The evolutionary process of masting behavior in a perennial herb, *Veratrum album*: its ecological significance and the physiological mechanism

（多年生植物バイケイソウにおける一斉開花結実現象の進化プロセス：

その生態的意義と生理的メカニズム）

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Summary

Synchronous and highly variable flower or seed production among years within/across populations (i.e., masting) has been reported in numerous perennial plants. Masting often affect population dynamics of specific species and community dynamics in the terrestrial ecosystems. For instance, it fluctuates the abundance of flower and seed consumers through a bottom-up effect that regulates the survivorship and fecundity of consumers by food resource limitation. To understand the mechanisms causing spatiotemporal dynamics of various organisms across the terrestrial ecosystems, therefore, it is crucial to clarify the ecological significance and the physiological mechanism of the masting phenomenon. Because the previous studies on masting ecology have often focused on interspecific variation in woody species, further studies focusing on herbaceous species and intraspecific variation are necessary.

The pattern of masting is comprised of the extent of reproductive synchrony among plants within or across populations and the periodicity and the interval of mating events at population and plant levels. As the ecological advantages of masting, numerous studies have demonstrated the possibility of the enhancement of pollination efficiency (the pollination efficiency hypothesis) and/or the escape from predator attack (the predator satiation hypothesis). As the physiological mechanisms, importance of resource dynamics stored within plants (the resource budget hypothesis) and/or environmental triggers causing simultaneous flowering onset, such as specific weather cues (the weather cue hypothesis), have been supported in the previous studies. These hypotheses suggest that masting behaviors may vary even among populations of the same species depending on the site-specific selective pressure and/or environmental conditions. However, its evolutionary process have rarely been studied. Some studies reported that masting patterns varied between high- and low-elevation populations of a single species. Because various biotic and abiotic conditions, such as temperature, precipitation, radiation, and plant-herbivore interaction, change steeply along the elevation gradient, masting patterns may exhibit diverse patterns depending on the

growing elevation even in a single species. In the present study, I examined the ecological significance and the physiological mechanism of masting behaviors (flowering synchrony and periodicity) of a perennial herb *Veratrum album* between lowland and alpine populations in Hokkaido, northern Japan, aiming to reveal the evolutionary process of the masting phenomenon in this species.

In Chapter 1, I investigated flowering synchrony within populations and its selective pressure in five lowland and six alpine populations to test the predator satiation hypothesis and the pollination efficiency hypothesis as the ecological significance of mast flowering. In most of the populations, floral abundance highly fluctuated between years, indicating that mast flowering is widespread in *V. album*. The intensity of flowering synchrony was higher in the lowland populations than in the alpine populations. Herbivory damage decreased in the year of intensive flowering synchrony at both of the lowland and alpine habitats. However, the ecological advantage of masting was greater in the lowland populations than in the alpine populations because floral-stem herbivory by dipteran larvae and seed predation by lepidopteran larvae were intensive at the lowland habitat, whereas herbivory damage by stem borers was absent and seed predation damage was relatively low at the alpine habitat. Improved pollination efficiency by mast flowering was detected only at the lowland habitat. As a result, the selective pressure acting on flowering synchrony was greater in the lowland populations than in the alpine populations. These results indicate that the pattern of flowering synchrony within populations reflects the habitat-specific selective forces.

In Chapter 2, I quantified the masting patterns of 23 populations (16 lowland and 7 alpine populations) using the herb-chronological method to evaluate the spatial patterns of flowering synchrony across populations and to test the weather cue hypothesis as a physiological mechanism of mast flowering. Masting events commonly occurred every two to six years in the lowland populations, whereas every two years in the alpine populations. Flowering intervals of individual plants were shorter in the alpine populations than in the lowland populations. Although mast flowering was triggered by a cool thermal condition during the growing season two years ago in both of the lowland and alpine populations, the

response to the thermal condition was more sensitive at the lowland habitat than at the alpine habitat. The degrees of flowering synchrony and the similarity of thermal condition declined greatly between populations locating at different elevations, whereas they were independent of simple geographic distance between populations. Because of the differences in the thermal conditions and the flowering sensitivity to thermal condition between the lowland and alpine habitats, habitat-specific patterns of flowering synchrony were formed.

In Chapter 3, I tested the resource budget hypothesis as a determinant factor of the variation in flowering intervals of individual plants between the lowland and alpine habitats. Firstly, I identified the important resource limiting the reproduction (carbon or nitrogen) in this species. Subsequently, I compared reproductive investment and annual carbon fixation between lowland and alpine populations to examine the relationship between the variation in masting intervals and carbon balance. Carbon storage was depleted greatly by a single reproductive event, whereas nitrogen storage was independent of reproduction. Total reproductive investment was smaller in the alpine populations than in the lowland populations, whereas annual carbon fixation per plant was greater at the alpine habitat than at the lowland habitat. This indicates that plants growing at the alpine habitat enable the flowering intervals shorter due to a faster recovery from the carbon depletion than plants growing at the lowland habitat. Thus, the variation in masting intervals can be explained by the habitat-specific carbon budget balances.

The previous studies on the evolutionary process of masting behavior have mainly been conducted based on the approach of interspecific comparisons, whereas few studies focused on the intraspecific variation. The present study demonstrated that flowering synchrony was advantageous for decreasing herbivorous damage on the reproductive organs (also for enhancing pollination efficiency only at the lowland habitat) and the flowering synchrony was regulated by the environmental trigger of cool thermal conditions. In the lowland populations suffering from intense herbivory pressure, highly synchronized flowering responding to the weather cue sensitively might be adaptive, indicating that the intensity of flowering synchrony varies depending on the habitat-specific selective forces.

Furthermore, the masting periodicity within populations and the flowering intervals of individual plants varied between the lowland and alpine habitats. These difference were related to the elevation-specific thermal conditions, sensitivity to the weather cue, and carbon budget balance. In conclusion, "elevation gradient" is a key driver creating various masting patterns within/across populations even in the same species.

General introduction

Mast flowering/seeding, or simply masting, is a phenomenon whereby synchronous highly variable reproduction occurs among years by a population of perennial plants (Kelly et al., 2008). Masting often causes the population dynamics of flower and seed consumers, and it creates cascade bottom-up effects on the terrestrial ecosystems (Ostfeld and Keesing, 2000; Satake et al., 2004; Koenig and Knops, 2005; Inari et al., 2012). Furthermore, it may influence various human activities, such as the management of rare plant/animal species, cultivation of agricultural crops, and zoonotic disease risk (Pesendorfer et al., 2021). Although masting has been reported in various plant species and populations worldwide, most of the studies have focused on woody species growing in tropical and temperate regions (Hacket-Pain et al., 2022). For a comprehensive understanding of masting phenomenon, further studies focusing on other biomes and herbaceous species are crucial.

Masting behavior is formed by the periodicity and the interval of mass reproductive outputs in individual populations and plants (Övrtgaard et al., 2007; Satake and Bjørnstad, 2008; Henkel and Mayor, 2019; Shibata et al., 2020) and the reproductive synchrony among plants within or among populations (Koenig and Knops, 2000; Mooney et al., 2011; Abe et al., 2016; Bogdziewicz et al., 2020c). Recent studies reported that climate change has modified masting behaviors of plants (Hacket-Pain and Bogdziewicz, 2021; Bogdziewicz, 2022). For instance, warm weather conditions have decreased the reproductive variability and synchrony in *Fagus sylvatica* (Bogdziewicz et al., 2020a) and shortened the masting periodicity in *Quercus crispula* (Shibata et al., 2020). These changes in masting patterns may modify the food availability for various animals, leading to outbreaks, shortages or extinction of some species. To understand the ecosystem responses to climate change, therefore, it is crucial to clarify how masting patterns vary responding to the changes in environmental conditions (Hacket-Pain and Bogdziewicz, 2021).

As the ecological advantages of masting (i.e., ultimate factors), improvement of pollination efficiency (Nilsson and Wästljung, 1987; Shibata et al., 1998; Kelly et al., 2001)

and escape from predator attack (Janzen, 1971; Silvertown, 1980; Zwolak et al., 2022) have been suggested in the previous studies. As the physiological mechanisms of masting occurrence (i.e., proximate factors), importance of resource budget of individual plants (Isagi et al., 1997; Satake and Iwasa, 2000; Crone and Rapp, 2014) and environmental triggers, such as specific weather cues (Norton and Kelly, 1988; Schauber et al., 2002; Kelly et al., 2013), have been reported. Because masting behavior is considered to be a life-history strategy of specific species, its evolutionary significance has been studied based on the interspecific comparison among populations (Herrera et al., 1998; Kelly and Sork, 2002; Koenig and Knops, 2003; Bogdziewicz et al., 2020b). Although the intraspecific variations in masting behavior are reported in some species (Sullivan and Kelly, 2000; Lázaro et al., 2006; Satake and Bjørnstad, 2008; Allen et al., 2012), little is known about the ecological and evolutionary background causing the variation within a species. Both of the ultimate and proximate factors can be related to the variations in masting behavior. If selective pressures acting on the masting behavior vary at a local scale within the distribution area of single species, it could generate different masting patterns among conspecific populations (Sullivan and Kelly, 2000). On the other hand, if environmental conditions affecting the physiological mechanism of masting behavior differ among populations, masting pattern may also vary among the populations (Kelly et al., 2008; Satake and Bjørnstad, 2008).

Elevation is an important environmental gradient for plant growth and distribution, and there is a variation in masting pattern between high- and low-elevation populations in some species (Sullivan and Kelly, 2000; Kelly et al., 2001; Allen et al., 2012). Various biotic and abiotic factors, such as temperature, precipitation, radiation, and plant-herbivore interaction, change steeply along the elevation gradient (Körner, 2007). Because these factors often affect reproductive activity of plants, masting patterns may vary among populations locating at different elevations. Furthermore, the elevation-specific masting behavior may disturb the spatial patterns of flowering synchrony across populations. Accordingly, comparisons of populations growing at different elevations are available to understand the intraspecific variation of masting behavior and its evolutionary process.

In the present study, I aim to clarify the ecological significance and the physiological mechanism of masting behavior of a perennial herbaceous species *Veratrum album* subsp. *oxysepalum* (Melanthiaceae) across populations locating at lowland and alpine habitats. The major issues of this study are the clarifications of (1) the ecological significance of mast flowering in terms of the pollination efficiency and the escape from predator attack, (2) the pattern of flowering synchrony across populations and the environmental trigger of masting, and (3) the contribution of carbon budget to masting intervals. Throughout the present study, I explore the evolutionary process of the masting behaviors (flowering synchrony and periodicity) in this species. The integrated approach of this study should contribute to further understanding of the evolutionary and ecological significances and the physiological mechanism of the masting phenomenon.

Material species and study sites

Veratrum album subsp. *oxysepalum* (Turcz.) Hultén (Melanthiaceae) is a clonal perennial herb growing at moist understory of deciduous forests and meadows from lowland to alpine zones in the central to northern Japan. Flowering of this species commonly occurs synchronously within a population after consecutive non-flowering years (Figure 0-1). Aerial shoot growth and leaf expansion start soon after snowmelt, and the length of the leafing period is approximately 1.5 months (Tani and Kudo, 2006). Even after the initiation of leaf senescence, reproductive plants continue to flower, and the fruits mature one month after the flowering initiation. Thus, the total growth period of reproductive plants is shorter than three months. This species develops thick lateral rhizomes on which annual growth scars are formed. The longevity of rhizomes is 5–30 years. When flowering occurs, the terminal growth of the rhizome is blocked, and the rhizome branches into one to three lateral parts at the apical growth point (Tani, 2005). This growth pattern enabled us to estimate the flowering years by counting the annual scars on the rhizomes from the current year to the branching point (Figure 0-2). Reproductive ramets produce a single floral stem on which 10–15 lateral inflorescences are arranged as a raceme. *Veratrum album* is an andromonoecious species that produces both male (staminate) and perfect (hermaphroditic) flowers (Nakagaki, 1997) that are pollinated mainly by dipteran, coleopteran, and hymenopteran insects (Kato et al., 2009). Several herbivorous insects attack the reproductive organs of *Veratrum* species 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 on vegetative ramets (Y. Ito and G. Kudo, personal observation).

Major field surveys were conducted in 11 *V. album* populations in Hokkaido during 2019–2023: seven sites in 2019 and all 11 sites in other years (Table 0-1, Figure 0-3). These

study sites were classified into five lowland populations (5–178 m elevation) and six alpine populations (1760–1990 m elevation). All of the lowland populations were located at 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 located at the snow-meadows of the Taisetsu Mountains, where plant growth started in late June, approximately two months later than the lowland populations, due to cool weather and late snowmelt time at higher elevations. Flowering occurred in early August, and fruits mature in mid-September in the alpine populations. In addition to the 11 sites, I selected more populations within Hokkaido for the short-time surveys of predation damage (Chapter 1) and annual scars on rhizomes (Chapter 2).

Table 0-1. Descriptions of the main study sites in Hokkaido. See Figure 0-3 for the location of each site.

Site name	Site cord	Elevation type	Elevation (m)	No. plots	Obs. year
Hoshioki	HO	Lowland	8	8	2019–2023
Nopporo	NP	Lowland	65	10	2019–2023
Tobetsu	TB	Lowland	5	8	2019–2023
Asahikawa	AS	Lowland	178	10	2019–2023
Obihiro	OB	Lowland	51	8	2019–2023
Hisago	HS	Alpine	1760	30	2020–2023
Mt. Kaun	KN	Alpine	1850	8	2020–2023
Mt. Goshiki	GS	Alpine	1865	8	2020–2023
Mt. Hakuun	HK	Alpine	1990	8	2020–2023
Mt. Kuro	KR	Alpine	1832	8	2020–2023
Mt. Asahi	MA	Alpine	1785	8	2020–2023

Figure 0-1. Images of a mast flowering year in *Veratrum album* populations at lowland (A) and alpine habitats (B), and a non-flowering year at the lowland (C) and alpine habitats (D).

Figure 0-2. Picture of *Veratrum album* rhizome from a lowland population in mid-June. This species develops thick lateral rhizomes on which annual growth scars are formed. In the flowering years, the rhizomes branch into one to three lateral parts from the apical growth point. Thus, flowering years can be tracked by counting the number of annual scars from the current year to the branching point. NF and F indicate non-flowering and flowering years, respectively.

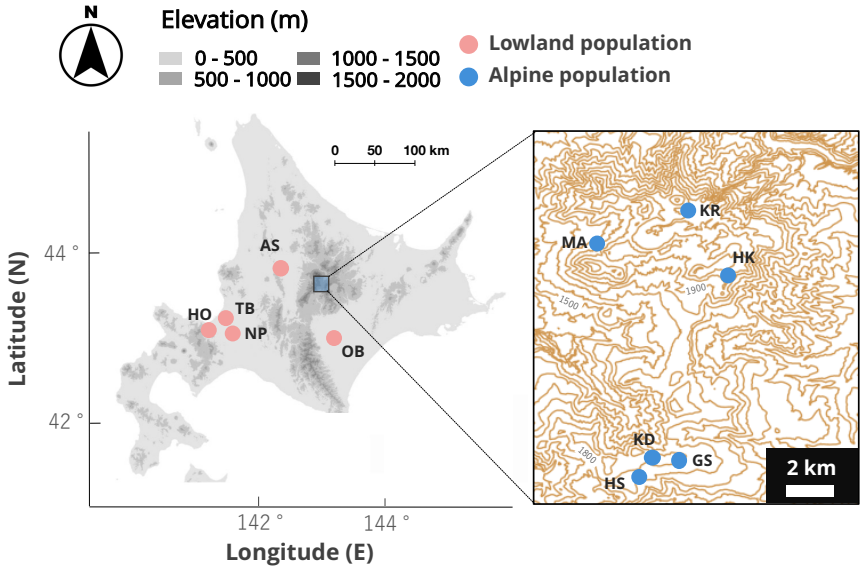


Figure 0-3. Locations of study sites in Hokkaido of northern Japan. The color difference indicates elevational habitat type. Red: lowland population, blue: alpine population. Refer to Table 0-1 for details of the sites.

Chapter 1: Ecological significance of mast flowering : a test of the pollination efficiency hypothesis and the predator satiation hypothesis

1.1 Introduction

Masting is a costly event if it causes a postponed reproduction (Waller, 1979) and/or a density-dependent mortality of offspring (Hett, 1971), but it provides fitness advantages if an occasional, synchronous, and large investment in reproduction results in a higher reproductive success than a stable and moderate resource allocation for reproduction (i.e., the economy of scale hypothesis; Norton and Kelly, 1988). 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., 2020b; Zwolak et al., 2022) and the pollination efficiency hypothesis (Nilsson and Wa¨stljung, 1987; Norton and Kelly 1988; Smith et al., 1990; Shibata et al., 1998; Kelly et al., 2001) have the most empirical support. The former hypothesis predicts the escape from intensive predation damage by the mast flowering and/or fruiting (Janzen, 1971), whereas the later hypothesis predicts higher pollination success by the mast flowering (Kelly et al., 2001). These hypotheses are applicable to individual populations of the same species in various environmental conditions.

Several studies reported the variations in masting behavior among populations of the same species (Sullivan and Kelly, 2000; Satake et al., 2004; Lázaro et al., 2006; Crone et al., 2011; Allen et al., 2012). Because ecological situations, such as the species composition 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). 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., 2020b), few studies have focused on intraspecific variation. Therefore, comparisons of populations in different ecological situations are crucial to understanding the evolutionary process of masting behavior in response to the local selective pressures.

The interaction between plants and herbivores often changes at different elevations (Hodkinson, 2005; Rasmann et al., 2014; Sakaguchi et al., 2017). For instance, the intensity of seed predation damage is often lower in populations at higher elevations in plant species having a wide distribution range of elevations (Lord and Kelly, 1999; Sullivan and Kelly, 2000). In this case, selective pressures on masting behavior may decrease along the elevation gradient, resulting in obscuring masting behaviors in high-elevation populations (i.e., less-synchronized or continuous reproduction). Accordingly, comparisons of populations at different elevations may improve our understanding of the ecological significance of masting in terms of plant–herbivore interactions.

This chapter aims to elucidate the variation in mast flowering characteristics (i.e., yearly variation in floral abundance and the intensity of flowering synchrony) and its ecological functions (i.e., enhancement of pollination efficiency and escape from herbivorous damage) in populations of *V. album* subsp. *oxysepalum* (here after *V. album*) growing at different elevations. Because of large differences in the growing environments between lowland and alpine habitats, such as meteorological conditions and growing season length, the 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, then mast flowering characteristics may also vary along the elevation gradient. Thus, I compared the mast flowering patterns and its selective pressure between lowland and alpine populations. Specifically, I addressed the following questions: (1) Is mast flowering a widespread behavior in this species across the distribution area, especially between lowland and alpine populations? (2) Which selective advantage, i.e., predation avoidance and pollination efficiency, is more closely related to the masting behavior in this species? (3) Does the intensity of selective pressure acting on flowering synchrony differ between lowland and alpine populations?

1.2 Methods

Data collection

I established 8 to 30 plots (5 × 1 m) in the 11 study site depending on the population size (Table 0-1). I recorded the numbers of flowering and non-flowering ramets within each plot to estimate the proportion of flowering ramets in each population and year. Then, I randomly selected 10 flowering ramets at each plot (or all flowering ramets if the number of flowering ramets was less than 10) and recorded the numbers of flowers and fruits every year. Flower number was recorded separately for perfect and male flowers of 16–35 plants in 2023 at KD site, and every year during 2021–2023 at other 10 sites. Using a correlation between total flower number and perfect flower number in each population (Appendix 1-1), I estimated the proportion of perfect-flowers in all flowering plants during 2019–2023.

I measured the degrees of floral-stem herbivory and seed predation in the natural populations. The presence of stem borers was detectable in a non-destructive manner as the existence of necrosis on inflorescences before flowering. For the floral-stem damage, therefore, I quantified the degree of necrotic stems for each ramet. I visually recorded the percentage of non-necrotic (i.e., survived) parts for individual lateral inflorescences on the floral stem and scored as follows: 0% non-necrotic part = score 0; 1–49% non-necrotic part = score 1; 50–99% non-necrotic part = score 2; 100% non-necrotic part = score 3 (Appendix 1-2). Scores of individual inflorescences were then averaged in each ramet to estimate the mean survived floral-stems per ramet. In the preliminary survey, I confirmed a negative correlation between average score and the number of stem borers (dipteran larvae) on floral stems (r = –0.79, N = 44; Appendix 1-2).

Regarding the degree of seed predation, I measured the proportion of intact fruits without seed predation to all fruits. The presence of seed-predator attack was also detectable in a non-destructive manner as the existence of small holes on fruits. To quantify the frequency of intact fruits without seed predation, I counted the numbers of intact and attacked fruits every year. Even in attacked fruits, there were some seeds without predation damage. Therefore, I randomly sampled five intact and five attacked fruits per ramet before fruit

dehiscence and counted the number of intact seeds for each fruit. This measurement was conducted during 2019–2023 for HS alpine population and during 2020–2023 for other 10 populations. The total seed production per ramet was estimated as follows: (mean seed number per intact fruit × intact fruit number) + (mean intact seed number per attacked fruit × attacked fruit number).

In this study, I excluded the data for plants on which inflorescences were damaged by strong wind or the herbivory of wild deer. When the total number of flowering ramets was less than 15 across the plots within a site, I added samples of flowering ramets outside of the plots to keep a minimum sample size of 15 ramets per population.

Identification and distribution of herbivorous insects

To determine the geographic distribution of herbivorous insects in Hokkaido, I surveyed 36 populations for floral-stem damage and 15 populations for seed predation (Table 1-1). When herbivorous damage was visible in either 2019 or 2020 within a population, I confirmed the presence of herbivorous insects in the population, and "absence" was defined as no sign of herbivorous damage both years. Herbivorous insects were collected and preserved in 100% ethanol for identification by DNA barcoding at the family level based on the sequencing of the *CO1* gene (Hebert et al., 2003). DNA was extracted from each insect using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The PCR mixture contained 1.0 μL DNA, 0.5 μM of each primer, 10.0 μL GoTaq DNA polymerase (Promega, Madison, WI, USA) in a total volume of 20 μL. The thermal profile of Geller et al. (2013) was used for the PCR. I used a BigDye Terminator Cycle Sequencing Kit ver. 3.1 (Applied Biosystems, Foster City, CA, USA) for terminator reaction. The amplicons were sequenced using an ABI 3100 automated sequencer (Applied Biosystems), and the sequence data were aligned using MEGAX (Kumar et al., 2018). The sequence data were compared with sequences from the NCBI database (https://www.ncbi.nlm.nih.gov) to identify taxa.

Statistical analyses

To evaluate the intensity of flowering synchrony within a population, I calculated pairwise Spearman's rank correlations for the proportion of flowering ramets between plots in every population (r_{plot}). To test the distance dependency of flowering synchrony between plots within a population, the correlation between the pairwise Spearman's rank correlation (r_{plot}) and the geographic distance between plots was analyzed by Mantel test with 9,999 permutations.

The effects of flowering synchrony (proportion of flowering ramets) on the avoidance of herbivorous damages (floral-stem damage and seed predation) and the pollination success (fruit-set rate) were analyzed by generalized linear mixed models (GLMMs) using the glmmTMB package (Brooks et al., 2017). The proportion of flowering ramets across plots in each site and year was used as the index of flowering synchrony in the analyses. The proportion of flowering ramets was the explanatory variable and site ID nested by plot ID was the random variable in every GLMM. In the analysis of avoidance of floral-stem damage (based on the non-necrotic score of inflorescences per ramet), GLMM postulating a gamma error distribution was used. Since the data of seed predation and fruit-set success contained numerous zero values, I used a 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 the probability of an extra zero (Brooks et al., 2017). In the analysis of seed predation, non-attacked (intact) fruit number was the response variable and total fruit number was set as an offset term after logarithmic transformation (Agresti, 1996). In the analysis of fruit-set success, fruit number was the response variable and perfect-flower number was set as an offset term after logarithmic transformation (Agresti, 1996).

For the quantification of the intensity of natural selection acting on phenotypes, a regression-based technique has been widely used (Lande and Arnold, 1983; Bogdziewicz et al., 2020b). This technique evaluates the strength of selective pressure from the regression coefficients of fitness variation responding to individual difference of the focal phenotype (Conner and Hartl, 2004). I constructed a linear mixed model (LMM) to compare the

selective pressure acting on flowering synchrony in each population. As an index of fitness in the analysis, I used the relative seed number, which was calculated by dividing the number of seeds in individual plants by mean number of seeds across the plots within a population over the observation years (2019–2023 or 2020–2023). In the LMM, relative seed number was the response variable and the proportion of flowering ramets across the plots within a population was the explanatory variable, and plot ID was set as the random variable. All analyses were performed in R version 3.6.0 (R Core Team, 2019).

1.3 Results

Mast flowering characteristics

The proportion of flowering ramets within plots fluctuated greatly across years (2019–2023 or 2020–2023) in most of the populations (Figure 1-1). Among the lowland populations, populations HO, TB, and AS had high floral abundancies in 2019 and 2022, but low floral abundancies in 2020 2021, and 2023. In contrast, populations NP showed high floral abundancies in 2020 and 2022, but low floral abundancies in 2019 2021, and 2023. Floral abundancies of the OB population were high in 2020 and 2023, but low in 2019 2021, and 2022. Among the alpine populations, populations KD, MA, KR, HK, and GS had high floral abundancies in 2021, but low floral densities in 2020 2022, and 2023. In contrast, floral abundancies of the HS population were consistently moderate over the 5-yr observation period, indicating an obscure masting pattern. Thus, mast flowering was observed in most of the *V. album* populations.

The site-averaged r_{plot} (intensity of flowering synchrony between plots within a population) was consistently larger than 0.8 in all lowland populations (0.92 in AS, 0.87 in TB, 0.85 in OB, 0.85 in NP, and 0.83 in HO). In contrast, it was 0.6 or less in many alpine populations (0.60 in HK, 0.53 in KR, 0.24 in MA, and 0.15 in HS), except for GS (0.88) and KD (0.85). These patterns indicate that the degree of flowering synchrony was intense in the lowland populations, while it was obscure in the alpine populations. Furthermore, any

significant relationship was not detected between plot distance and r_{plot} in every population (Figure 1-2).

Distribution of herbivorous insects

I 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, floral-stem borer was identified as dipteran larvae of Lonchaeinae sp. with 96% sequence matching. Seed predator was identified as lepidopteran larvae of *Eupithecia veratraria* (Geometridae) with a 99% sequence matching.

In the extensive observation of herbivorous damages over 36 populations (Table 1-1), floral-stem damages by dipteran larvae were detected in all lowland populations (22 populations), whereas there was no damage in all alpine populations (14 populations; Figure 1-3). Seed predation by lepidopteran larvae was detected in some of the lowland and alpine populations. Three out of seven lowland and three out of eight alpine populations were free from seed predation damages (Figure 1-4).

Ecological advantage of mast flowering

The GLMM results analyzing the effects of flowering synchrony (i.e., the proportion of flowering ramets) on the avoidance of herbivorous damage and the pollination efficiency are summarized in Table 1-2. Floral-stem herbivory by stem borers was observed only in the lowland populations, where undamaged inflorescences (averaged non-necrosis score per ramet) significantly increased with higher flowering synchrony (Figure 1-5A).

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 non-attacked fruits by seed predators significantly increased with flowering synchrony with a significant interaction between habitat type and flowering synchrony. Additionally, the proportion of non-attacked fruits was lower in the lowland populations than in the alpine populations (conditional model; Table 1-2). The frequency of completely predated fruits decreased with

flowering synchrony in both habitats, but it was lower in the lowland populations than in the alpine populations (zero-inflation model; Table 1-2). These indicate that the proportion of survived seeds in the alpine populations was consistently high and could be maximized even at a lower level of the flowering synchrony (Figure 1-5B).

Fruit-set rate, i.e., pollination success, was higher in the alpine populations than in the lowland populations independent of flowering synchrony, but a significant interaction was detected between habitat type and flowering synchrony (conditional model; Table 1-2). These patterns indicate that fruit-set success in the lowland populations increased with higher flowering synchrony to some extent, whereas fruit-set success in the alpine populations was consistently high regardless of the flowering synchrony (Figure 1-5C). The frequency of complete failure in fruit production was independent of the flowering synchrony in both habitat types, but it was significantly higher in the lowland populations than in the alpine populations (zero-inflation model; Table 1-2).

Selective pressure acting on flowering synchrony

Relative seed production increased significantly with higher flowering synchrony in all lowland and three alpine populations (MA, GS, KD), whereas it decreased (HS) or did not differ (KR, HK) responding to the flowering synchrony in other alpine populations (Figure 1-6). The regression coefficients ranged from 4.7 to 13.8 in the lowland populations, whereas ranged from –8.3 to 6.5 in the alpine populations (Table 1-3). Thus, the intensity of natural selection acting on flowering synchrony was greater in the lowland populations than in the alpine populations.

1.4 Discussion

The proportion of flowering ramets fluctuated greatly between years in all populations, indicating that masting is a widespread phenological trait in this species. However, the intensity of flowering synchrony was higher in the lowland populations than in the alpine populations. Floral-stem herbivory was observed only at the lowland habitat, while seed

predation was found at both the lowland and alpine habitats. With increasing flowering synchrony, the herbivorous damages decreased at both habitats, and the fruit-set success increased in the lowland populations. These results support the predator satiation hypothesis in both habitats and the pollination efficiency hypothesis only in the lowland habitat (Janzen, 1971, Kelly et al., 2001).

The dominant herbivorous insects in *V. album* were stem borers (dipteran larvae) and seed predators (lepidopteran larvae). As the damage by stem borers progresses during the pre-flowering period and it causes a decay of inflorescences before flowering onset (Figure 1-3A, Appendix 1-2), not only seed production but also pollen donation are greatly restricted by the stem herbivory. The present study revealed that herbivorous damage of floral stems was effectively decreased with higher flowering synchrony within a population (Figure 1-5A). Additionally, the contribution of flowering synchrony was greater for the avoidance of floral-stem herbivory compared with the avoidance of seed predation (Figure 1-5). While most previous studies testing the predator saturation hypothesis focused on seed predation (Janzen, 1971; Kelly et al., 2008; Bogdziewicz et al., 2020b), the present study is the first report demonstrating the importance of floral-stem herbivory before flowering as a selective pressure on mast flowering.

Although floral-stem herbivory was common in the lowland populations, it was absent in the alpine populations (Figure 1-3B), indicating that the herbivory pressure by stem borers is specific to the low-elevation habitat. In contrast, seed predation by *E. veratraria* larvae occurred at both high and low elevations (Figure 1-4B). This moth species is the main seed predator of *Veratrum* species also in western Europe and it greatly reduces seed production (Hesse et al., 2008). Because intact fruits without seed predation increased with higher flowering abundance in the lowland and alpine populations (Figure 1-5B), mast flowering is beneficial for avoiding seed predation. However, the intensity of seed predation was milder in the alpine populations, where even in a moderate flowering synchrony might be effective for predator satiation compared with the lowland populations (Figure 1-5B). Plant–herbivore interactions (Lord and Kelly, 1999; Sullivan and Kelly, 2000) and the distribution patterns of

herbivorous insects (Hodkinson, 2005) often vary between different elevations. Also in the present study, the selective pressure of herbivorous damage was smaller in the alpine populations than in the lowland populations.

The advantage of pollination efficiency by mast flowering was specific to the lowland populations (Figure 1-5C). Because *V. album* is self-incompatible in both of the lowland and alpine populations (Kato et al., 2009; Kudo et al., 2022), pollinator visits are crucial to set fruits. Flowers of *V. album* in alpine meadows are frequently visited by various insects, such as dipteran, coleopteran, and hymenopteran insects (Y. Ito and G. Kudo, personal observation), resulting in a high pollination success regardless of floral density. In contrast, those in lowland forests are visited by coleopteran and dipteran insects at low frequency, and they often suffer from low pollination success due to pollen limitation, especially at low floral density (Kato et al., 2009). Thus, mast flowering was beneficial in the lowland populations for increasing pollinator visits to improve pollination success.

Synchronized flowering at a population level was obvious in the lowland populations, where great advantages of mast flowering were detected. In contrast, flowering synchrony was clear at local patch scale, but sometimes obscure at a whole population level in the alpine sites, where a moderate advantage of masting was detected. Thus, the pattern of flowering synchrony may reflect site-specific selective pressures at different elevations. On the other hand, less distinct synchrony across patches in the alpine populations might be related to the clonal structure and heterogeneous environmental conditions specific to alpine environments. In the masting of a bamboo species, for instance, variation in flowering cycle among patches within a population was explained by the difference in clone structures (Isagi et al., 2004). As clonal ramet production by rhizome branching is common in *V. album* (Kleijn and Steinger, 2002), flowering synchrony at a local patch scale might be caused among ramets of the same genets.

Furthermore, yearly cycle of flowering event of individual plants often synchronizes within a microhabitat with similar environmental conditions (Crone and Rapp, 2014). Snowmelt time in the alpine site varies across local patches owing to the heterogeneity of

microtopography, and it may cause a large variation in growing period of alpine plants (Kudo, 2006). A previous study reported that the mast flowering of a *Veratrum* species growing in subalpine environment in North America was triggered by cool summer temperature two years before (Iler and Inouye, 2013). Because temperature conditions perceived by plants vary across the patches having different snowmelt times and meltwater supply periods, the activation of environmental trigger for floral-bud formation may not be uniform across patches within a population. Therefore, flowering synchrony at local patch scale and a whole population scale at alpine habitat might be caused by the spatial heterogeneity of environmental conditions (Crone and Rapp, 2014).

The present chapter revealed that the selective pressures acting on the mast flowering in *V. album* varied among populations depending on the growing elevations. Mast flowering was more beneficial in the lowland populations for the enhancement of pollination success and for the decrease in predation damages than in the alpine populations, resulting in more strict flowering synchrony in the lowland populations. In conclusion, variation in the masting patterns among *V. album* populations can be explained by the difference in local selective forces.

Table 1-1. Distribution of herbivorous damages in *Veratrum album* across 36 populations in Hokkaido. Presence or absence of damages by floral-stem borers and seed predators in each population are expressed by ○ or ×, respectively.

Pop. ID	Elevation type	Elevation (m)	Habitat type	Latitude (N°)	Longitude (E°)	Stem borer	Seed predator
1	Lowland	8	Understory	43.0750	141.1215	○	×
2	Lowland	5	Understory	43.1117	141.2707	○	○
3	Lowland	4	Understory	43.1050	141.2531	○	○
4	Lowland	65	Understory	43.0317	141.3052	○	×
5	Lowland	18	Understory	42.5246	141.2649	○	×
6	Lowland	39	Understory	42.5207	141.3439	○	no data
7	Lowland	178	Understory	43.4732	142.1816	○	○
8	Lowland	51	Understory	42.5443	143.0933	○	○
9	Lowland	49	Understory	42.5418	143.1034	○	no data
10	Lowland	50	Understory	42.5413	143.1020	○	no data
11	Lowland	61	Understory	42.5423	143.0806	○	no data
12	Lowland	58	Understory	42.5309	143.4735	○	no data
13	Lowland	10	Understory	42.4723	143.4612	○	no data
14	Lowland	90	Understory	42.0753	143.5450	○	no data
15	Lowland	11	Understory	43.0444	141.2018	○	no data
16	Lowland	8	Meadow	45.0542	141.3754	○	no data
17	Lowland	9	Understory	45.0745	142.2050	○	no data
18	Lowland	5	Meadow	45.0900	142.2208	○	no data
19	Lowland	184	Meadow	44.0048	143.2109	○	no data
20	Lowland	62	Understory	42.3921	140.1837	○	no data
21	Lowland	68	Understory	43.0126	144.5022	○	no data
22	Lowland	1102	Understory	43.3908	142.4758	○	no data
23	Alpine	1363	Meadow	43.6106	142.8236	×	○
24	Alpine	1482	Meadow	43.5981	142.8327	×	no data
25	Alpine	1760	Meadow	43.5565	142.8678	×	○
26	Alpine	1850	Meadow	43.5605	142.8763	×	×
27	Alpine	1865	Meadow	43.5656	142.8871	×	×
28	Alpine	1701	Meadow	43.6159	142.9026	×	no data
29	Alpine	1730	Meadow	43.6241	142.9022	×	no data
30	Alpine	1900	Meadow	43.6538	142.9120	×	no data
31	Alpine	1990	Meadow	43.6583	142.9140	×	×
32	Alpine	1843	Meadow	43.6878	142.9085	×	no data
33	Alpine	1832	Meadow	43.6902	142.9104	×	○
34	Alpine	1985	Meadow	43.6872	142.8911	×	no data
35	Alpine	1785	Meadow	43.6806	142.8575	×	○
36	Alpine	1380	Meadow	43.4319	141.3131	×	no data

Table 1-2. Summary of the GLMMs conducted for the avoidance of herbivorous damages (floral-stem damage and seed predation) and the fruit-set rate responding to the proportion of flowering ramets (PFR) and habitat type (alpine and lowland habitats). For the GLMM of floral-stem herbivory, data of alpine populations are excluded because stem borers were absent in the alpine habitat. The conditional and the zero-inflation models are shown in the GLMM of the damaged-fruit rate and the fruit-set rate, respectively.

Variable		Coefficient	SE	*z* value	*P* value
Objective	Explanatory				
Undamaged floral-stems (non-necrotic score)	Intercept	0.361	0.06525	5.535	**< 0.001**
	PFR	3.630	0.35858	10.124	**< 0.001**
Non-attacked fruit rate	**Conditional model**				
	Intercept	–0.090	0.047	–1.901	0.0573
	PFR	0.404	0.101	3.989	**< 0.001**
	Elevation (low)	–0.329	0.073	–4.519	**< 0.001**
	Elevation (low) × PFR	1.360	0.245	5.546	**< 0.001**
	Zero-inflation model				
	Intercept	–2.034	0.688	–2.955	**0.003**
	PFR	–3.882	1.669	–2.326	**0.012**
	Elevation (low)	1.620	0.725	2.235	**0.025**
	Elevation (low) × PFR	2.648	1.680	1.576	0.115
Fruit-set rate	**Conditional model**				
	Intercept	–0.496	0.091	–5.445	**< 0.001**
	PFR	0.046	0.292	0.157	0.875
	Elevation (low)	–0.569	0.136	–4.184	**< 0.001**
	Elevation (low) × PFR	1.959	0.493	3.97	**< 0.001**
	Zero-inflation model				
	Intercept	–3.234	0.266	–12.14	**< 0.001**
	PFR	–1.347	1.995	–0.675	0.5
	Elevation (low)	1.690	0.334	5.056	**< 0.001**
	Elevation (low) × PFR	–3.639	2.507	–1.452	0.147

Table 1-3. Results of the LMMs for relative seed production responding to the proportion of flowering ramets (PFR) in each population. Refer to Table 0-1 for the site code.

Site code	Explanatory variable	Coefficient β	SE	z value	P value
TB	Intercept	–0.055	0.1893	–0.289	0.773
	PFR	13.782	1.9914	6.921	**< 0.001**
OB	Intercept	0.094	0.1757	0.536	0.593
	PFR	7.107	1.1829	6.008	**< 0.001**
HO	Intercept	0.064	0.2528	0.254	0.8
	PFR	6.822	1.5205	4.487	**< 0.001**
NP	Intercept	0.165	0.1591	1.035	0.304
	PFR	5.158	1.0388	4.966	**< 0.001**
AS	Intercept	0.313	0.3148	0.995	0.3229
	PFR	4.054	2.2371	1.812	**0.077**
MA	Intercept	0.658	0.1202	5.478	**< 0.001**
	PFR	6.071	1.7108	3.549	**< 0.001**
GS	Intercept	0.638	0.1557	4.102	**< 0.001**
	PFR	2.071	0.7964	2.6	**0.012**
KD	Intercept	0.590	0.1871	3.154	**< 0.001**
	PFR	2.011	0.8092	2.485	**0.015**
HK	Intercept	0.870	0.1104	7.875	**< 0.001**
	PFR	0.906	0.7356	1.231	0.22
KR	Intercept	0.915	0.1375	6.657	**< 0.001**
	PFR	0.713	0.4946	1.441	0.152
HS	Intercept	1.261	0.151	8.35	**< 0.001**
	PFR	–8.038	3.176	–2.531	**0.012**

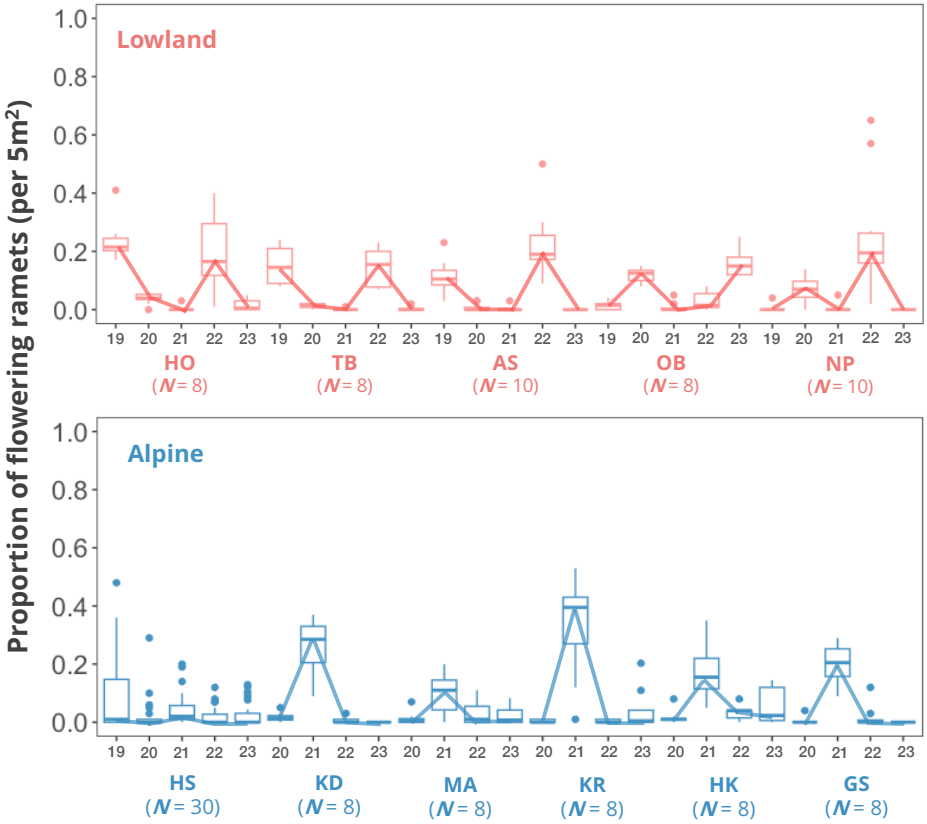


Figure 1-1. Yearly variation in the proportion of flowering ramets in each population (per 5 × 1 m plot) during 2019–2023 (HO, TB, AS, OB, NP, and HS) and 2020–2023 (KD, MA, KR, HK, and GS). A box-and-whisker plot is shown for each year for each population. Red: lowland population; blue: alpine population. The site codes are listed in Table 0-1.

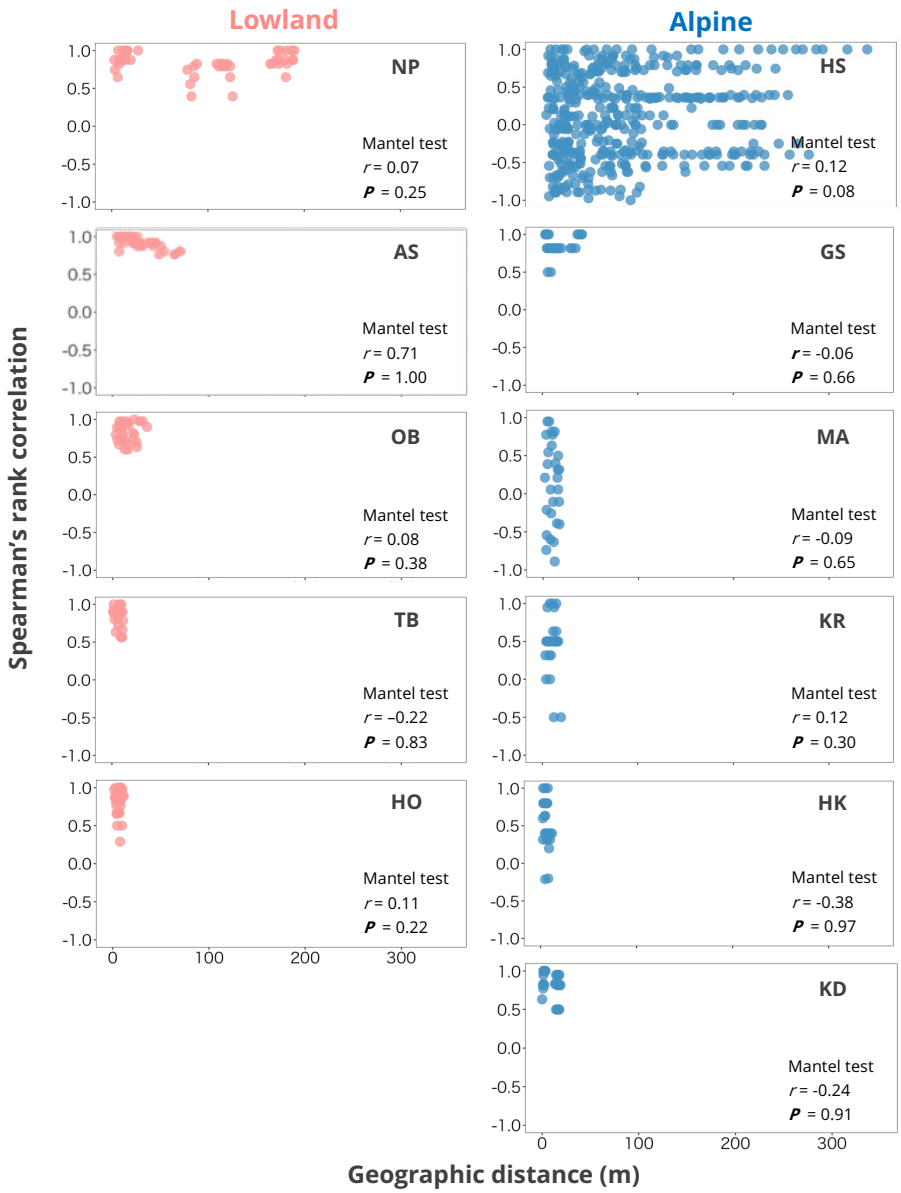


Figure 1-2. Relationships between plot–plot distance and pairwise Spearman's rank correlation for flowering synchrony. Red: lowland population; blue: alpine population. No significant correlations were found in the Mantel test for every population. Site code is listed in Table 0-1.

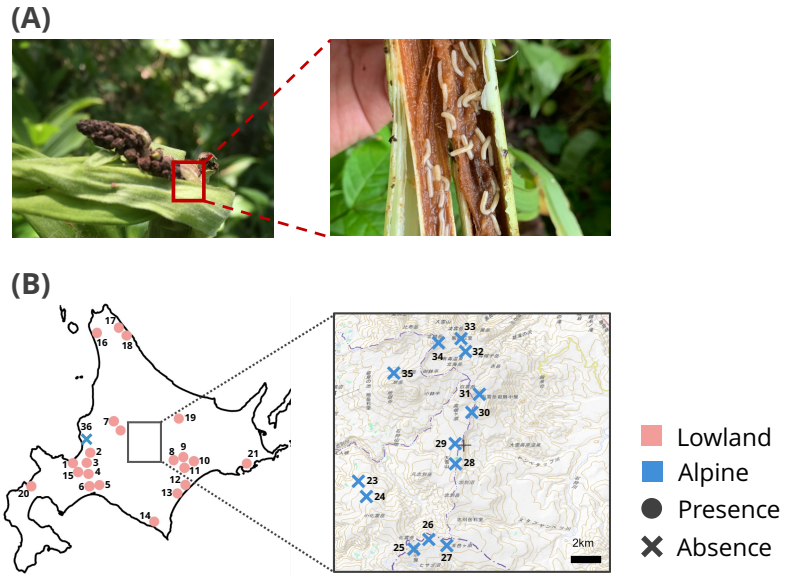


Figure 1-3. (A) Images of floral-stem herbivory by dipteran larvae (Lonchaeidae, Diptera). (B) Distributions of the floral-stem borer. The color indicates the difference in elevational habitat types (red: lowland habitat, blue: alpine habitat). Presence or absence of herbivorous damages in floral stems across 36 populations was shown as ○ or ×, respectively. The assigned number corresponds to population ID in Table 0-1.

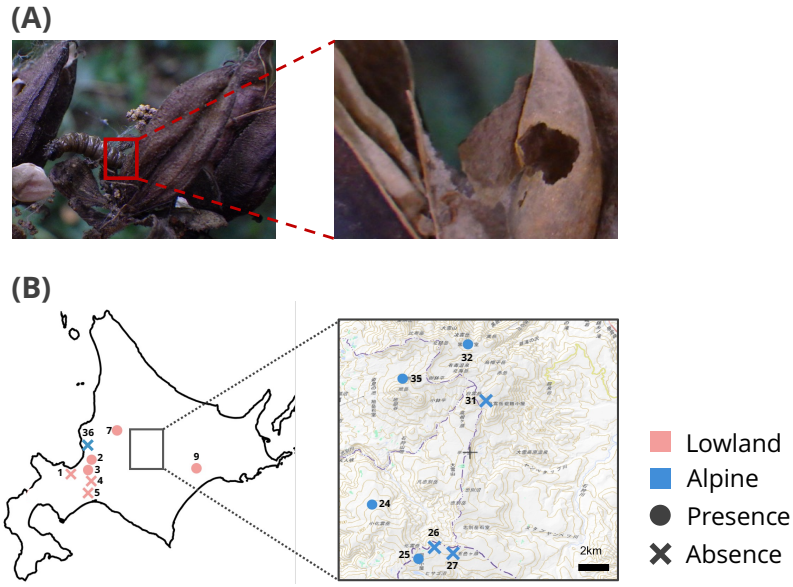


Figure 1-4. (A) Images of seed predation by lepidopteran larvae *Eupithecia veratraria* (Geometridae, Lepidoptera). (B) Distributions of seed predator. The color indicates the difference in elevational habitat types (red: lowland habitat, blue: alpine habitat). Presence or absence of seed predation damages in fruits across 15 populations was showed as ○ and ×, respectively. The assigned number corresponds to population ID in Table 0-1.

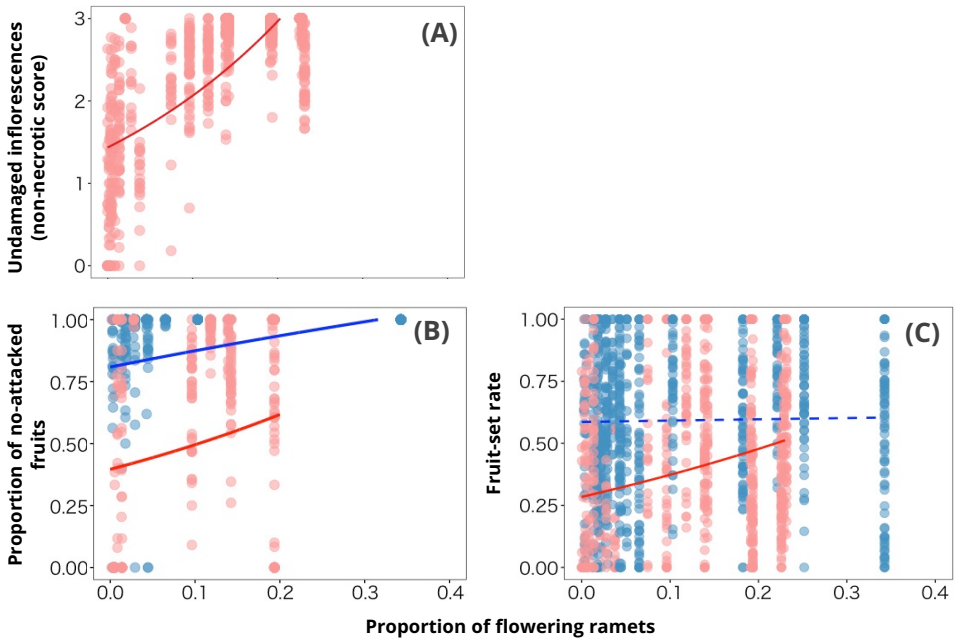


Figure 1-5. The relationships between flowering synchrony (proportion of flowering ramets) and undamaged inflorescences by stem borers (A), between flowering synchrony and non-attacked fruits by seed predators (B), and between flowering synchrony and fruit-set success (C) in the lowland populations (red points and line) and the alpine populations (blue points and line). Each point indicates each ramet. Solid lines indicate the predicted relationships by the GLMMs. Fruit-set rates in the alpine populations were independent of flowering synchrony (dotted line).

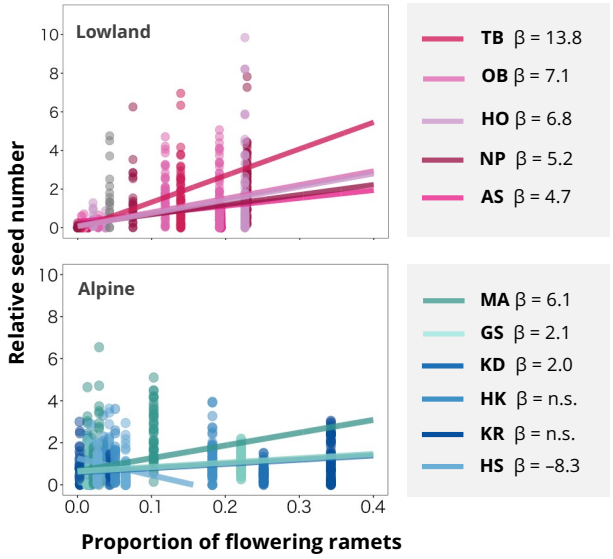


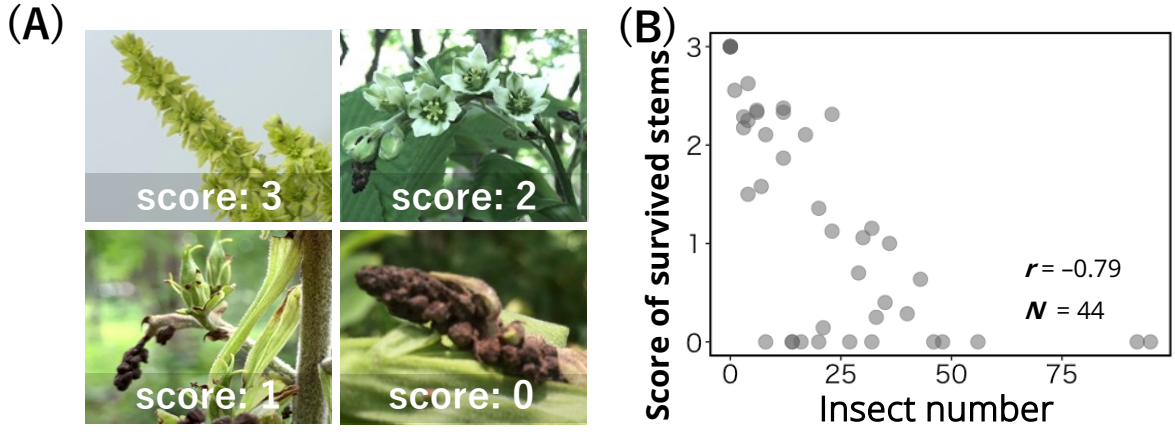
Figure 1-6. Relationship between flowering synchrony (proportion of flowering ramets) and relative seed number in each population. Results for the lowland habitat (5 populations) and the alpine habitat (6 populations) are illustrated separately. β shows the coefficient size of the LMM in each population. Lines indicate the predicted relationships by the LMMs. HK and KR

sites were independent of flowering synchrony (no lines). Site code is listed in Table 0-1.

Appendix 1-1. The summary of the GLMs conducted for perfect-flower production responding to total flower production in each site. In the GLMs with Poisson distribution, perfect flower number was a response variable and total flower number was an explanatory variable. Significant positive correlations were detected in all sites. Site code is listed in Table 0-1.

Site ID	Explanatory variable	Coefficient	SE	*z* value	*P* value
TB	Intercept	3.630	0.1629	22.28	**< 0.001**
	Total flower No.	0.002	0.0001	32.23	**< 0.001**
OB	Intercept	3.407	0.1977	17.24	**< 0.001**
	Total flower No.	0.004	0.0002	24.21	**< 0.001**
HO	Intercept	4.324	0.0518	83.43	**< 0.001**
	Total flower No.	0.002	0.0001	28.4	**< 0.001**
NP	Intercept	3.617	0.2048	17.658	**< 0.001**
	Total flower No.	0.001	0.0002	6.889	**< 0.001**
AS	Intercept	3.705	0.1820	20.36	**< 0.001**
	Total flower No.	0.003	0.0001	19.28	**< 0.001**
MA	Intercept	4.635	0.0588	78.86	**< 0.001**
	Total flower No.	0.002	0.0001	42.04	**< 0.001**
GS	Intercept	3.784	0.1185	31.942	**< 0.001**
	Total flower No.	0.004	0.0004	8.972	**< 0.001**
KD	Intercept	3.291	0.0873	37.69	**< 0.001**
	Total flower No.	0.006	0.0005	12.22	**< 0.001**
HK	Intercept	4.069	0.0609	66.85	**< 0.001**
	Total flower No.	0.004	0.0002	16.66	**< 0.001**
KR	Intercept	4.135	0.0704	58.7	**< 0.001**
	Total flower No.	0.003	0.0002	15.6	**< 0.001**
HS	Intercept	3.692	0.0500	73.82	**< 0.001**
	Total flower No.	0.005	0.0002	28.61	**< 0.001**

Appendix 1-2. (A) Examples of the damage score of inflorescence necrosis caused by floral-stem borers. (B) Relationship between the number of floral-stem borers and the score of survived floral stems. There was a significant negative correlation between the number of insects and score ($r = –0.790$, $N = 44$, $P < 0.001$).



Chapter 2: Spatial patterns of flowering synchrony across populations: a test of the weather cue hypothesis

2.1 Introduction

Synchronized reproductive events (flowering and/or fruiting) often occur not only within a single population but also across multiple populations in species having a masting behavior (Koenig and Knops, 2000). The magnitude of reproductive synchrony may affect the population dynamics and migration pattern of animals that consume flowers or fruits of masting plants as food resources. If the spatial scale of reproductive synchrony is smaller than the home range of consumers, the consumers move to the sites with rich resources (Curran and Webb, 2000; Satake and Bjørnstad, 2004). If the reproductive synchrony occurs at a larger scale than the home range of consumers, on the other hand, the populations of consumers may vary depending on the fluctuation of reproductive output of plants due to a bottom-up effect (Satake et al., 2004). The degree of synchrony across populations of a masting species tends to decrease with an increase in the geographical distance between populations (Koenig and Knops, 2000; Liebhold et al., 2004; Mooney et al., 2011; Koenig and Knops, 2013). The most of previous studies on the relationship between reproductive synchrony and geographic distance were conducted on the basis of horizontal distance, whereas there are few studies focusing on the elevational distance of reproductive synchrony (Mooney et al., 2011, Masaki et al., 2020). For a comprehensive understanding of the spatial scale of the occurrence of reproductive synchrony, therefore, clarification of the synchronous pattern along elevation gradients is crucial.

Among the physiological mechanisms causing mast flowering, one of the empirically supported hypotheses is the weather cue hypothesis, which predicts that specific environmental cues, such as temperature and precipitation, act as triggers stimulating floral-bud formation, resulting in the occurrence of synchronous flowering (Ashton et al., 1988; Norton and Kelly, 1988). Because various abiotic conditions change steeply along the

elevation gradient (Körner, 2007), timing of the trigger activation may be different between elevations, resulting in asynchronous masting patterns between low- and high-elevation populations. Furthermore, if the selective pressure acting on masting behavior varies between low- and high-elevation habitats, the sensitivity to weather cues may differ between the habitats, resulting in less synchrony of masting events between populations growing at different elevations. In order to clarify the physiological mechanism determining flowering synchrony, therefore, comparisons of masting patterns, weather conditions, and their relationships between lowland and alpine habitats are necessary.

In Chapter 1, I revealed that the degree of flowering synchrony and the selective pressure acting on masting behavior differed between the lowland and alpine populations. In the previous chapter, I focused on flowering synchrony at a site level, but other masting behaviors, such as the periodicity of masting event and flowering interval of individual plants, and flowering synchrony across populations have not been assessed. Furthermore, physiological mechanisms causing flowering synchrony have not been examined. If there is a clear differences in masting patterns and/or sensitivity to weather cues between lowland and alpine populations, flowering synchrony between populations inhabiting different elevations will be smaller than that between populations inhabiting the same elevations. This chapter aims to reveal the spatial patterns in relation to geographic and elevational distances and the physiological mechanisms of flowering synchrony in *V. album* populations. Specifically, I addressed the following questions: (1) Does the periodicity of masting events and/or the flowering interval of individual plants differ between lowland and alpine populations? (2) What is the important weather cue(s) of mast flowering in *V. album*? (3) How does the degree of flowering synchrony vary along the geographic and/or elevational distances? (4) Are weather conditions and/or sensitivity to weather cues different between lowland and alpine habitats?

2.2 Methods

Measurements of masting events

As mentioned above, it is possible to measure the previous flowering years in *V. album* by counting the annual scars on the rhizomes from the current year to the branching point (Figure 0-2). To obtain the time-series data for masting events within a population using the herb-chronological method, I established a 20-m × 20-m plot in the 16 lowland populations and in seven alpine populations in 2022 (Table 2-1, Figure 2-1). I randomly selected 20 or 30 ramets within the plots, irrespective of the presence or absence of flowering, and measured previous flowering events (during the last 6–25 years). Additionally, I estimated the flowering intervals of individual plants by counting the number of annual scars between branching segments in which flowering occurred. Then, I calculated the proportion of flowering ramets in each year for each population. Because the frequency of countable scars decreased with backward processing (due to the decay of old tissues), I used annual data in which the sample size (i.e., the number of measurable plants) was greater than 15. As a result, I obtained a time-series data for approximately last ten years at each site (Figure 2-2). In the time series data, high-flowering years were defined as the flowering occurrence of >15% ramets in the population. The intervals between high-flowering years were then recorded, and the coefficient of variation (CV; the ratio of the standard deviation to the mean value of the proportion of flowering ramets across years) was calculated for each population.

Collection of weather data

Because mean air-temperature and total precipitation during the growing season are effective masting cues for several plant species (Koenig and Knops, 2000; Kelly et al., 2013; Iler and Inouye, 2013), I focused on these variables to explore the masting cue(s) in *V. album*. I collected the weather data from the Agro-Meteorological Grid Square Dataset (https://amu.rd.naro.go.jp/wiki_open/doku.php). I calculated mean air-temperature and total precipitation from April to June for the lowland habitat, and from June to September for the alpine habitat. The time frames include the period from growth initiation to fruit maturation.

The previous study reported that the masting behavior of *Veratrum* species growing in the subalpine meadow of Rocky Mountains in USA sensitively responded to the meteorological conditions during the growth period (Iler and Inouye, 2013).

Statistical analysis

The abundance of flowering plants often decreases in years following masting events due to a resource depletion (Satake et al., 2004; Moreira et al. 2019). For instance, in populations exhibiting a 2-year cycle of masting events, negative autocorrelations are detected at lags of odd numbers (e.g., lags of 1, 3, and/or 5) in a time series of reproductive abundance (Satake et al., 2004). To judge the periodicity of masting in each population, I estimated the autocorrelation function (ACF), which is the correlation function between a given year and a lagged year over successive time intervals, using standard autocorrelation analysis for the time series of each population.

I compared flowering intervals of individual plants between the lowland and alpine habitats by a generalized linear mixed model (GLMM) postulating a Poisson error distribution using the lme4 package (Bates et al., 2015). In the GLMM, the flowering interval was the response variable, habitat type was the explanatory variable, and site ID nested by plant ID was set as the random variable.

To explore the effective weather variable(s) that triggers masting in *V. album* populations, I constructed GLMMs postulating a binomial error distribution at each lowland and alpine habitat. In the GLMMs, the proportion of flowering ramets (flowering abundance in current year) was the response variable, air-temperatures and precipitations two years ago and three years ago, and the proportion of flowering ramets in the previous year were the explanatory variables, and site ID was set as random variable. Because floral bud-formation in this species has been completed one year before the flowering, similar to other *Veratrum* species (Iler and Inouye, 2013), I assumed that environmental triggers would act two or three years before the flowering. I included flowering abundance in the previous year as an explanatory variable in the GLMM because the expenditure for large flower and fruit

production in a certain year may cause a resource depletion for reproduction in the following year. I standardized all explanatory variables to be a mean of zero and a standard deviation of one for comparing the effect size (regression coefficient) among the variables. The model with the lowest AIC was selected as a best-fitting model (Akaike, 1974).

To assess the spatial patterns of flowering synchrony and the similarity of weather conditions across populations, I calculated pairwise Spearman's rank correlations between populations for the time series data of flowering abundance and air-temperature that triggered masting (see 2.3 Results). Then, I compared the correlations among three categories based on the elevation habitats, i.e., (1) pairs between the lowland populations, (2) pairs between the alpine populations, and (3) pairs between the lowland and alpine populations. Furthermore, the correlation between geographic distance and flowering synchrony or air-temperature similarity was analyzed using Mantel test in each lowland and alpine habitat. All statistical analyses were performed using R version 3.6.1 (R Core Team, 2019).

2.3 Results

Masting behavior within a population

The proportion of flowering ramets estimated from rhizome morphology fluctuated greatly across years in most of the populations (Figure 2-2). High-flowering years (>15 % flowering) commonly occurred every two years in the alpine populations, while every two to six years in the lowland populations. The CV in the proportions of flowering plants ranged from 0.91 to 1.52 in the lowland populations, whereas it ranged from 0.64 to 1.06 in the alpine populations (Table 2-1). Thus, the abundance of flowering plants in the lowland populations was more variable across years than that in the alpine populations.

Autocorrelation analysis of flowering synchrony showed a clear difference between the lowland and alpine habitats. In most of the alpine populations, the autocorrelation function showed positive values for lags of 2, 4, and 6 years, and negative values for lags of 1, 3, and 5 years (Figure 2-3), indicating masting behavior with an alternate year cycle, i.e., one-year

intervals of flowering. In contrast, the autocorrelation pattern was less clear in the lowland populations (Figure 2-3), indicating no consistent periodicity of masting events in this case.

The average of flowering intervals in individual plants ranged from 3.5 to 8.5 years in the lowland populations and from 3.4 to 6.6 in the alpine populations (Table 2-1). Plants in the lowland populations exhibited significantly longer masting and flowering intervals than those in the alpine populations (Figure 2-4).

Weather cues of masting

The effects of weather variables and the previous flowering event on flowering abundance by the GLMMs are summarized in Table 2-2. At the lowland habitat, all explanatory variables were selected in the best-fit model, but the negative effect of air-temperature two years ago was the most prominent among the variables (see coefficient in Figure 2-5). This indicates that cool conditions might act as a trigger of simultaneous floral-bud formation in the lowland populations.

The best-fit model at the alpine habitat included all explanatory variables, except for precipitation three years ago. The negative effect of air-temperature two years ago was most extensive, although its impact was milder in comparison with the lowland habitat (see coefficient in Figure 2-5). Additionally, flowering abundance in the previous year was negatively, and air-temperature three years ago was positively related to flowering abundance (Figure 2-5). These results indicate that masting events in the alpine populations were regulated by both of temperature conditions and resource depletion in the previous year.

Spatial synchrony of flowering abundance and weather cue

The correlations of flowering abundance between population pairs across all populations (among-population flowering synchrony) ranged from –0.47 to 0.98, and the median value was 0.43. Flowering synchrony was smaller in the pairs of lowland and alpine populations (median, 0.22) than in the pairs of lowland populations (median, 0.57) or alpine populations (median, 0.70) (Figure 2-6). Furthermore, mantel tests revealed that flowering synchrony was

independent of geographic distance between populations at both of the lowland habitat (r = –0.34, P = 0.99) and the alpine habitat (r = –0.12, P = 0.61; Figure 2-7).

The correlations of air-temperatures during the growing season between population pairs across all populations (among-population air-temperature similarity) ranged from –0.45 to 1.00, and the median value was 0.67. The similarity of air-temperature was smaller in the pairs of lowland and alpine populations (median, –0.08) than in the pairs of lowland populations (median, 0.80) or alpine populations (median,1.00) (Figure 2-8). Furthermore, mantel tests revealed that the similarity of air-temperature was independent of geographic distance between populations at both of the lowland habitat (r = –0.59, P = 1.00) and the alpine habitat (r = –0.78, P = 1.00; Figure 2-9). Thus, the spatial pattern of flowering synchrony strongly reflected that of air-temperature.

2.4 Discussion

The present study revealed that the masting behaviors (i.e., the flowering intervals of individual plants and the masting periodicity of individual populations) were very different between the lowland and alpine populations. Mast flowering was mainly triggered by a cool thermal condition during the growing season two years ago at both of the lowland and alpine habitats. However, the sensitivity to thermal conditions was higher in the lowland populations than in the alpine populations. The degrees of flowering synchrony and the similarity of thermal condition were independent of geographic distance when populations of the same habitat type (lowland or alpine) were compared.

Flowering intervals of individual plants and the masting periodicity of populations were shorter at the alpine habitat than at the lowland habitat (Figures 2-2, 2-3; Appendix 2-1). The mast flowering of alpine populations occurred every other year, while the flowering of individual plants occurred approximately at three-year intervals. However, about 20 % plants had flowering intervals shorter than two-year (Appendix 2-1), and these plants might contribute to the alternate-year masting cycle in the alpine populations. This indicates that the

masting cycle is regulated by the flowering interval of individual plants in the alpine environment.

The coefficient of variation (CV) has often been used as an index of the intensity of masting behavior in individual populations. For instance, Herrera et al. (1998) reported that the CV values of 144 masting species mostly varied between 0.4 and 1.8. In turn, in my study, the CV values of all *V. album* populations were within the range of the masting species, indicating that *V. album* is a typical masting species. Additionally, a study focusing on the intraspecific variation in masting patterns indicated that the large CV in annual flower production was common in populations, where small reproductive outputs lasts several years between the masting events (Crone et al., 2011). Also in the present study, the CV values in the lowland populations (0.91–1.52) were larger than in the alpine populations (0.64–1.06).

Cool thermal conditions during the growing season two years ago was the most important cue of mast flowering at the lowland and alpine habitats. However, the sensitivity to cool conditions clearly differed between the lowland and alpine populations. Masting in the lowland populations was constrained more strictly under warm conditions in comparison with the alpine populations (Figure 2-5), suggesting less opportunity of weather cues in the lowland habitat. Previous studies predicted that if the occurrence of weather cues is less frequent, the masting pattern will be less frequent, more chaotic and synchronized (Crone et al., 2014; Bogdziewicz, 2022). This is consistent with the masting patterns at the lowland habitat, which occurred irregularly at longer intervals.

In contrast, masting events in the alpine populations exhibited an alternative pattern, suggesting that the regulation by weather cues is not intense in cool alpine environments. In the alpine populations, the alternative masting pattern may be constrained only at extreme warmer conditions. It is predicted that if reproductive events of many plants are prevented by some external factors in a given year, resource dynamics used for reproduction become to synchronize among plants within the population, resulting in a synchronous and periodic reproduction (Bogdziewicz et al., 2018). Thus, the alternate-year masting pattern in the alpine population may be regulated by some unpredictable factors, such as extremely warm weather,

that synchronize resource dynamics among plants. Further study is needed to clarify the mechanism of alternate-year masting event in the alpine populations.

In the present study, relatively high flowering synchrony was detected across populations within each habitat type independent of geographic distance. Generally, the geographic patterns of flowering synchrony is regulated by the degree of spatial similarity of weather cues regulating the masting occurrence (LaMontagne et al., 2020; Bogdziewicz et al., 2021). In my study sites, the similarity of thermal conditions was consistently high between sites within the lowland habitat and the alpine habitat, respectively, regardless of the geographic distance. Thus, the mast flowering of *V. album* tends to synchronize throughout the Hokkaido scale, especially for the lowland habitat. In contrast, less flowering synchrony between the lowland and alpine populations may be caused by the habitat specific masting patterns, i.e., unpredictable masting with longer intervals in the lowland populations vs. alternate-year masting in the alpine populations. Because the growing season at the alpine habitat progresses approximately two months later than that at the lowland habitat due to late snowmelt, thermal conditions during the leafing period differed clearly between the habitats, suggesting that the masting trigger acts asynchronously in the lowland and alpine populations. Additionally, previous study supposed that plants may adapt their temperature sensitivity of flowering to each habitat in order to optimize their masting behavior (Kelly et al., 2008). Because the selective pressure acting on masting behavior differed between the lowland and alpine habitats as shown in Chapter 1, temperature sensitivity may also differ between the lowland and alpine populations.

Table 2-1. Descriptions of the study sites in Chapter 2. See Figure 2-1 for the location of each site. CV indicates the coefficient of variation for the proportion of flowering ramets.

No.	Site code	Latitude (N °)	Longitude (E °)	Elevation type	habitat	Elevation (m)	Flowering interval (year)	CV
1	KRM	42.3921	140.1837	Lowland	Understory	62	8.4 ± 3.1	1.22
2	SPD	42.5539	141.0954	Lowland	Understory	504	8.1 ± 2.9	1.25
3	HOK	43.0750	141.1215	Lowland	Understory	8	6.9 ± 1.9	1.01
4	TBH	43.1117	141.2707	Lowland	Understory	5	8.2 ± 1.0	1.28
5	TBA	43.1050	141.2531	Lowland	Understory	4	7.3 ± 1.4	1.23
6	NPO	43.0317	141.3052	Lowland	Understory	65	8.5 ± 1.7	1.50
7	NPT	43.0134	141.3129	Lowland	Understory	89	7.8 ± 2.0	1.25
8	KNN	42.0753	143.545	Lowland	Understory	90	6.2 ± 2.4	1.37
9	OBI	42.5443	143.0933	Lowland	Understory	51	6.0 ± 2.2	1.15
10	OBM	42.5408	143.0840	Lowland	Understory	65	6.4 ± 1.5	1.52
11	ASH	43.4732	142.1816	Lowland	Understory	178	7.0 ± 1.8	1.11
12	MRS	44.0047	143.2108	Lowland	Meadow	184	4.7 ± 2.2	0.91
13	KCR	45.0745	142.2050	Lowland	Meadow	9	5.8 ± 3.1	1.26
14	BNY	45.0900	142.2208	Lowland	Meadow	5	3.5 ± 1.9	0.94
15	TYT	45.0542	141.3754	Lowland	Meadow	8	4.0 ± 2.4	0.98
16	RBN	45.2555	141.0146	Lowland	Meadow	3	5.3 ± 2.6	1.01
17	DCK	43.6106	142.8236	Alpine	Meadow	1363	6.6 ± 3.1	1.02
18	HSG	43.5565	142.8678	Alpine	Meadow	1760	4.8 ± 1.6	0.64
19	MKD	43.5605	142.8763	Alpine	Meadow	1850	4.4 ± 2.2	0.89
20	MHK	43.6583	142.914	Alpine	Meadow	1990	5.4 ± 3.2	0.87
21	MKR	43.6902	142.9104	Alpine	Meadow	1832	4.8 ± 2.5	0.93
22	MTS	43.9625	142.8803	Alpine	Meadow	1396	3.4 ± 1.7	0.93
23	MSK	43.4318	141.3131	Alpine	Meadow	1379	5.0 ± 3.1	1.06

Table 2-2. Summary of the GLMMs for the proportion of flowering ramets (PFR_i) responding to the proportion of flowering ramets in the previous year (PFR_{i-1}), mean air-temperatures two years ago (T_{i-2}) and three years ago (T_{i-3}), and total precipitations two years ago (P_{i-2}) and three years ago (P_{i-3}). The GLMMs were conducted separately for lowland habitat and alpine habitat.

Habitat	Variable	Coefficient	SE	*z* value	*P* value
Lowland	Intercept	–2.248	0.282	–7.974	**> 0.0001**
	PFR_{i-1}	–0.517	0.084	–6.168	**> 0.0001**
	T_{i-2}	–2.706	0.193	–14.011	**> 0.0001**
	T_{i-3}	1.300	0.208	6.245	**> 0.0001**
	P_{i-2}	–0.324	0.097	–3.346	**0.0014**
	P_{i-3}	–0.15	0.092	–1.618	0.1057
Alpine	Intercept	–2.046	0.087	–23.619	**> 0.0001**
	PFR_{i-1}	–0.814	0.099	–8.261	**> 0.0001**
	T_{i-2}	–1.122	0.291	–3.852	**> 0.0001**
	T_{i-3}	0.883	0.276	3.196	**0.0014**
	P_{i-2}	–0.337	0.105	–3.218	**0.0013**

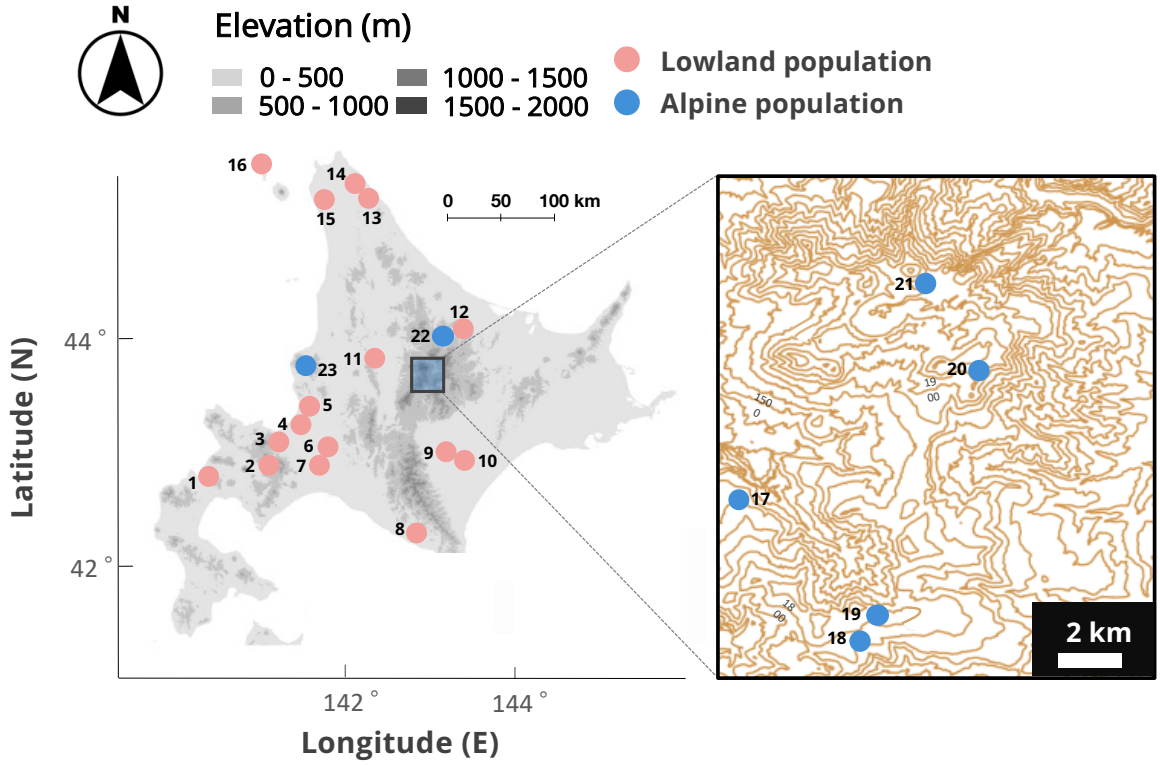


Figure 2-1. Locations of study sites in Chapter 2. The color difference indicates elevation type. Red: lowland population, blue: alpine population. Refer to Table 2-1 for details of the sites.

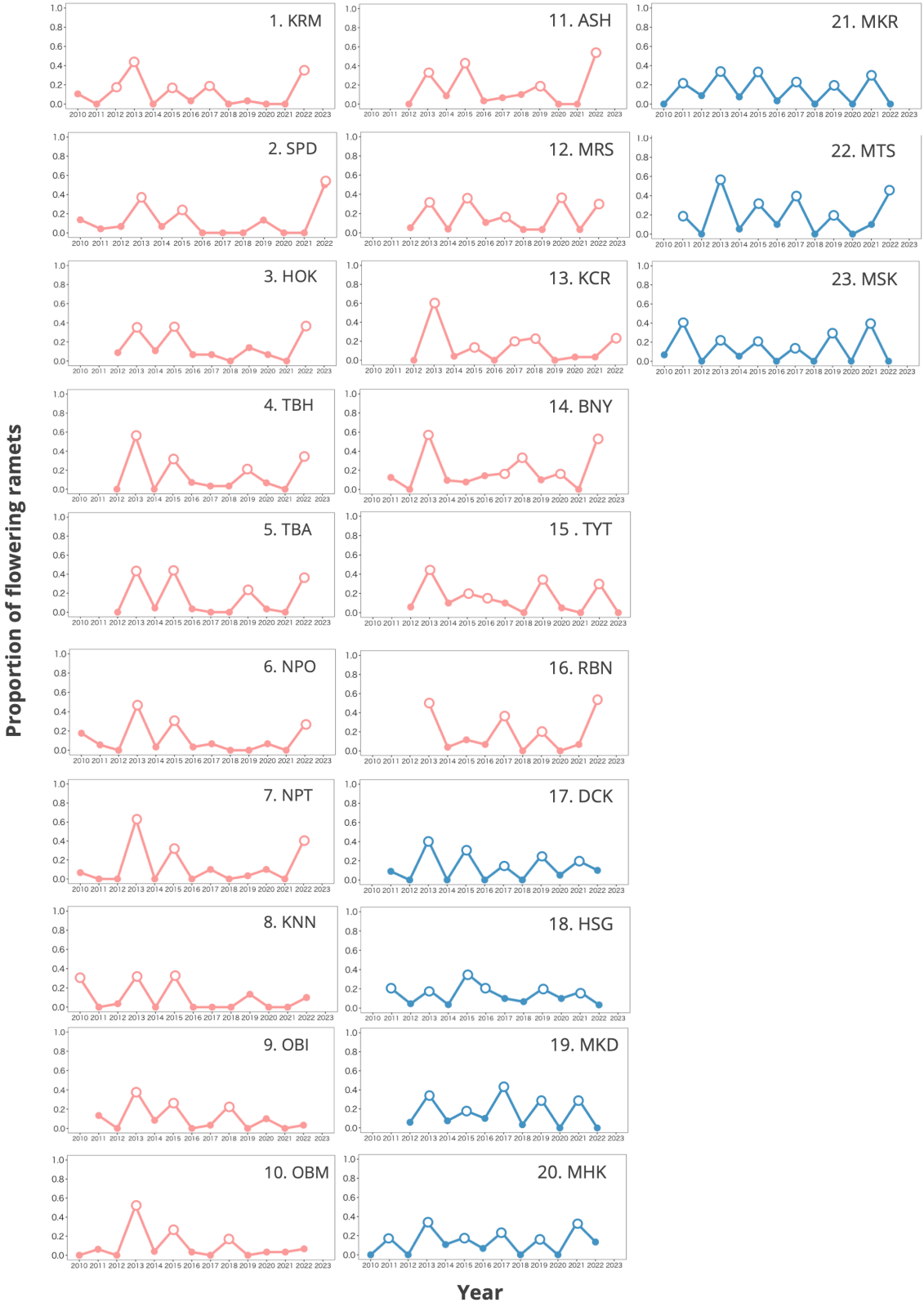


Figure 2-2. Annual fluctuation of the proportion of flowering plants in each population. The occurrence of flowering in individual plants was measured using annual growth scars on rhizomes. Open circles indicate the masting year (> 15% of individuals flowering). Red: lowland populations; blue: alpine populations. Table 2-1 lists the site code.

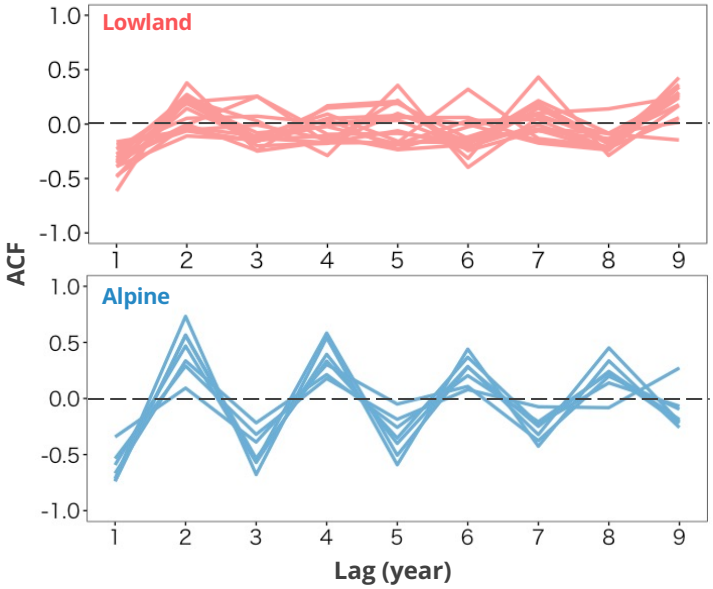


Figure 2-3. Autocorrelation function (ACF) of the time-series data for individual populations. Each line represents each population. Results for the lowland and alpine habitats are illustrated separately.

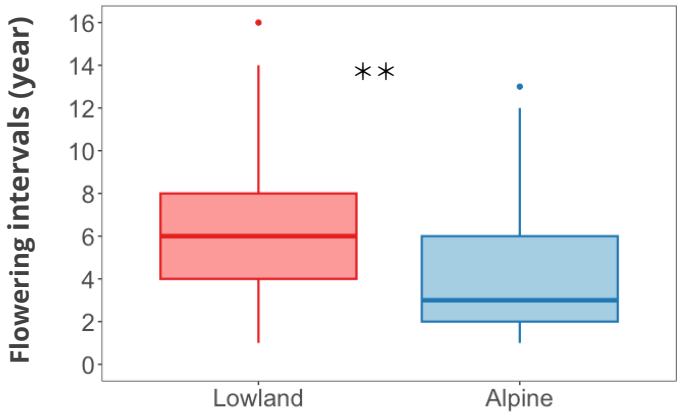


Figure 2-4. Comparisons of flowering intervals (year) of individual plants between the lowland (red) and alpine habitats (blue). ** $P < 0.01$

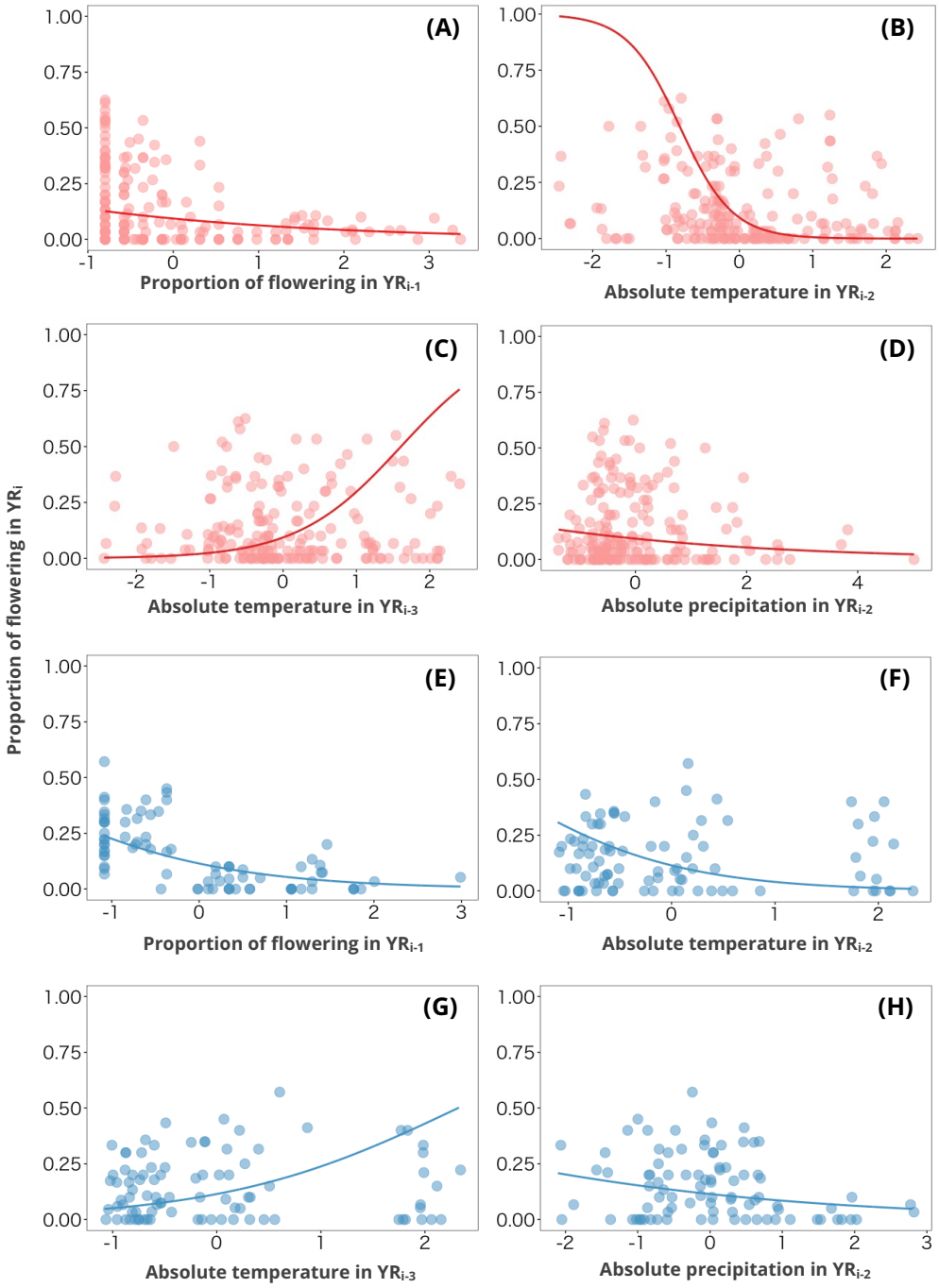


Figure 2-5. Relationships between the proportion of flowering ramets and the variables that had a significant correlation with the flowering abundance at each habitat. At the lowland habitat (red points and lines), (A) flowering abundance in the previous year, (B) mean air-temperature two years ago, (C) mean air-temperature three years ago, and (D) total precipitation three years ago. At the alpine habitat (blue points and lines), (E) flowering abundance in the previous year, (F) mean air-temperature two years ago, (G) mean air-temperature three years ago, and (H) total precipitation two years ago. All explanatory variables were standardized to be a mean of zero and a standard deviation of 1. Lines indicate the relationships predicted by GLMMs (see Table 2-2 for details).

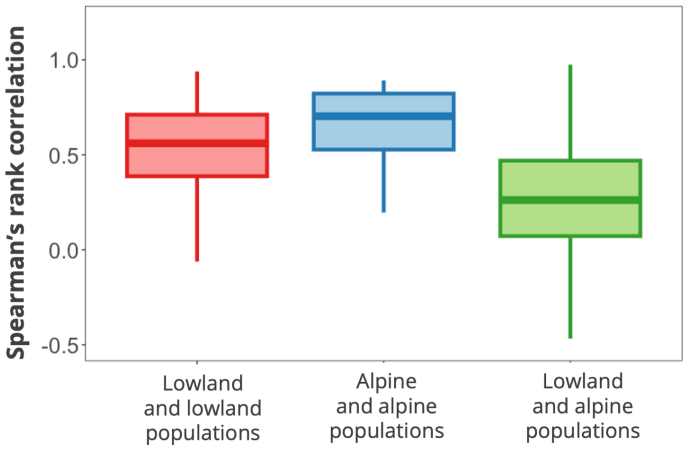


Figure 2-6. Comparison of the Spearman's rank correlation of flowering synchrony among three categories based on the elevation habitats, i.e., the pairs between lowland populations, between alpine populations, and between lowland and alpine populations.

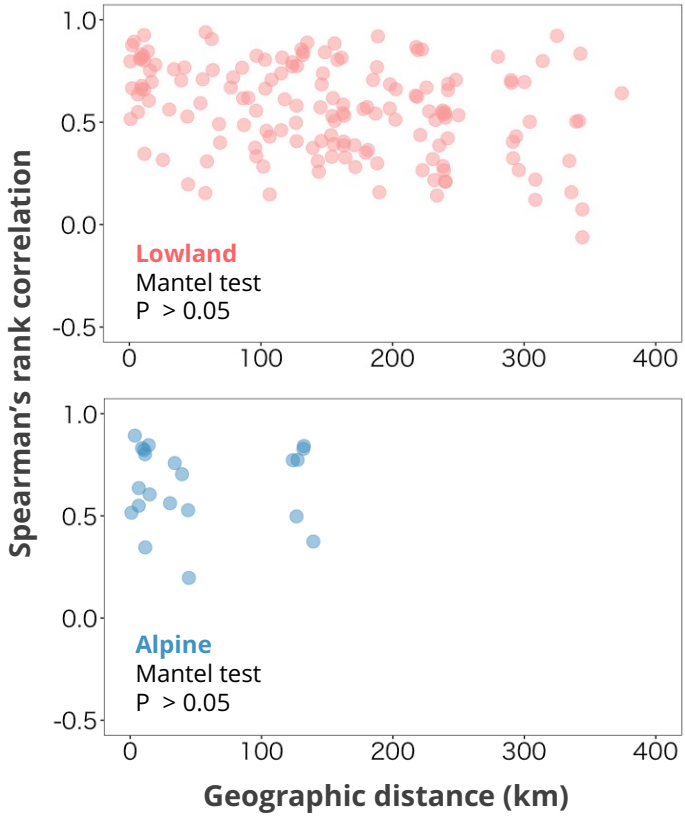


Figure 2-7. Relationships between geographic distance and pairwise Spearman's rank correlation of flowering synchrony at the lowland habitat (red) and the alpine habitat (blue). No significant correlations were found by Mantel test at both habitats.

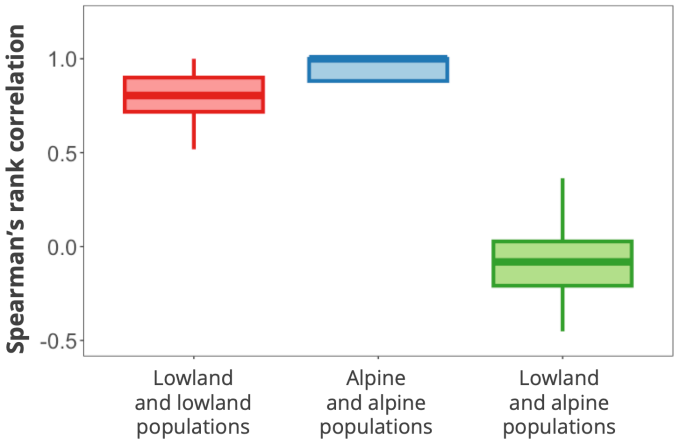


Figure 2-8. Comparison of the Spearman's rank correlations of air-temperatures among three categories based on the elevation habitats, i.e., the pairs between lowland populations, between alpine populations, and between lowland and alpine populations.

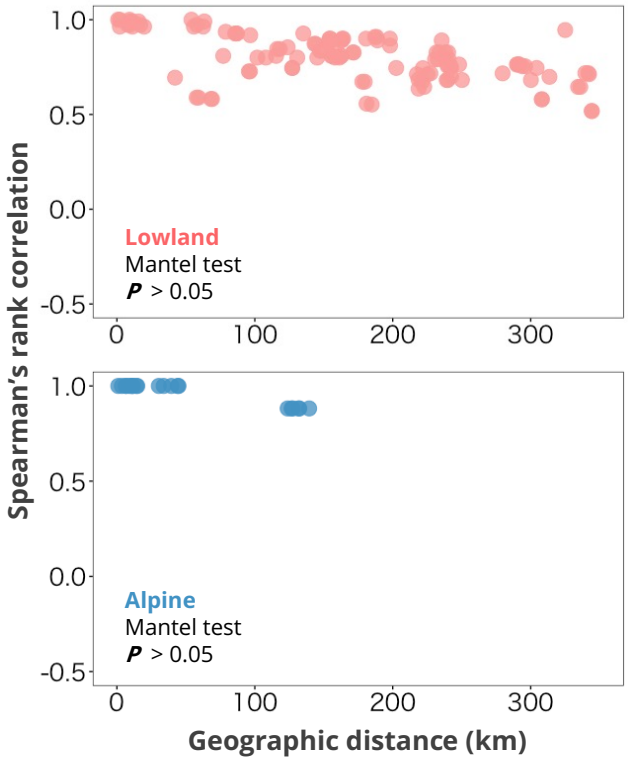
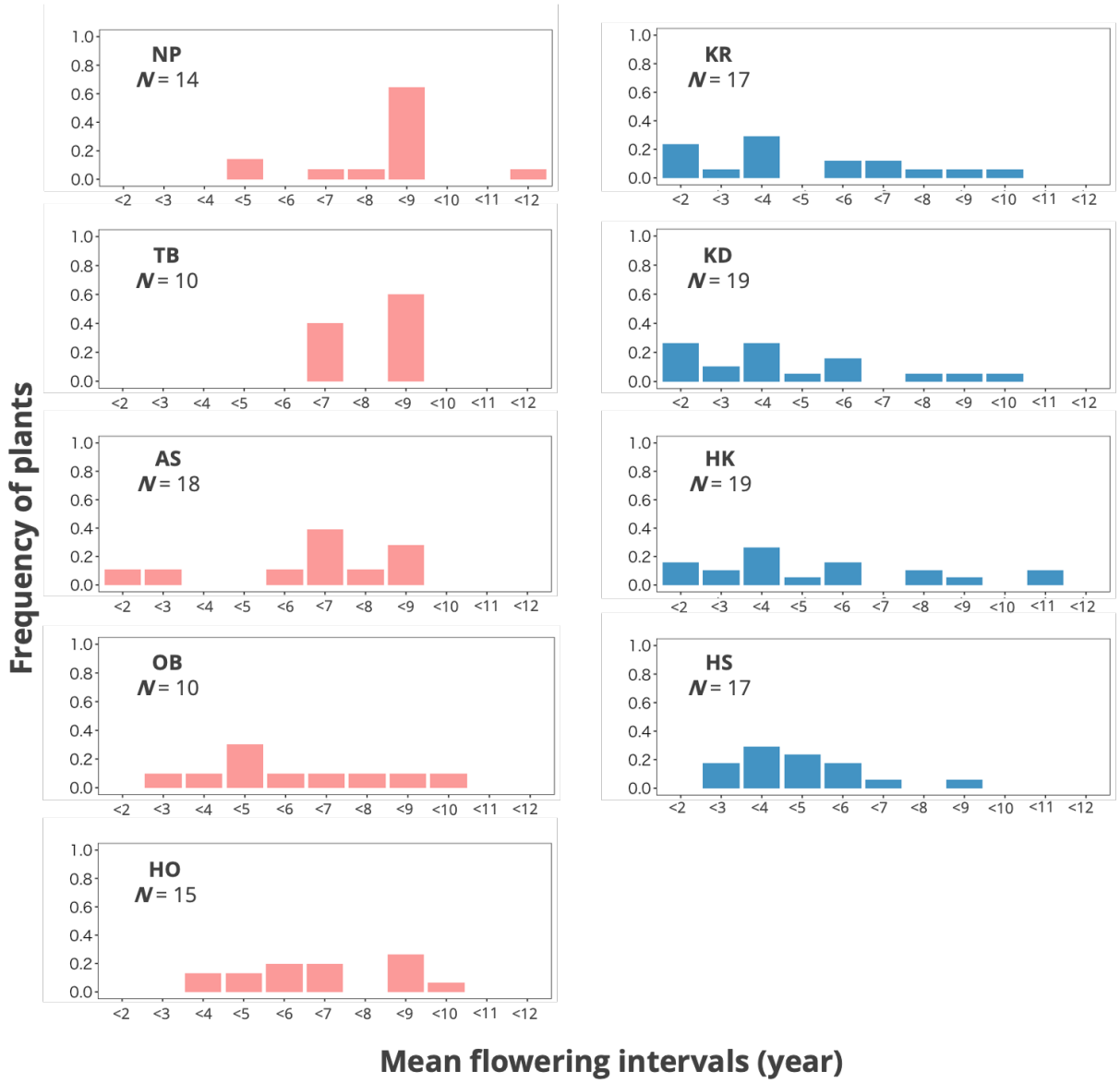


Figure 2-9. Relationships between geographic distance and pairwise Spearman's rank correlation of air-temperatures at the lowland habitat (red) and the alpine habitat (blue). No significant correlations were found by Mantel test at both habitats.

Appendix 2-1. Frequency of mean flowering intervals within plants in each population. Red: lowland population; Blue: alpine population. Refer to Table 1 for the site code.



Chapter 3: Physiological mechanism determining masting intervals: a test of the resource budget hypothesis

3.1 Introduction

The periodicity of masting may affect the population and community dynamics of various animal species in the terrestrial ecosystems (Satake et al., 2004; Touzot et al. 2020). For instance, infrequent masting events can decrease the numbers of flower and seed consumers, extensively (Ostfeld and Keesing, 2000; Koenig and Knops, 2005; Inari et al., 2012). The masting periodicity is often explained by the frequency of weather cues (Crone et al., 2014; Bogdziewicz, 2022). However, temporal patterns of seed production are often infrequent and highly variable more than the pattern of weather cues, suggesting that the periodicity of masting events cannot be explained only by the weather cue hypothesis (Kelly and Sork, 2002). As the physiological mechanisms regulating masting events, the resource budget hypothesis has been studied (Isagi et al., 1997; Satake and Iwasa, 2000; Rees et al., 2002; Crone and Rapp, 2014). It predicts that if a plant invests excess resource for a single reproductive event, i.e., more than the resource level gained during one growing season, next reproductive event will not occur until resource recovery exceeds a threshold level, resulting in an intermittent mass production of flowers and/or fruits (Crone and Rapp, 2014). For a comprehensive understanding of the proximate mechanisms of masting periodicity, therefore, it is necessary to test not only the weather cue hypothesis but also the resource budget hypothesis.

Among studies testing the resource budget hypothesis, several studies reported the importance of nitrogen as a limiting resource for masting event in tree species, such as, *Fagus crenata*, *Nothofagus solandri*, and *Pinus albicaulis* (Han et al., 2008; Smaill et al., 2011; Sala et al., 2012). In contrast, there is only one study indicating the importance of carbon storage in a herbaceous species, *Astragalus scaphoides* (Crone et al., 2009). Although the studies of resource budget in herbaceous plants are limited, carbon storage may be crucial in

herbaceous species because of their smaller storage function in comparison with tree species (Han and Kabeya, 2017). Furthermore, relative importance of carbon budget may vary among populations of single species depending on the growing habitats. Therefore, the contribution of carbon budget to masting periodicity needs to be evaluated at an inter-population scale in herbaceous species.

Chapter 2 revealed that the flowering intervals of *V. album* were significantly shorter in the alpine populations than in the lowland populations, and this difference was related to the sensitivity to cool temperature cues specific to the habitat type. However, the masting cues sometimes occurred at consecutive years (Appendix 3-1), whereas flowering events at plant level consistently occurred at intervals longer than one year (Figure 2-4). This suggests that flowering intervals of individual plants is determined not only by the weather cue but also by the internal resource conditions.

The resource budget hypothesis predicts that the intraspecific variation in masting intervals is related to the productivity and the resource requirement for reproduction of individual populations, namely, (1) the recovery of resource level after reproduction is faster in the populations with high productivity, resulting in a shorter masting interval, and (2) the recovery of resource level takes longer time in the populations with a high threshold level of resource requirement for reproduction, resulting in a longer masting interval (Satake and Bjørnstad, 2008). These predictions may explain the different flowering intervals between the lowland and alpine habitats in *V. album*. Plants growing at higher elevations tend to be smaller in size at a flowering stage in comparison with the conspecific plants growing at lower elevations (Giménez-Benavides et al., 2007; Takahashi and Matsuki, 2017). Because reproductive plant size is positively correlated to flower and seed production in *Veratrum* species (Liao et al., 2006; Hesse et al., 2008), absolute reproductive investment in *V. album* may be smaller in alpine populations in comparison with lowland populations. Additionally, as plants grow under brighter conditions in alpine meadows than in understory of forests, plants growing in alpine meadows may acquire larger amounts of photosynthetic products during a growing season than plants growing in understory. If alpine populations can

accumulate carbon more efficiently and invest smaller amounts of resources in a single reproductive event than understory populations, the recovery of resource level after reproduction should be faster, resulting in a shorter masting interval in alpine populations.

Chapter 3 aims to test whether carbon budget balance can explain the variation in masting intervals between *V. album* populations locating at different elevations. First, I analyzed the adequacy of the resource budget hypothesis in this species using a long-term record of plant size and presence or absence of flowering. Then, I examined the key resources limiting the reproduction (carbon or nitrogen) in this species. Subsequently, I compared dry-weight allocation to reproduction and annual carbon fixation between lowland and alpine populations. As this species is an andromonoecious herb in which inflorescences are composed of perfect flowers and male flowers, it may be possible to regulate resource investment in fruit production by changing the proportion of perfect and male flowers (Spalik, 1991; Liao et al., 2006), in which case, resource-limited populations may have longer masting intervals and/or male-biased floral sex expression. To test this hypothesis, I compared the numbers of perfect and male flowers and the size dependency of perfect flowers between lowland and alpine habitats. Finally, I discussed whether the variation in flowering intervals among populations can be explained by the carbon balance between single reproductive investment and annual carbon fixation.

3.2 Methods

Size and flowering occurrence in plants

It is difficult to quantify the interannual variation in resource storage of plants by a non-destructive manner. Thus, I used stem volume as a substitute of resource levels, which is employed in several studies (e.g., Shibata and Kudo, 2016). In the masting year of 2013, 52 flowering *V. album* were tagged in NP site, and they were recorded every year for the presence or absence of flowering and stem volume (maximum height × basal diameter^2) during 2013–2023.

Resource currency of reproduction

I collected eight plants in each flowering and non-flowering in NP site in early June 2022 (a pre-flowering period). All plants were sampled, separated into above- (leaf, leaf sheath, floral-stem, flower) and belowground organs (rhizome, root), and dry weights were measured after drying for 48 h at 80°C. Then, these samples were completely ground into powdered state. Sugar and starch concentrations of the above- and belowground organs were analyzed following the procedure described by Kabeya et al. (2021). In brief, each sample was dissolved in water, then extracted three times with 80% ethanol. In the resulting solutions, ethanol was evaporated off, and the precipitate was redissolved in deionized water. In that resulting solution, soluble sugars were measured using the phenol-sulfuric acid method. In turn, for extracting starch, the precipitate from the ethanol extractions was boiled with 500µl KOH for 30 minutes, then it was neutralized with 200µl CH_3COOH. Starch in the solutions was hydrolyzed into glucose for 30 min at 55 °C by adding amyloglucosidase, then glucose content was measured using a glucose-peroxidase testing kit (glucose C-II test, Wako corp., Japan). Lastly, nonstructural carbohydrate (NSC) concentrations were calculated by adding sugar and starch concentrations. Furthermore, the powder samples were sent to the laboratory, Creative Research Institution of Hokkaido University, and total carbon and nitrogen concentrations were measured using a CHN analyzer (Model 932; LECO instruments, St Joseph, MI, USA). Total carbon, nitrogen, and NSC dry weights on above- and belowground organs of flowering and non-flowering plants were calculated by multiplying the concentrations by total dry weights.

Reproductive investment

To compare the reproductive investment between the lowland and alpine habitats, I estimated dry weights of floral stem, flowers, and fruits per plant using data of the reproductive performance within the 5-m × 1-m plots in 11 sites (five lowland populations and six alpine populations, see Table 0-1). To evaluate the potential reproductive performance, plants damaged by mechanical accident or herbivore attack were excluded from the measurements.

As mentioned in Chapter 1, abundance of flowering ramets varied greatly between years in most of the study sites. Furthermore, the numbers of flowers and fruits also varied between years depending on flowering abundance within populations. Therefore, I used the data of high-flowering years (the proportion of flowering ramets was > 15 %) from each site to compare the reproductive investment between habitat types (Figure 1-1).

To quantify individual flower mass and fruit mass, I randomly harvested 50 flowers and fruits outside the plots in TB site (as a representative of lowland habitat) and HS site (as a representative of alpine habitat) and measured their dry weights. Using the mean values of individual flowers and fruits, total flower mass and total fruit mass per plant were estimated by multiplying the mean values by the number of flowers and fruits in each case. To estimate a floral stem mass, I randomly harvested 10 floral stems at the same sites soon after fruit maturation and measured stem volume ($d^2 \times h$ cm^3) and dry weight. Floral stem mass of individual ramets within the plots was calculated using the correlation between floral stem mass and volume at each habitat (Appendix 3-2).

Photosynthetic capacity and carbon fixation

To compare annual carbon fixation between the lowland and alpine habitats, I measured the photosynthetic capacity of non-flowering plants (in a non-masting year), light availability throughout the leafing period, and total leaf area per plant in 2021. I measured the light responses of photosynthetic rates per unit leaf area in NP site as a representative of lowland habitat. Measurement of photosynthetic rates at the alpine habitat were conducted in SG site on Mt. Asahidake (43.66122° N, 142.8262° E, 1620 m of elevation), located close to MA site due to easy access to the alpine zone using a gondola cable. The measurements at the lowland and alpine sites were conducted three times throughout the leafing period: soon after the completion of leaf development (early May at the lowland site and early July at the alpine site), at the middle stage (late May at the lowland site and late July at the alpine site), and immediately before leaf senescence (early June at the lowland site and early August at the alpine site). Photosynthetic rates were measured in three randomly selected individuals, using

a portable LI–6400 photosynthesis system (Li-Cor, Lincoln, NE, USA). Ten light levels (2,000, 1,500, 1,000, 800, 500, 300, 100, 50, 10, and 0 μmol·m^{-2}·s^{-1}) of photosynthetically-active radiation were irradiated using a red-blue LED light source. The humidity of the incoming air during the measurement was maintained such that the vapor pressure deficit (kPa) was 1.1; leaf temperature was set at 15°C, and ambient CO_2 concentration at 380 μmol·m^{-2}. Net photosynthetic rate per unit area as a function of photon irradiance (I, mol·m^{-2}·s^{-1}) normally follows the shape of a non-rectangular hyperbola, as indicated by the following equation:

$$P_{area} = \frac{\alpha I + P_{max} - \sqrt{(\alpha I + P_{max})^2 - 4 \alpha I \theta P_{max}}}{2\theta} - R_d \qquad \text{Eq. (1)}$$

where, P_{max} is a light-saturated photosynthetic rate per unit area (μmol·m^{-2}·s^{-1}), α is the initial slope (μmol·m^{-2}·s^{-1}), θ is the degree of curvature, and R_d is the dark respiration rate (μmol·m^{-2}·s^{-1}; Marshall and Biscoe 1980). The data obtained from individual plants were fitted into this equation. The mean values of the individual parameters obtained for each population were used as a representative of plant photosynthetic capacity. Photosynthetic photon flux density (PPFD, μmol·m^{-2}·s^{-1}) at a height of 2 m above the ground was recorded in NP site (as a representative of lowland habitat) and HS site (as a representative of alpine habitat), at 1-min intervals using a data logger connected to a solar radiation sensor (S-LIA-M003; Onset Computer Corp., Bourne, MA, USA) during the growing period. The PPFD data recorded at one-hour intervals throughout the leafing period were used for the estimation. To estimate the leaf area (m^2) of non-flowering ramets in the plots, we measured the total leaf area per plant and stem volume for 15 and 18 non-flowering ramets selected from outside the plots in NP and HS site, respectively. Using the correlations between total leaf area and stem volume at each habitat (Appendix 3-3), I calculated the total leaf area of the non-flowering plants within the 5-m × 1-m plots.

Daily CO_2 fixation per unit area (μmol·day^{-1}·m^{-2}) was expressed as a sum of hourly photosynthetic rates, which were calculated for each of the lowland and alpine populations by

Eq. (1), using hourly mean PPFD and photosynthetic parameters throughout the leafing period. Then, annual carbon fixation per unit area (g $C \cdot m^{-2}$) was estimated by adding the daily CO_2 fixation throughout the leafing period. Lastly, annual carbon fixation per plant was estimated by multiplying the annual carbon fixation per unit area (g $C \cdot m^{-2}$) by total leaf area (m^2) of plant in each population. In most plant tissues, the energy efficiency of conversion of glucose or sucrose into the different cell components is assumed to be approximately 70% (Poorter and Villar,1997). Thus, the amount of actual available carbon fixation was calculated as 70% of the total amount of photosynthetically fixed carbon (g C) per year.

Because photosynthetic rate is influenced by ambient temperature (Berry and Björkman, 1980), consideration of both light and temperature conditions is preferable for an accurate estimation of annual carbon fixation. However, it was difficult to measure the photosynthetic response to ambient temperature in the field, especially in alpine sites, because of the unstable weather condition and no power-supply facility. Instead, I compared the daily-mean temperature during the leafing period between the lowland and alpine habitat using the data of air-temperature recorded during the five years (2017–2021) in NP and HS sites, respectively (G. Kudo, unpublished data; see Figure 3-6B).

Statistical analysis

To test the resource budget hypothesis, I postulated a following procedure; (1) stem volume, that is a substitute of resource level, will be minimum in the following year of flowering, (2) stem volume will gradually increase from year to year after flowering, and (3) next flowering event will occur when stem volume attains at a certain threshold level. I constructed a GLMM postulating a gamma error distribution to explain this procedure, in which log-transformed stem volume was the response variable, current year flowering, last year flowering, and passed years after flowering were the explanatory variables, and plant ID was set as the random variable.

To identify the essential resource for reproduction, i.e., carbon or nitrogen, I predicted that resource concentration and mass of aboveground organs will be larger in flowering

plants than in non-flowering plants, whereas resource concentration and mass of belowground organs will be smaller in flowering plants than in non-flowering plants. Thus, I compared the concentration and mass of carbon and nitrogen in aboveground and belowground organs between flowering and non-flowering plants. Because carbon resource for reproduction is stored in rhizomes as NSC, I compared NSC contents for the belowground organs. Resource investments in aboveground organs were compared using GLMs postulating a gamma error distribution, in which each of the concentration and mass of total carbon or total nitrogen was the response variable, respectively, and the presence or absence of flowering was the explanatory variable. The same analyses were performed also for the comparison of belowground organs between flowering and non-flowering plants in which the concentration and mass of NSC were used instead of total carbon.

Reproductive investments, i.e., flower, fruit, and floral-stem production, were compared between the lowland and alpine habitats using GLMMs postulating a gamma error distribution, in which each of flower mass, fruit mass, floral-stem mass, and total mass of reproductive organs was the response variable, respectively, habitat type was the explanatory variable, and site ID nested by plot ID was set as the random factor. To examine the differences in floral sexual allocation between the lowland and alpine habitats, the numbers of perfect and male flowers, and the proportion of perfect flowers were compared using GLMMs. In the GLMMs for flower number postulating a Poisson error distribution, perfect or male flower number was the response variable, habitat type was the explanatory variable, and site ID nested by plot ID was set as the random factor. In the GLMM for floral sex expression postulating a binomial error distribution, the ratio of perfect flowers to male flowers was the response variable, habitat type was the explanatory variable, and site ID nested by plot ID was set as the random factor. The size dependency of perfect-flower production, which is a potential investment in fruit production, was analyzed using a GLMM postulating a Poisson error distribution, in which the number of perfect flowers was the response variable, log-transformed stem volume (cm^3), habitat type, and their interaction were the explanatory variables, and site ID nested by plot ID was set as the random factor.

Seasonal pattern of photosynthetic capacities was compared between the lowland and alpine habitats using a GLM postulating a gamma error distribution, in which the light-satiated photosynthetic rate (P_{max}) was the response variable, seasonal stage (early, middle, and late), habitat type, and their interaction were set as the explanatory variables. Total leaf area per plant was compared between the lowland and alpine habitats using a GLMM postulating a gamma error distribution, in which leaf area (m^2) was the response variable, habitat type was the explanatory variable, and site ID nested by plot ID was set as the random factor. The same analysis was performed for the comparison of annual carbon fixation (g C) between the lowland and alpine habitats. For each GLM or GLMM, a best-fit model was selected based on the lowest AIC value. All statistical analyses were performed using R version 3.6.1 (R Core Team, 2019).

3.3 Results

Interannual variation in plant size

Table 3-1 summarized the result of the GLMM regarding interannual variations in plant size. Stem volume, as a substitute for resource level, declined sharply in the following year of reproduction (Figure 3-1). Then, it gradually recovered from year to year, and next flowering occurred at a large size (Figure 3-1). These results support the adequacy of the resource budget hypothesis in this species.

Currency of the cost of reproduction

The concentration and mass of total carbon in aboveground parts were larger, and those of NSC in belowground organs were smaller in flowering plants compared to non-flowering plants (Figure 3-2), indicating that stored carbon was depleted greatly by reproduction. In contrast, the mass of total nitrogen in aboveground organs was larger in flowering plants probably due to larger plant size, while nitrogen concentration was greater in non-flowering plants (Figure 3-3). Furthermore, the concentration and mass of total nitrogen in belowground organs did not differ between the flowering and non-flowering plants (Figure 3-

3). These results indicate that nitrogen is not a limiting resource for reproduction. Thus, carbon resource is an important currency limiting the masting occurrence in this species.

Reproductive investment at the lowland and alpine habitats

Individual dry weight of flowers was 7.2 ± 3.7 mg and 7.3 ± 3.3 mg in the lowland (TB) and alpine (HS) populations, respectively, and individual dry weight of fruits was 56.0 ± 21.4 mg and 53.1 ± 16.1 mg, respectively. Floral stem mass and total flower mass per plant were significantly greater at the lowland habitat than at the alpine habitat (Figure 3-4A,B), whereas total fruit mass per plant did not differ significantly between the habitats (Figure 3-4C). Total reproductive investment (total mass of reproductive organs per year in flowering plants) was approximately 1.7 times larger at the lowland habitat than at the alpine habitat (Figure 3-4D). Additionally, there was no significant difference in the number of perfect flowers per plant between the habitats (Figure 3-5A), whereas the number of male flowers per plant was significantly larger at the lowland habitat than at the alpine habitat (Figure 3-5B). As a result, the proportion of perfect flowers was higher at the alpine habitat than at the lowland habitat (Figure 3-5C). The number of perfect flowers increased with stem volume (i.e., plant size), and the alpine populations tended to produce more perfect flowers at smaller size than the lowland populations (Table 3-2; Figure 3-5D).

Carbon fixation at the lowland and alpine habitats

The seasonal transition of air-temperature during the leafing period differed between NP (lowland) and HS (alpine) sites (Figure 3-6B). In the lowland site, daily-mean temperature was 10.4°C in the early season (April 28–May 14), increased gradually to 13.6°C in the middle season (May 15–May 31), and attained at 15.5°C in the late season (June 1–16). In the alpine site, it was 12.5°C in the early season (July 5–18) and increased to 14.8°C in the middle season (July 19–August 1), then decreased to12.6°C in the late season (August 2–15). Thus, plants growing at the alpine habitat experienced warmer conditions during the early to

middle season than plants growing at the lowland habitat despite of the higher elevation due to later growth initiation.

Seasonal patterns of daily-maximum light intensity during the leafing period were different between the habitats (Figure 3-6B). Light intensity in the lowland site fluctuated in the range of 500–1000 μmol·m^{-2}·s^{-1} in the early season and decreased rapidly due to the progress of overstory canopy closure. On the other hand, the light intensity in the alpine site fluctuated in the range of 1000–2000 μmol·m^{-2}·s^{-1} throughout the leafing period. Thus, cumulative light intensity throughout the leafing period was larger at the alpine habitat than at the lowland habitat.

The light-saturated photosynthetic rates (P_{max}) at the lowland and alpine habitats were 11.61 and 14.36 μmol·m^{-2}·s^{-1} in the early season, 10.05 and 16.94 μmol·m^{-2}·s^{-1} in the middle season, and 5.07 and 9.20 μmol·m^{-2}·s^{-1} in the late season, respectively (Figure 3-6A, Appendix 3-4). The P_{max} value was significantly larger at the alpine habitat than at the lowland habitat, and it decreased in the late stage at both habitats (Table 3-3). Although total leaf area per plant was significantly larger at the lowland habitat (Figure 3-7A), annual carbon fixation was greater at the alpine habitat (Figure 3-7B). As a result, mean annual carbon fixation at the alpine habitat (17.2 g C) was approximately 1.5 times larger than that at the lowland habitat (11.5 g C).

3.4 Discussion

The present chapter demonstrated the importance of carbon as a currency of the cost of reproduction. The alpine population enabled shorter masting intervals because of smaller reproductive investments and greater carbon fixation in comparison with the lowland populations. These results strongly support the resource budget hypothesis based on the carbon balance. As predicted, plant size of *V. album* declined greatly after reproduction, and next flowering did not occur until plant size exceeds a threshold level. Many perennial plants changes the stage class from vegetative stage (absence of reproduction) to reproductive stage depending on the plant size (Kachi and Hirose, 1985). Once becoming a reproductive stage,

some plants continue to flower every year, whereas others regress to growth stage in the following year of reproduction. The life history of *V. album* is consistent with the latter case, resulting in the intermittent flowering occurrence. This is because carbon storage is intensively depleted by a large reproductive investment. The largest fraction of carbon on aboveground parts was used as the structural carbohydrate (approximately 80 % of total carbon mass; Appendix 3-5). As dry weight allocation was the largest in floral stems (Figure 3-4), carbon depletion after reproduction may be caused by the great investment in the supporting organ of floral stems.

In contrast, nitrogen budget was independent of masting occurrence. Because *V. album* commonly grows in the environments with moist and fertile soil conditions and has a developed rhizome system, the nitrogen limitation may not be serious in comparison with the carbon limitation, especially in the lowland forests, where the shading by canopy trees restricts the photosynthesis of understory vegetation. However, the present study analyzed the amount of nitrogen resource only during a pre-flowering period, but not during post-fruiting periods. Because several studies reported that nitrogen storage depleted after seed production (e.g., Sala et al., 2012; Han et al., 2014), further studies on nitrogen costs of seed production are necessary.

The total reproductive investment was larger in the lowland populations than in the alpine populations because of the larger floral-stem production (Figure 3-4A, B). Plants having large inflorescences often attract numerous pollinators, resulting in larger success of male as a pollen donor and female as seed producer (O'Connell and Johnston, 1998; Gómez, 2003; Sletvold and Ågren, 2010; Diniz et al., 2019). As shown in Chapter 1, fruit-set success in the lowland populations was considerably lower than that in the alpine populations due to low frequency of pollinator visits. Thus, taller floral stems producing many flowers may be advantageous at the lowland habitat in terms of pollinator attraction.

Although total flower production was larger in the lowland populations (Figure 3-4B), the number of perfect flowers per plant did not differ significantly between the lowland and alpine habitats (Figure 3-5A), resulting in a similar level of fruit production in both of the

lowland and alpine populations (Figure 3-4C). Although the number of perfect flowers increased with plant size in every population, furthermore, the alpine populations produced more perfect flowers in small-sized plants than the lowland populations (Table 3-2, Figure 3-5D). These results suggest that individuals at the alpine habitat preferentially invested the available resources in perfect-flower production rather than male-flower production, enabling fruit production similar to that at the lowland habitat.

The number of male flowers was considerably larger in the lowland populations (Figure 3-5B), resulting in a male-biased flower production. Male-biased resource allocation may be a strategy for pollinator attraction because plants with numerous flowers often have higher pollination success owing to the attraction of many pollinators (Chen and Zhao, 2019). As the cost of male-flower production is often smaller than that of perfect-flower production in animal-pollinated plants (Solomon, 1986; Vallejo-Marín and Rausher, 2007), higher resource allocation to male flowers may enable an increase in total flower production per plant. Under severe pollen limitations, male-biased resource allocation may be beneficial for pollination success. Furthermore, male-biased flower production may contribute to a decreased in the total cost of reproduction (Dorken and Barrett, 2004; Han et al., 2011; Zhang et al., 2014). Because the lowland populations had smaller carbon fixation compared with the alpine populations, they may invest more resources in male-flower production (Figure 3-5B).

Higher photosynthetic capacity under bright conditions resulted in 1.5 times larger carbon fixation at the alpine havitat than at the lowland habitat regardless of the smaller leaf area and shorter growth period. Previous studies have reported that the photosynthetic capacity of *V. album* inhabiting the understory of a deciduous forest decreases rapidly with canopy closure (Tani and Kudo, 2006), as observed in this study. Thus, the major photosynthetic period of the lowland habitat is determined by the time of canopy closure. The alpine population showed the maximum P_{max} values in the middle season under warm conditions, whereas the maximum P_{max} values of the lowland populations appeared early in the season under cool conditions. Thus, there is a trade-off between bright period and warm period at the lowland habitat.

Although the present study focused on annual carbon fixation during the leafing period, respiratory loss during the non-leafing period might affect the seasonal dynamics of carbon storage. In *Veratrum* species growing in western Europe, stored carbon in rhizomes declined considerably during the non-leafing season, especially in autumn, owing to high respiration rates under warm conditions (Kleijn et al., 2005). As leaf senescence occurs in late June at the lowland habitat, there is no photosynthetic carbon gain during the large part of summer. In contrast, the alpine habitat was covered with snow during the most of the non-leafing period, during which soil temperature remained close to 0°C. Thus, carbon decline due to respiratory loss should be significantly greater in the lowland populations, resulting in slower accumulation of carbon resources.

A decrease in annual productivity with increasing elevations is reported in some masting plants, such as *Chionochloa* and *Nothofagus* species (Kelly and Sork, 2002; Richardson et al., 2005). However, the decreasing carbon gain along the elevation gradient is not a consistent pattern in mountain ecosystems (Körner, 2003). For instance, studies of interspecific comparisons of herbaceous species reported that there were no significant differences in the photosynthetic capacities between different elevations or even higher photosynthetic capacity at higher elevation (Körner and Diemer, 1987; Diemer and Körner, 1996). This is consistent with my result, i.e., a summer green herb *V. album* exhibited a greater photosynthetic capacity and annual carbon fixation at the alpine habitat than at the lowland habitat.

In conclusion, shorter masting intervals in the alpine populations can be explained by habitat-specific carbon budget balances. Plants growing at alpine habitat invested smaller amount of carbon resource in a single reproductive event and fixed more carbon by annual photosynthesis than plants growing at lowland habitat, resulting in a faster recovery from the previous reproductive event.

Table 3-1. Summary of the GLMM for the changes in stem volume (as a substitute for resource level) in response to years passed after flowering, current-year flowering, and last year flowering.

Variable	Coefficient	SE	*z* value	*P* value
Intercept	4.111	0.104	39.708	**> 0.0001**
Years after flowering	0.085	0.007	11.619	**> 0.0001**
Current-year flowering	2.226	0.068	32.794	**> 0.0001**
Last year flowering	–0.308	0.063	–4.881	**> 0.0001**

Table 3-2. Summary of the GLMM for the number of perfect flowers in response to stem volume (as a representative of plant size), habitat type (lowland, alpine) and their interaction. A result of the best-fit model by AIC is shown.

Variable	Coefficient	SE	*z* value	*P* value
Intercept (lowland)	1.118	0.379	2.952	**0.003**
ln (stem volume)	0.644	0.0593	10.861	**> 0.0001**
Elevation (alpine)	0.230	0.113	2.027	**0.0426**

Table 3-3. Summary of the GLM for the light-saturated photosynthetic rate (P_{max}) in response to seasonal stage (early, middle, late), habitat type (lowland, alpine) and their interaction. A result of the best-fit model by AIC is shown.

Variable	Coefficient	SE	*z* value	*P* value
Intercept	2.249	0.128	17.577	**> 0.0001**
Elevation (alpine)	0.354	0.128	2.766	**0.015**
Seasonal stage (middle)	0.025	0.157	0.161	0.875
Seasonal stage (late)	–0.734	0.157	–4.685	**> 0.0001**

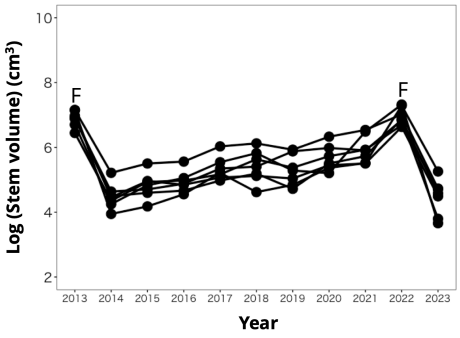


Figure 3-1. Interannual patterns of log-transformed stem volumes (cm^3) in the tagged plants in NP population. To visualize the size variation between flowering events, this figure indicates only plants that flowered in both 2013 and 2022. Each line represents each plants. F indicates flowering years.

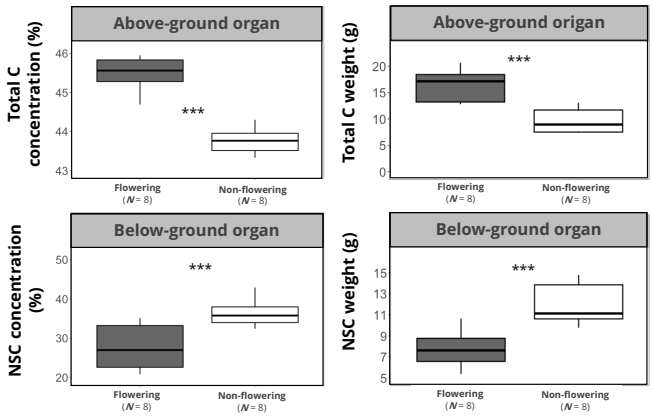


Figure 3-2. Comparisons of the concentration and mass (g) of total carbon and nonstructural carbohydrate (NSC) between flowering (black) and non-flowering plants (white) in aboveground and belowground organs. *** $P < 0.001$

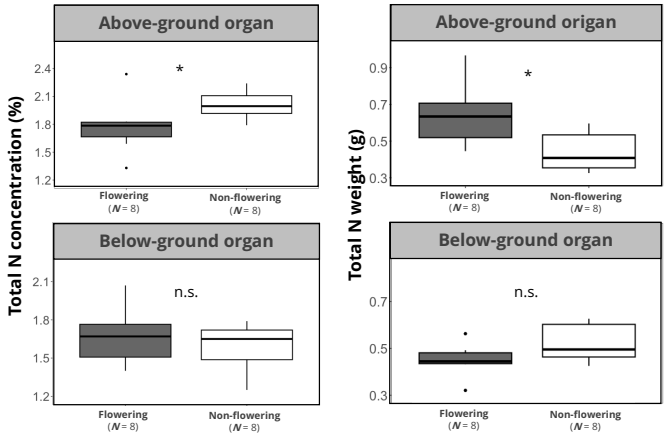


Figure 3-3. Comparisons of the concentration and mass (g) of total nitrogen between flowering (black) and non-flowering plants (white) in aboveground and belowground organs. * $P < 0.05$; n.s. $P > 0.1$

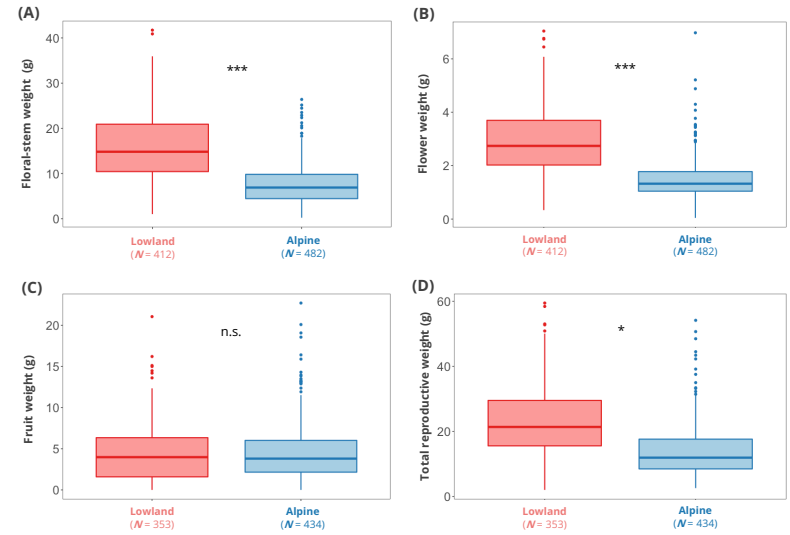


Figure 3-4. Comparisons of (A) floral stem mass, (B) total flower mass, (C) total fruit mass, and (D) sum of reproductive organs per plant between the lowland (red) and alpine habitats (blue). *** $P < 0.001$; * $P < 0.05$; n.s. $P > 0.05$

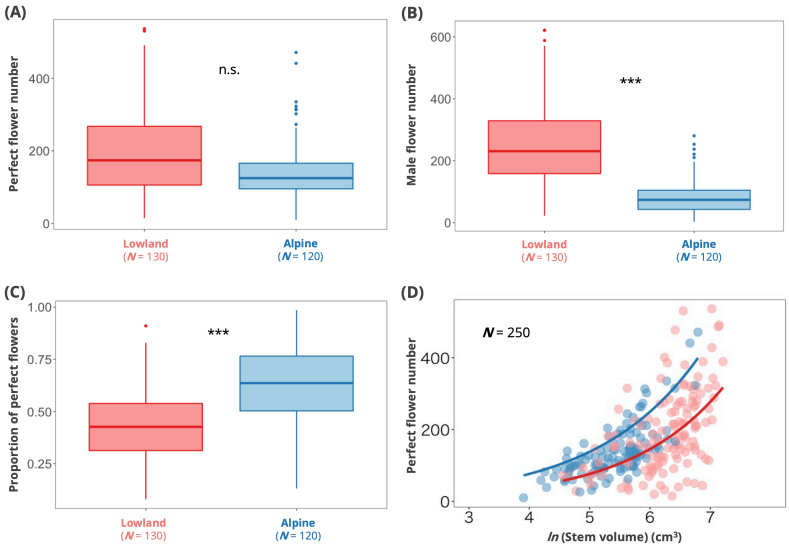


Figure 3-5. Comparisons of (A) perfect-flower number, (B) male-flower number, and (C) the proportion of perfect flowers per plant in the lowland (red) and alpine populations (blue). *** $P < 0.001$; n.s. $P > 0.05$. (D) Size dependency of perfect flower production per plant. The regression line indicates the relationships predicted by the GLMMs (see Table 3-2).

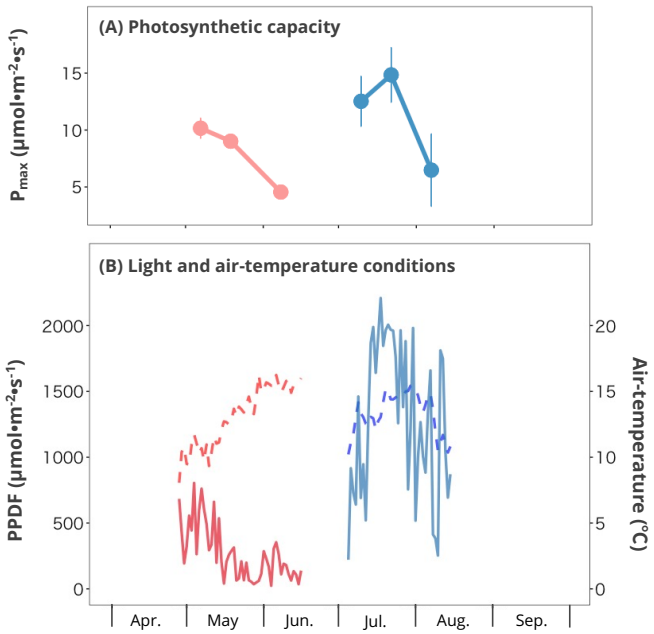


Figure 3-6. (A) Seasonal changes in maximum photosynthetic rate, P_{max}, and (B) daily-maximum photosynthetic photon flux density (PPFD; solid line) and daily-mean air-temperature (dashed line) during the leafing periods at the lowland site (NP) and the alpine site (A: SG, B: HS). Red: lowland site; Blue: alpine site. P_{max} values represent means ± SD of three plants. Daily-maximum PPFDs were measured in 2021. Daily-mean temperatures of 5-year average (from 2017 to 2021) are shown.

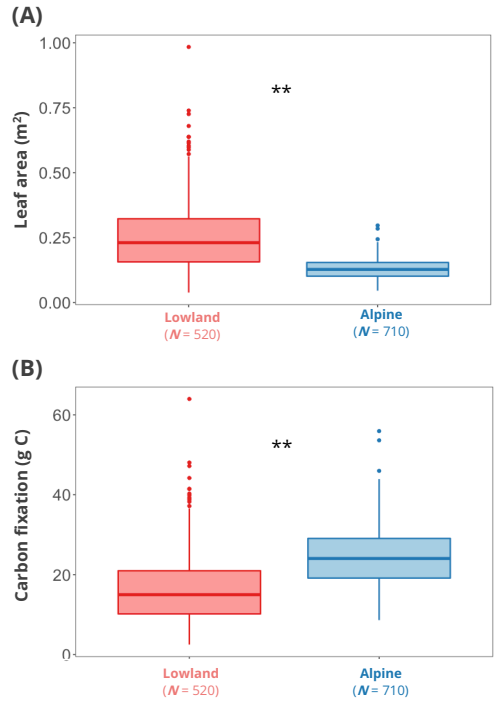
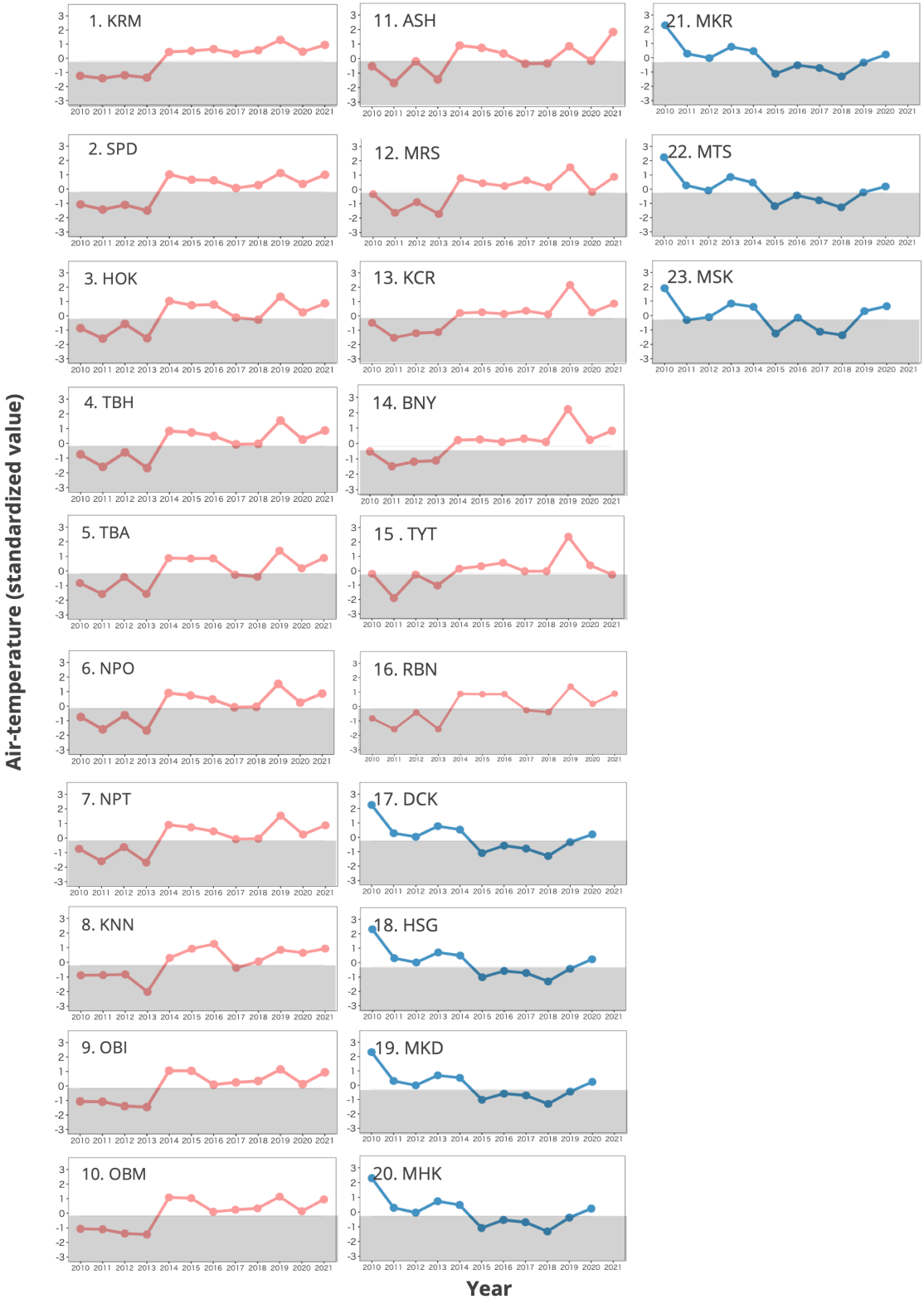


Figure 3-7. Comparisons of (A) total leaf area (m^2) and (B) annual carbon fixation (g C) per plant between the lowland (red) and alpine populations (blue). ** $P < 0.01$

Appendix 3-1. Annual fluctuation of the mean air-temperature during the growing season of *V. album* in each site. Grey area indicates the temperature level that enables masting occurrence (more than 15% of individuals flowering). Red: lowland population; blue: alpine population. See Table 2-1 for site codes.



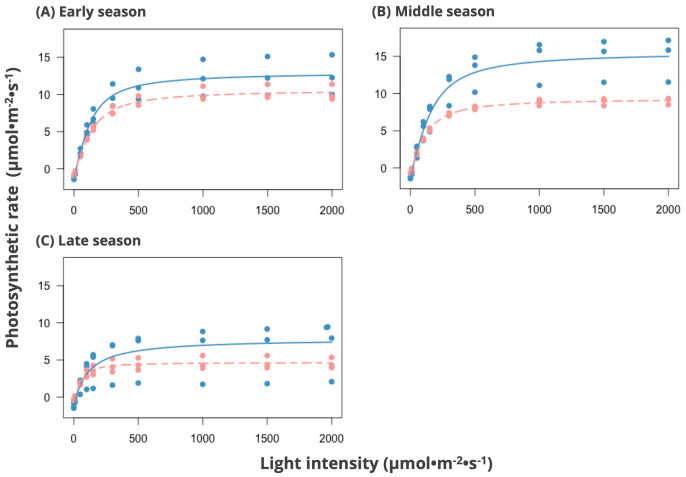
Appendix 3-2. Summary of GLMs for the relationship between floral-stem weight (g) and stem volume (cm^3) in TB (lowland) and HS (alpine) sites. In the GLMs, floral stem mass was the response variable and stem volume was the explanatory variable. There was a significant positive correlation between floral-stem mass and stem volume in each site. The site codes are listed in Table 1.

Variable		Coefficient	SE	*t* value	*P* value
Objective	Explanative				
TB (lowland) (N = 10)	Intercept	–3.738	2.286	–1.635	0.141
	ln (Stem volume)	1.015	0.361	2.81	**0.023**
HS (alpine) (N = 10)	Intercept	–2.718	0.656	–4.146	**0.003**
	ln (Stem volume)	0.882	0.124	7.128	**< 0.001**

Appendix 3-3. Summary of GLMs for the relationship between total leaf area per plant (m^2) and stem volume (cm^3) in NP (lowland) and HS (alpine) sites. In the GLMs, leaf area was the response variable and stem volume was the explanatory variable. There was a significant positive correlation between floral stem mass and stem volume in each site.

Variable		Coefficient	SE	*t* value	*P* value
Objective	Explanative				
NP (lowland) (N = 18)	Intercept	–5.372	0.272	–19.79	**< 0.001**
	ln (Stem volume)	0.734	0.051	14.43	**< 0.001**
HS (alpine) (N = 15)	Intercept	–3.932	0.374	–10.524	**< 0.001**
	ln (Stem volume)	0.415	0.086	4.857	**< 0.001**

Appendix 3-4. Light response curves of photosynthetic rates in (A) early, (B) middle, and (C) late growing seasons in the lowland NP site (red dashed line) and the alpine SG site (blue solid line).



Appendix 3-5. Mass (g) and concentrations of total nitrogen (N), total carbon (C), structural carbohydrate (SC), nonstructural carbohydrate (NSC), starch, and sugar in aboveground and belowground organs of flowering and non-flowering plants.

Plant part	Plant type	Total dry weight	Total N	Total C	SC	NSC	Starch	Sugar
Above	Flowering	36.0 ± 6.4 (g)	0.6 ± 0.2 (g)	16.38 ± 2.84 (g)	13.3 ± 2.2 (g)	3.1 ± 1.3 (g)	0.3 ± 0.1 (g)	2.7 ± 1.2 (g)
		NA	1.8 ± 0.3 (%)	45.5 ± 0.4 (%)	45.4 ± 0.5 (%)	8.3 ± 2.6 (%)	0.9 ± 0.3 (%)	7.5 ± 2.5 (%)
	Non-flowering	22.0 ± 4.9 (g)	0.4 ± 0.1 (g)	9.6 ± 2.2 (g)	7.1 ± 1.7 (g)	2.5 ± 0.8 (g)	0.2 ± 0.1 (g)	2.3 ± 0.8 (g)
		NA	2.0 ± 0.2 (%)	43.8 ± 0.3 (%)	43.7 ± 0.3 (%)	11.4 ± 2.4 (%)	1.1 ± 0.1 (%)	10.3 ± 2.4 (%)
Below	Flowering	29.3 ± 9.1(g)	0.5 ± 0.2 (g)	13.0 ± 3.8 (g)	5.2 ± 2.8 (g)	7.8 ± 1.7 (g)	6.0 ± 1.4 (g)	1.8 ± 0.3 (g)
		NA	1.7 ± 0.2 (%)	44.6 ± 0.8 (%)	44.3 ± 0.8 (%)	27.6 ± 5.5 (%)	21.2 ± 4.6 (%)	6.4 ± 1.3 (%)
	Non-flowering	32.8 ± 3.4 (g)	0.5 ± 0.1 (g)	14.1 ± 1.8 (g)	2.2 ± 0.9 (g)	12.0 ± 1.9 (g)	10.8 ± 1.7 (g)	1.2 ± 0.4 (g)
		NA	1.6 ± 0.2 (%)	43.0 ± 1.6 (%)	42.6 ± 1.6 (%)	36.4 ± 3.2 (%)	32.6 ± 2.7 (%)	3.7 ± 0.9 (%)

General discussion

The present study reported several important findings regarding the ultimate and proximate factors of masting behavior in *V. album*. First, the intensity of flowering synchrony varied responding to the habitat-specific selective forces between the lowland and alpine populations in terms of the predator satiation hypothesis and the pollination efficiency hypothesis (Chapter 1). Second, the occurrence of masting event was largely related to a specific weather cue (cool temperature), but the sensitivity to the weather cue and the periodicity of masting events differed between the lowland and alpine habitats (Chapter 2). Third, the variation in masting intervals among populations can be explained by the habitat-specific carbon budget balances (Chapter 3). In this section, I discuss how the synchrony and periodicity have been developed in *V. album* in terms of the ecological and evolutionary significances and the physiological mechanisms.

Evolutionary process of flowering synchrony

Among the hypotheses that explain the ecological and evolutionary significances of flowering synchrony within and among populations, there are many empirical supports in the predator satiation hypothesis (Janzen, 1971; Silvertown, 1980; Zwolak et al., 2022) and the pollination efficiency hypothesis (Nilsson and Wästljung, 1987; Shibata et al., 1998; Kelly et al., 2001) as the ultimate factors, and in the weather cue hypothesis as the proximate factors (Norton and Kelly, 1988; Schauber et al., 2002; Kelly et al., 2013). The present study supports the adequacy of these hypotheses; i.e., mast flowering in *V. album* was advantageous for decreasing herbivorous damages (for both of lowland and alpine habitats) and enhancing pollination efficiency (only for lowland habitat), thereby the synchronized flowering behavior triggered by cool temperature (as a weather cue) has been selected. At the lowland habitat, where the selective pressure is more intense (Chapter 1), flowering sensitivity to the weather cue was more intense (Chapter 2), resulting in a higher flowering synchrony in the lowland populations than in the alpine populations. Although various

phenotypic traits often vary among the populations of a single species depending on the local selective pressures (e.g., Kudo and Shibata, 2021; Chen and Pannell, 2022), most of the previous studies reported the intraspecific variations in the intensity of flowering synchrony within sites focusing on plant size, age, and local neighborhood density (Minor and Kobe 2017; Wion et al., 2020; Bogdziewicz et al., 2020c; Wion et al., 2023). In my knowledge, this is the first study demonstrating the mechanisms of intraspecific variation in flowering synchrony among populations responding to the habitat-specific selective pressure.

Evolutionary process of flowering periodicity

The alpine populations exhibited shorter flowering intervals at both population and plant levels than the lowland populations because they had a faster carbon recovery and less reproductive constraint by weather cues. Although the present study focused on the physiological mechanisms of masting periodicity within populations and flowering intervals of individual plants, its evolutionary significance, i.e., fitness advantages of masting periodicity, has not been tested precisely. According to the predator satiation hypothesis, the existence of years with low reproductive output contributes to starve and reduce their predators effectively, enabling escape from predation attack in the subsequent masting years (Silvertown, 1980). Because herbivorous damages in the lowland populations are rather intense (Chapter 1), longer low-flowering years may be necessary to keep the herbivore density at a low level before the occurrence of high-flowering year. In contrast, alternate-year occurrence of low-flowering event may be sufficient to suppress the herbivore density in the alpine populations, where herbivorous pressure is not serious (Chapter 1). To clarify this prediction, further studies on the habitat-specific masting intervals and evaluation of fitness are necessary.

In plant species having wide elevation ranges, high-elevation populations often show unpredictable and/or less frequent masting behavior (Webb and Kelly, 1993; Kelly et al., 2001; Kelly and Sork, 2002), presumably because annual productivity decreases with increasing elevation owing to the cooler and shorter growing conditions, resulting in a longer

filling period of resources before the following reproductive event (Kelly and Sork, 2002). Interestingly, the results of this study showed an opposite elevation pattern of the masting trend, i.e., less variable and more frequent reproduction at higher elevations. This is because the alpine populations had smaller resource investment in a single reproduction and higher annual carbon fixation capacity than the lowland populations.

Spatial scale of synchrony and its determinants

Owing to the distinct masting behaviors at the lowland and alpine habitats, elevational difference was the important spatial scale affecting the flowering synchrony across *V. album* populations in Hokkaido. To my knowledge, there are only two studies reporting the elevational patterns of flowering synchrony (Mooney et al., 2013; Masaki et al., 2020). One study indicated that the degree of flowering synchrony decreased along the elevation gradient (Mooney et al., 2013), whereas the other study reported the pattern of synchrony independent of elevations (Masaki et al., 2020). The maximum elevation difference between sites was > 1200 m in the former study and my study, whereas it was less than 600 m in the latter study, indicating that the elevational scale can affect the synchrony in plant species having a wide vertical distribution.

Spatial similarity in weather conditions is a key driver of reproductive synchrony across populations (Bogdziewicz et al., 2021; Bogdziewicz et al., 2023). In *Fagus sylvatica*, the similarity of air-temperature conditions explained well the synchrony in seed production across populations, and the synchrony was maintained at a geographic scale of 1500 km (Bogdziewicz et al., 2021). In contrast, flowering synchrony in *V. album* declined greatly between the lowland and alpine habitats irrespective of geographic distance because of the elevation-specific thermal conditions and flowering sensitivity to the weather cues. This indicates that spatial patterns of flowering synchrony can be regulated not only by the similarity of weather conditions but also the habitat-specific masting strategy in individual populations.

The predator satiation hypothesis can explain the ecological significance of flowering synchrony not only within a local population but also across a large-scale populations. If the spatial scale of reproductive synchrony of host plants is smaller than the migration ability of predators, predators will move to other populations that produce more flowers and fruits, reducing the effectiveness of masting as a strategy of predator starvation (Curran and Webb, 2000; Satake and Bjørnstad, 2004). Thus, spatial scale of flowering synchrony should be selected depending on the mobility of herbivorous insects. The evolution of synchronous masting across populations is possible only when herbivores are specialist of a specific host plant and the mobility of herbivores is not limited to a local population scale. Although previous studies have reported that floral-stem borers of the Lonchaeinae family (Diptera) and the seed predator *Eupithecia veratraria* (Lepidoptera) are highly dependent on *Veratrum* species in western Europe (Schaffner et al., 2001; Hesse et al., 2008; Ozerov and Krivosheina, 2017), the feeding habits of stem borers and seed predators in Hokkaido are unknown. Therefore, further studies on the life histories of these herbivorous insects are needed for the further understanding of the ecological significance of flowering synchrony in *V. album*.

Intraspecific variation in masting behavior

The evolutionary process of masting behavior has mainly been studied by the approach of interspecific comparisons (Kelly and Sork, 2002; Koenig and Knops, 2003; Bogdziewicz et al., 2020b). The previous studies revealed that masting patterns varied by the species-specific selective pressures, such as pollination modes (wind pollination or animal pollination) and life history of herbivores (generalist or specialist, mobile or immobile). In contrast, I studied the evolutionary process of masting behavior by the approach of inter-population comparisons of a single species. As a result, the variation in masting behavior was generated by the site-specific selective pressures, such as the intensity of herbivorous damage and the degree of pollinator limitation. This indicates that plants can evolve the masting behavior responding the local scale selective forces in order to optimize their reproductive success.

Recent climate change may modify the masting pattern of *V. album* populations because the physiological mechanisms of masting were closely related to abiotic conditions, such as air-temperature and light conditions. As flowering of *V. album* was triggered by cool conditions during the growing period, global warming may suppress the frequency of masting events. Because climate change often causes the upward advance of timberline (Harsch et al., 2009; Vitasse et al., 2012; Sakio and Masuzawa, 2020), shading by canopy trees may restrict photosynthesis and flowering events in the alpine populations. Previous study reported that the variation in masting patterns caused by climate change has decreased the reproductive success of individual plants because of less-effective pollination and more seed predation damage (Bogdziewicz et al., 2020a). Since climate change has been progressing acceleratory, it may be difficult for plants to adapt their masting strategy responding to climate change, resulting in population collapse and species extinction. Although the present study focused on the spatial variation in masting behavior, future studies focusing on the temporal variation are also necessary.

Conclusion

The present study successfully revealed that the flowering synchrony and periodicity in *V. album* clearly varied between the lowland and alpine populations responding to the habitat-specific selective forces and environmental conditions. Because of the habitat-specific masting patterns, the spatial pattern of flowering synchrony across populations changed greatly by the elevation scale. In conclusion, "elevational difference" is an important factor creating diverse masting patterns across populations even in a single species. However, it is not clear in the present study how masting intervals in each population and flowering synchrony among populations are related to the fitness advantage in *V. album*. Furthermore, climate change impacts on the masting behavior will be a central issue to predict the effects of global warming on the biodiversity of terrestrial ecosystems. Further studies focusing on these issues are necessary for the comprehensive clarification of the masting phenomenon in plants.

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