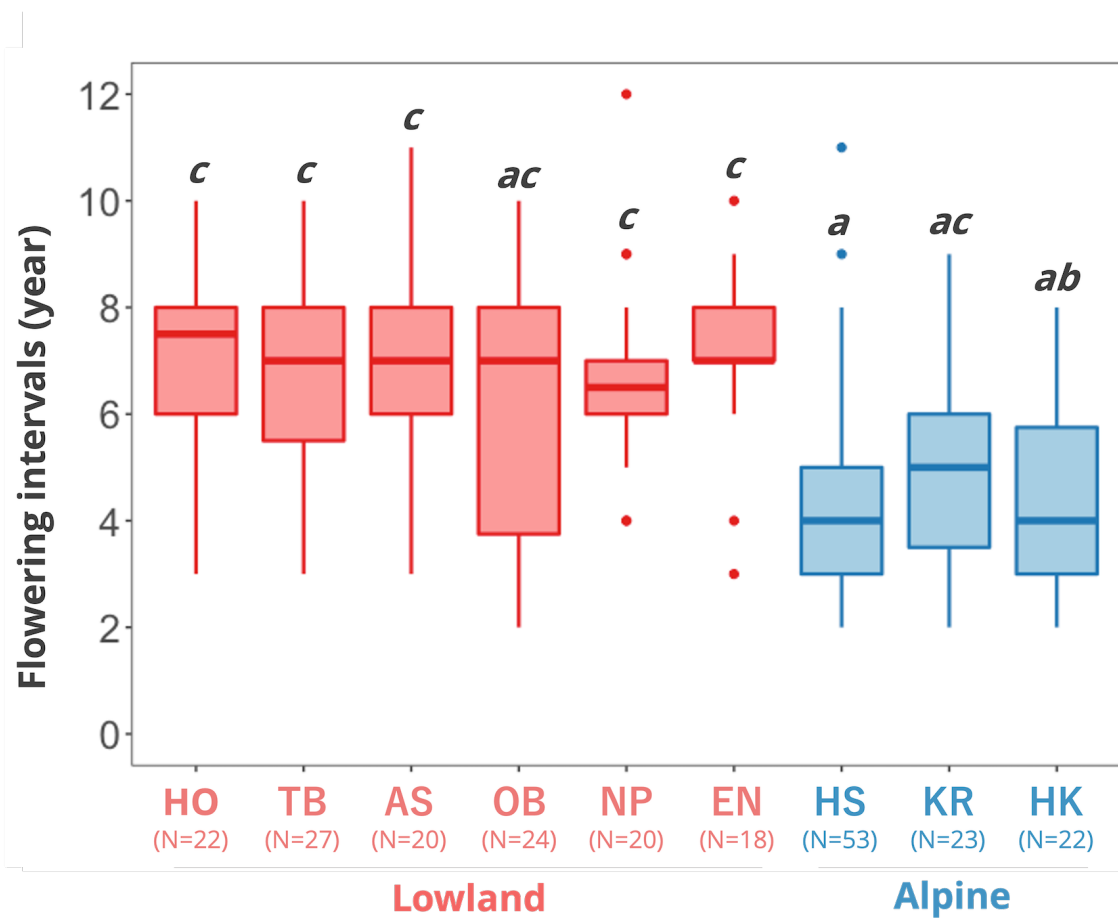


FIGURE 3. Comparison of flowering intervals (year) estimated from the growth scars on rhizomes among populations. *a–d* indicates significant differences by Turkey's multiple comparison test ($P < 0.05$). Red: lowland population; blue: alpine population. Refer to Table 1 for site cords.

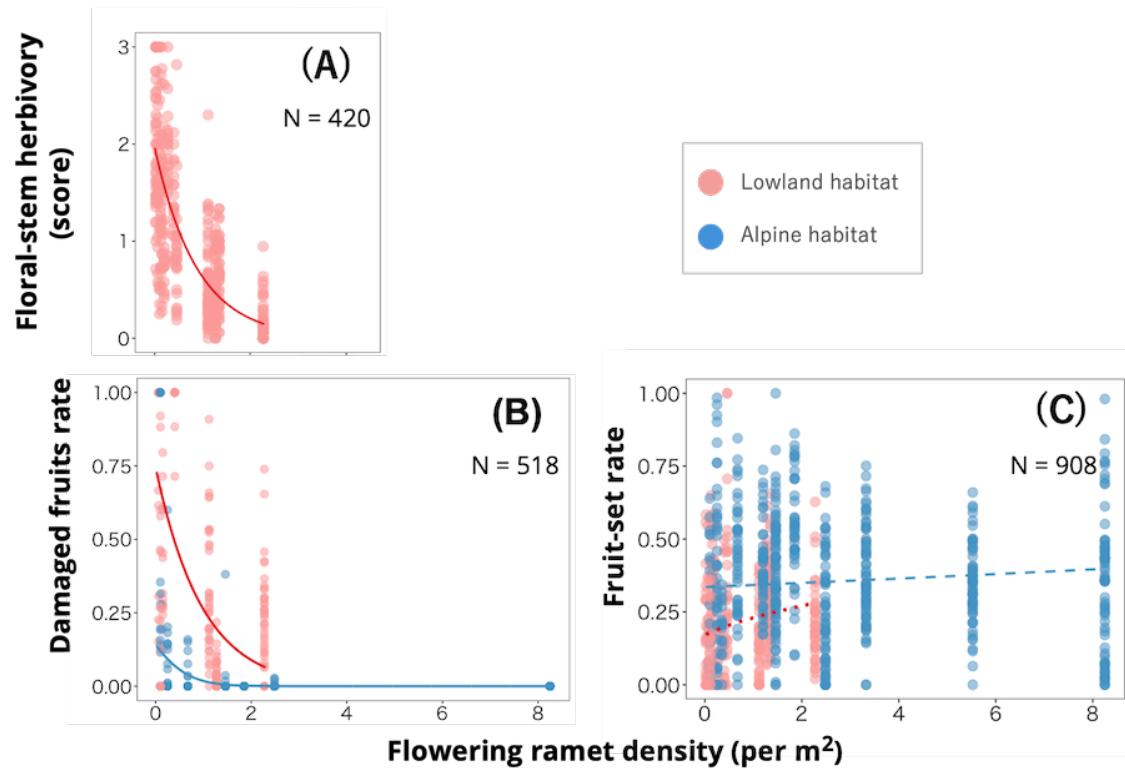


FIGURE 4. Relationships between flowering ramet density (per m²) and herbivorous damage by stem borers (A) and seed predators (B), and between flowering ramet density and fruit-set rate (C) for lowland populations (red point and line) and alpine populations (blue point and line). Each point indicates each ramet. The solid line indicates the predicted relationships by the GLMMs. Fruit-set rates were independent of flowering ramet density (dotted line).