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Vulnerability of phenological synchrony between plants and pollinators in an alpine ecosystem

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Abstract

The relationship between flowering phenology and abundance of bumble bees (*Bombus* spp.) was investigated using two years of phenological data collected in an alpine region of northern Japan. Abundance of *Bombus* species was observed along a fixed transect throughout the flowering season. The number of flowering species was closely related to the floral resources for pollinators at the community scale. In the year with typical weather, the first flowering peak corresponded to the emergence time of queen bees from hibernation, while the second flowering peak corresponded to the active period of worker bees. In the year with an unusually warm spring, however, phenological synchrony between plants and bees was disrupted. Estimated emergence of queen bees was 10 days earlier than the first flowering date owing to earlier soil thawing and warming. However, subsequent worker emergence was delayed, indicating slower colony development. The flowering season finished two weeks earlier in the warm-spring year in response to earlier snowmelt. A common resident species in the alpine environment, *B. hypocrita sapporoensis*, flexibly responded to the yearly fluctuation of flowering. In contrast, population dynamics of other *Bombus* species were out of synchrony with the flowering: their frequencies were highest at the end of the flowering season in the warm-spring year.

Therefore, phenological mismatch between flowers and pollinators is evident during warm years, which may become more prevalent in a warmer climate. To understand the mechanism of phenological mismatch in the pollination system of the alpine ecosystem, ground temperature, snowmelt regime, and life cycle of pollinators are key factors.

Keywords: alpine plants, bumble bee, flowering phenology, phenological mismatch, temperature

Introduction

Phenological modification is the most common climate change impact on natural ecosystems (Fitter and Fitter 2002; Root et al. 2003; Parmesan 2007). Climate change may affect not only flowering phenology of individual plant species but also flowering abundance and phenology of whole plant communities in northern ecosystems. This is because plant growth and development under cold climate are strongly determined by ambient thermal conditions (Diekman 1996; Hülber et al. 2010). In addition to the direct effects of atmospheric temperature, snow depth during winter and snowmelt timing in spring also indirectly affect thermal conditions at a micro scale. Because most alpine plants retain overwintering meristems around ground level, snowmelt pattern strongly affects plant phenology (Kudo 1991; Wipf 2010; Iler et al. 2013).

Changes in flowering phenology and abundance at the community scale should influence resource availability for pollinators and their foraging behavior. Furthermore, the life cycle of pollinators may be influenced by weather variation. For instance, spring temperature is the most important determinant of pollinator emergence (Høye and Forchhammer 2008; Gordo and Sanz 2003; Ellwood et al. 2012; Iler et al. 2013). For pollinators overwintering

in the ground, snow conditions during winter also influence the emergence schedule (Alford 1969; Høye and Forchhammer 2008; Iler et al. 2013) because soil temperature is strongly influenced by the insulation effect of snow cover. Therefore, biological interactions in an alpine ecosystem are sensitive to phenological variations of individual organisms. If flowering phenology and pollinator emergence respond differently to climate fluctuation, their mutualistic relationships may be disrupted, i.e. creating phenological mismatch (Thomson 2010; Kudo and Ida 2013).

Bumble bees (*Bombus* spp.) are the most effective pollinators in alpine ecosystems (Bergman et al. 1996; Bingham 1998). They are generalist pollinators and utilize many plant species throughout the flowering season (Heinrich 1979). In the early season, only overwintered queen bees are available and their foraging activity is commonly much lower than worker bees that appear in mid-summer (Kameyama and Kudo 2009; Kudo et al. 2011). Nevertheless, many early-blooming plants depend on pollination by queen bees. Because queen bees overwinter in the ground and emerge from hibernation when soil temperature reaches a threshold in spring (Alford 1969), soil thermal conditions are an important determinant of bee phenology. Experimental studies revealed that diapause duration and thermal conditions affect the preoviposition period and subsequent colony development of bumble bees (Beekman et al. 1998; Amin et al. 2007). Therefore, winter climate may influence the activity and availability of bees in alpine ecosystem through snow accumulation and melting patterns. However, the relationship between thermal environment and bee activity has scarcely been studied in alpine ecosystems.

Clarification of climate impacts on the relationship between flowering phenology and bee activity is very important to understand the dynamics of plant-pollinator interactions in alpine ecosystems. Alpine ecosystems are composed of fellfield and snowbed habitats (Kudo and Hirao 2006; Kudo 2006). In wind-blown fellfield sites where little snow accumulates during winter,

flowering starts in early June and lasts until mid-summer. In contrast, snowbeds accumulate thick snow cover during winter, the onset of flowering is usually delayed until mid-summer, depending on snowmelt time, and flowering lasts until late in the growing season. Fellfield flowers are an important nectar resource for queen bees after hibernation in early season. Snowbed habitat is an important foraging site for worker bees during colony development and for new queen bees before hibernation. These functional roles of alpine habitats, however, may be disrupted if phenological mismatch between plants and bees occurs owing to climate variations.

The purpose of this study is to evaluate the sensitivity of phenological synchrony between plants and pollinators under variation in weather in an alpine ecosystem. First, I quantify how weather fluctuation affects flowering phenology at a regional scale. In a previous study on flowering phenology (Kudo and Yokosuka 2012), we revealed that thermal requirements for flowering onset were strongly species-specific. However, the impact of weather variation on phenology at the community scale has been scarcely studied (e.g. Kudo and Suzuki 1999; Aldridge et al. 2011). Second, I evaluate how flowering phenology is related to the floral resource level at the community scale. Regional flowering phenology may reflect the temporal transition of floral resources for generalist pollinators such as bumble bees. To evaluate the phenological linkage between flowers and pollinators, the relationship between the flowering overlap among species and flower abundance at community scale is important. Third, I compare how the extent of seasonal activity and life cycles of major *Bombus* species vary between years. The effects of weather variation on life cycle may be different between resident bee species in the alpine environment and migrant bee species that visit the alpine ecosystem for foraging from lower elevations. I expect that the life cycle of resident bees is more sensitive to yearly weather fluctuation if environmental conditions acting on colony development become harsher with elevation. Finally, I analyze the relationship between flowering phenology and bee abundance over years. Especially, I focus

on the responses of bees, whether bees adjust their life cycle in accordance with the variation in flowering phenology or whether they conservatively retain their life cycles irrespective of the fluctuation of flowering phenology, resulting in the increasing risk of phenological mismatch. To answer these questions, I synthesize the relationship between flowering phenology and bee abundance between years with a typical spring and an unusually warm spring using a phenological data set collected by volunteer efforts in the Taisetsu Mountains, northern Japan.

Materials and methods

Study sites

The main body of this study was conducted on Mt. Akadake (summit elevation is 2078 m) in the Taisetsu Mountains (Daisetsuzan National Park), in Hokkaido, northern Japan. The tree line in this area is 1600–1650 m elevation. One fellfield site and one snowbed site were selected as major monitoring plots for the quantification of flowering phenology in this area. The fellfield site (N 43°41'42", E 142°54'46") is on a plateau of 1850 m elevation, where soil surface commonly has no snow cover during the winter and soil is usually frozen from November to May. The snowbed site (N 43°40'36", E 142°56'49") is on an east-facing valley slope of 1970 m elevation, where thick snow usually remains until late June or early July. The distance between the fellfield and snowbed sites is 1.1 km. This area has been registered as a long-term monitoring site of Japanese alpine ecosystems (Monitoring Sites 1000 Project) by the Ministry of the Environment Biodiversity Centre since 2010 in which weather conditions, vegetation dynamics, species composition, flowering phenology, and shoot growth of alpine dwarf pine (*Pinus pumila*) are measured over five mountain regions in Japan (<http://www.biodic.go.jp/moni1000/index.html>). Air temperature at 1.5 m height at the fellfield site, and surface and soil temperatures at 10 cm depth at both sites have been recorded throughout the

year since 2010. Annual mean temperature at the fellfield site is -3.2 °C and summer temperature (June to August) is 9.6 °C.

Supplementary data of the relationship between the number of flowering species and floral density (as an index of floral resources) at the landscape scale were collected on an east-facing slope (1790–1900 m elevation) of Mt. Kaun located 14 km southwest of Mt. Akadake in 2012.

Flowering phenology and floral resources

Flowering phenology was monitored within a 20-m x 10-m plot established in the fellfield and snowbed sites. Flowering conditions of every entomophilous plant species have been recorded by volunteer activity throughout the growth season since 2006 (Kudo and Yokosuka 2012). Flowering condition in each species was classified into four stages; ‘initial stage’ in which fewer than 50% of the plants have started opening flowers (stage A), ‘peak stage’ in which more than 50% of the plants (50–100%) have open flowers (stage B), ‘later stage’ in which 10–50% of the plants retain flowers after peak flowering (stage C), and ‘terminal stage’ in which most flowers have finished (>90 %) and only a small portion of plants retain open flowers (stage D). The period from stages A to C represents the major flowering period in each species.

Our previous studies on flowering phenologies over multiple sites (Kudo and Suzuki 1999; Kudo and Hirao 2006) demonstrate that regional flowering of this mountain area can be represented by the combination of flowering phenologies of fellfield and snowbed communities. In this study, floral resources were expressed qualitatively based on the species composition of flowering plants as the number of overlapping flowering species during the growth season (late May to early September) in the fellfield and snowbed plots. Although the size of the monitoring plots was relatively small, major plant species in this area were included within the plots and flowering periods within plots largely represented the flowering season of this area (G. Kudo, personal observation).

In this study, phenological records in 2011 and 2012 were used for analyses. In 2011, 46 records of flowering phenology were accumulated from 1 June to 15 September (2.3-day intervals in average). In 2012, 52 records were accumulated from 30 May to 7 September (1.9-day intervals in average). In both years, flowering phenologies of 14 common species were recorded (see Fig.1). Our previous report revealed that first flowering date of individual species could be expressed as the species-specific temperature sum of soil surface above 5 °C (Kudo and Yokosuka 2012), indicating that flowering phenology at a regional scale can be determined by the thermal conditions and snowmelt regime.

To test the validity of using the number of overlapping flowering species as an index of floral resource level, the relationship between the number of flowering species and floral density was measured among five plots (20-m x 20-m in each plot) that were arranged along a snowmelt gradient (about 400 m in length) from the exposed fellfield (1900 m elevation) to the bottom of snowbed (1790 m) on an east-facing slope of Mt. Kaun. This site is suitable for the measurement of floral resources at a community scale because flowering periods last from the beginning to the ending of growing season due to the formation of the deepest snow patch in the Taisetsu Mountains (Kudo and Hirao 2006). Flowering plant species within the plots were recorded 13 times during the flowering period from late May to early September, and floral density was counted eight times (on 8, 18 June, 4, 17 July, 2, 12, 22 August, and 1 September). For the floral density measurements, ten 2-m x 2-m quadrats were arbitrarily set on floral patches in each plot and the flower number of every species visited by insects within a quadrat was counted. I counted the number of heads for Asteraceous flowers and the number of umbels for Apiaceous flowers instead of the number of florets. The average flower number of 10 quadrats in each plot was used to represent floral density (per 4 m²) at the time of measurement, and the sum of the average flower number over five plots was used as available floral resources (per 20 m²) at the

community scale along the snowmelt gradient. For this measurement, I targeted only entomophilous flowers visited by *Bombus* spp.

Bumble bee monitoring

Monitoring of bee activity was conducted in 2011 and 2012 along the hiking trail from the sub-alpine forest (1490 m elevation) through the fellfield site to the snowbed site (total length = 3.4 km, difference in elevation = 330 m). By walking at a constant speed (about 1.5 hours from the sub-alpine forest to the snowbed plot), species and caste of bees and plant species of visited flowers were recorded along the trail within about 2-m width of both sides. Flying and resting bees around the trail were also recorded. In the summary report of the 2011 monitoring (Kudo and Imoto 2012), five bee species were recorded visiting 37 plant species in this area: *Bombus hypocrita sapporoensis* Cockerell, *B. hypnorum koropokkrus* Sakagami et Ishikawa, *B. beaticola moshkarareppus* Sakagami et Ishikawa, *B. yezoensis* Matsumura, and *B. diversus tersatus* Smith. Only data recorded in the alpine zone above the tree line (1650 m elevation) were used for analysis (trail length = 2.2 km) in this study because the focus is on the relationship between alpine plant phenology and bee activity. Monitoring was conducted over 12 days from 4 June to 27 August in 2011 (7.6-day intervals in average), and 17 days from 1 June to 9 September in 2012 (6.3-day intervals in average). Time, temperature, and cloud cover (0 to 10 at 10% intervals) were also recorded at the beginning and end of each observation. Observation was not conducted on rainy days.

Data analysis

Flowering onset date (day of year) and major flowering period (day) of individual plant species on Mt. Akadake were compared between habitat types (fellfield vs. snowbed) and between years (2011 vs. 2012) by linear mixed-effect models (LME) postulating Gaussian error distribution using the lme4 package by R ver.3.0.1 (R Development Core Team 2009) in which flowering onset date

or flowering period was set as the objective variable, habitat and year were included as explanatory factors, and plant species as a random factor. Effective variables were selected based on the Akaike's information criterion (AIC) in each LME. The relationship between the number of flowering species and floral density (after logarithmic transformation) along the snowmelt gradient on Mt. Kaun was analyzed by Pearson's correlation test throughout the flowering period.

Seasonal patterns of bee abundance (the number of bees observed in each census) were compared between years for individual *Bombus* species using generalized linear models (GLM) of negative-binomial distribution with log-link function using the MASS package by R ver.3.0.1. In the GLM, bee abundance is an objective variable and year (2011, 2012), day of year (d), cloud cover (1–10), and temperature are explanatory variables. Because bee abundance commonly showed a unimodal distribution as seasonal progress (see results), the effect of d was included as a quadratic function in the model, i.e., d^2 and d . Because the seasonal pattern of overwintered queen bees of *B. hypocrita sapporoensis* was apparently different from bees of the current generation (workers, males and new queens), they were analyzed separately. Overwintered queens of other *Bombus* species were rarely observed, and GLMs were performed only for current generation in which all castes were pooled because workers were dominant in every species. For individual GLMs, effective variables were selected based on AIC. To evaluate the phenological synchrony between flowers and bee activity, Pearson's correlation test was performed between the number of flowering species and bee abundance throughout the season in each year.

Results

Weather condition

Monthly air temperature from April to September ranged from -6.2 to 12.8°C in

2011 and from -4.3 to 13.4°C in 2012 (Table 1). Air temperature was noticeably higher in 2012 throughout the period, especially in May and September, than in 2011 except for July. A long-term climate record (2003-2012) from a weather station (1700 m elevation) close to Mt. Kaun indicated that mean temperature from April to September was 0.8°C warmer in 2011, versus 2.2°C warmer in 2012 than the long-term mean, indicating that 2012 was an unusually warm year in the Taisetsu Mountains (G. Kudo, unpublished data).

Soil temperature at the fellfield plot showed a similar trend as air temperature, although April soil temperature was lower in 2012 than in 2011 (Table 1). Soil thawing at the fellfield plot occurred on 23 May in 2011, while it was three weeks earlier in 2012 (1 May). Snowmelt in the snowbed plot progressed from 10 to 31 July (21 days melting period) in 2011, while from 25 June to 20 July (25 days) in 2012. Earlier snowmelt initiation and slower melting period in 2012 reflects the warmer spring but cooler July temperatures. Soil temperature in the snowbed plot remained around -2 to 0°C during the snow-covered period, and showed a similar trend to the fellfield after snowmelt.

Flowering phenology

Flowering onset of every fellfield species occurred earlier in 2012 (8.6 days on average, ranging from 3 to 14 days) than in 2011 (Fig.1a). For snowbed plants, 13 of 14 species showed earlier flowering onset in 2012 (6.7 days on average, ranging from -2 to 14 days) than in 2011 (Fig.1b). LME result indicated that flowering onset was significantly earlier in the fellfield plot than in the snowbed plot ($t_{26} = 10.98$, $P < 0.001$) and in 2012 than in 2011 ($t_{26} = 8.17$, $P < 0.001$) with no significant interaction between plot and year ($t_{26} = 1.38$, $P = 0.18$). Major flowering periods (stage A to C) of individual species were 21–22 days in the fellfield plot and 25–28 days in the snowbed plot. LME result indicated that flowering period was significantly longer in the snowbed plot than in the fellfield plot ($t_{27} = 2.12$, $P = 0.043$) but year effect was not significant ($t_{27} = -1.67$, $P = 0.11$) and the interaction effect between plot and year was

excluded by AIC. Longer flowering period in the snowbed plot relative to the fellfield plot reflected gradual snowmelt progress within the plot.

Total flowering period including both fellfield and snowbed plants was 104 days in 2011 (from 4 June to 15 September), and 96 days in 2012 (from 30 May to 2 September; Fig.2). Peak flowering period, expressed as flowering overlap of >50% species, occurred from late July to early August in both years. However, the number of flowering species sharply declined after mid-August in 2012.

Relationship between flowering overlap and floral density

Throughout the flowering period along the snowmelt gradient on Mt. Kaun (late May to early September in 2012), flowering of 41 species was recorded over five plots. The number of flowering species increased towards late July, and decreased rapidly after that (Fig.3). The sum of floral densities over plots was highly variable, responding to the seasonal pattern of the number of flowering species; it attained maximum levels in early August and rapidly decreased after that. A small peak appeared in the middle of June irrespective of smaller number of opening species. This reflected the dense flowering of mat forming fellfield dwarf shrubs (*Loiseleuria procumbens* and *Diapensia lapponica*). There was a strong positive correlation between the number of flowering species and the sum of floral densities over plots ($r = 0.85$, $t_6 = 3.99$, $P = 0.007$). This result indicates that the number of flowering species at the community scale is a good indicator of regional floral resources in the Taisetsu Mountains.

Bumble bee phenology

During the 12 observations in 2011, 456 bees composed of five species were recorded; *B. hypocrita sapporoensis* (58%), *B. hypnorum koropokkrus* (20%), *B. beaticola moshkarareppus* (14%), *B. yezoensis* (8%), and *B. diversus tersatus* (<1%). During the 17 observations in 2012, 588 bees composed of four species were recorded; *B. hypocrita sapporoensis* (22%), *B. hypnorum koropokkrus*

(2%), *B. beaticola moshkarareppus* (29%), and *B. yezoensis* (47%).

Overwintered queens of *B. hypocrita sapporoensis* emerged in the beginning of June, decreased in number after mid-June but were sporadically observed through mid- to late July. GLM results indicate a significant difference in the pattern of abundance between years in which the high abundance period came earlier in 2012 than in 2011 (Fig.4a, Table 2). Furthermore, queen abundance was positively influenced by cloud cover and temperature, indicating that queen activity tended to high on warm and cloudy days. The abundance of workers was also significantly different between years (Table 2); they emerged in early July in 2011 at low frequency, increased in number toward mid-August, and decreased in late August. In 2012, however, the first workers were observed in late July, increased rapidly in early August, then decreased by late August. Thus, the active worker period was much shorter in 2012 in comparison with 2011. Reproductive castes (males and new queens) appeared in late August in both years.

Overwintered queens of *B. beaticola moshkarareppus* were observed only in the subalpine forests in mid-June and they seldom came to the alpine zone (data not shown). Seasonal trend of workers was similar between years and year effect was excluded in GLM (Table 2); they appeared in mid-July, increased in number toward late August, then decreased in early September (Fig.4b). Reproductive castes (males and new queens) appeared from late August to early September at low frequency.

The abundance of *B. hypnorum koropokkrus* varied between years (Fig.4c, Table 2) and a very small number of bees was observed in 2012 (90 bees in 2011 and 11 bees in 2012). The number of *B. yezoensis* workers varied highly between years (36 bees in 2011 and 277 bees in 2012) but a significant year effect was not detected by GLM (Table 2). Similar to *B. hypocrita sapporoensis* queens, the abundance of *B. yezoensis* was positively influenced by cloud cover and temperature. Reproductive castes were observed only in early September and at low frequency.

Relationship between flowering phenology and bee frequency

The abundance of bees was largely concordant with the number of flowering species at a regional scale in 2011 (Fig.2), indicating a positive correlation between them ($r = 0.81$, $t_{11} = 4.53$, $P < 0.001$; Fig.5). In 2012, however, bee abundance obviously deviated from the flowering phenology; the period of low bee activity lasted through late July, then bee abundance attained a maximum level in late August, after the peak flowering period (Fig.2). Even after the flowering season in the snowbed plot, bees (mainly *B. beaticola*, *moshkarareppus* and *B. yezoensis*) continuously visited remaining flowers (*Cirsium amorensense*, *Sausurea riederi* var. *yezoensis* and *Gentiana nipponica*) outside of the plot. There was no correlation between the number of flowering species and bee abundance in 2012 ($r = -0.01$, $t_{15} = -0.045$, $P = 0.97$; Fig.5). When only *B. hypocrita sapporoensis* was compared, however, significant correlations were detected in both years ($r = 0.72$, $t_{10} = 3.30$, $P = 0.008$ in 2011; $r = 0.54$, $t_{15} = 2.47$, $P = 0.026$ in 2012). This indicates a robust phenological matching between flowering phenology and seasonal activity of *B. hypocrita sapporoensis* in the alpine ecosystem.

Discussion

Flowering phenology and seasonal pattern of bee abundance varied between years, and a phenological synchrony between them was disrupted in the warm-spring year. Flowering onset time of alpine plant species is commonly determined by the species-specific daily temperature sum above a threshold value, i.e. growing degree days (GDD; Kudo and Suzuku 1999; Hülber et al. 2010; Wipf 2010). In the fellfield habitat with little snow cover during winter, temperature regime is the most important determinant factor of flowering onset time. Although there was a three-week difference in soil thawing date (ST) between 2011 and 2012, the difference in the first flowering date (FF) was

only five days in this area (Fig.2). This is because accumulation of GDD progresses slowly in the early season (Kudo and Suzuki 1999). Therefore, flowering onset time of fellfield plants is relatively resistant to climate variation as reported before (Kudo and Hirao 2006), which might help protect flower buds from exposure to frost (Inouye 2008). In contrast, the flowering season was completed two weeks earlier in the warm year. This is because snowmelt in the snowbed plot started 15 days earlier in 2012. Because flowering periods of individual species did not differ in length between years, flowering onset time of snowbed plants determines the total flowering period of the alpine ecosystem. Recently, snowmelt time has been advancing in many alpine regions (Mote et al. 2005; Rixen et al. 2012) including the Taisetsu Mountains (Kudo and Hirao 2006). Under warm weather, therefore, alpine plant communities tend to shorten the whole flowering period due to the earlier completion of flowering season, especially in snowbed habitats.

Seasonal dynamics of bee abundance were highly species-specific. Among four common *Bombus* species, only *B. hypocrita sapporoensis* showed a clear bimodal frequency in early June and August in both years; first peak corresponded to queen bees after hibernation and second peak to worker bees. This species has a wide altitudinal distribution range from lowland to high mountains in Hokkaido, but its life cycle pattern suggests that populations of this site may remain in the alpine environment for the whole life cycle from overwintering to breeding, i.e. it is a resident pollinator. *B. beaticola moshkarareppus* was commonly observed in alpine sites from late June to early September but queen bees were rare in the early season. This species can also breed in alpine habitats but its major overwintering site may be in the subalpine habitat because overwintered queens are commonly observed in subalpine forests in mid-June (G. Kudo, personal observation). The other two species (*B. hypnorum koropokkrus* and *B. yezoensis*) showed large fluctuations in abundance between years. It is possible that they only visit the alpine habitat for foraging, i.e. be migrant pollinators, because their active periods in

alpine sites were short.

The flowering pattern of alpine plant communities indicated a bimodal distribution with a small peak in early to mid-June and a large peak in late July to early August in both years. The first peak corresponds with the emergence time of overwintered queen bees, while the second peak corresponds to the active season of worker bees. Plant-pollinator interactions in alpine ecosystems vary greatly, reflecting flowering phenology and life cycles of bees (Kameyama and Kudo 2009; Kudo et al. 2011). Early bloomers in the fellfield, such as *Arctous alpinus*, *Loiseleuria procumbens* and *Diapensia lapponica*, provide important nectar resource for queen bees after hibernation. Generally, the pollination service of queen bees is insufficient because of low visitation frequency and cool temperatures in the early season, resulting in low pollination success (Kudo and Suzuki 2002). On the other hand, longer flight distance between inflorescences and shorter sequential flower visits within patches by queen bees contribute to reduced geitonogamous pollination in comparison with worker bees that intensively collect nectar and pollen within floral patches (Kudo et al. 2011). Therefore, there is a trade-off between qualitative and quantitative pollination service between early- and late bloomers dependent on queen and worker bees, respectively. Because many early bloomers in the fellfield habitat are dwarf shrubs forming dense floral patches, pollination by queen bees may be more beneficial for outcross pollination than by worker bees.

A positive correlation between the number of flowering species and bee abundance in a normal year indicates the importance of ecological linkage between flowering phenology and life cycles of bumble bees in the alpine ecosystem where bumble bees are the most effective pollinators (Bingham 1998). In the warm year, however, the positive correlation disappeared due to phenological mismatch. In the early season, phenological mismatch between plants and bees may be caused by the different thermal requirements for flowering onset and termination of hibernation (Kudo and Ida 2013). As

mentioned before, preflowering periods of alpine plants are determined by species-specific GDD. On the other hand, bumble bees hibernating in the ground tend to emerge when the soil temperature reaches 5–9 °C (Alford 1969). In the fellfield site, soil temperature reached this range on 1–2 June in 2011, but 21–22 May in 2012. Thus, first bee emergence might occur 10 days earlier in 2012. First flowering of nectar plants occurred on 4 June in 2011 and 30 May in 2012. Therefore, expected phenological mismatch was only 2–3 days in the normal year, whereas it was 8–9 days in the year with a warm spring. Emerging from hibernation too early before flowering may starve bees, resulting in high mortality or failure to reproduce. Lower frequency of *B. hypocrita sapporoensis* queens in 2012 might be caused by the early season mismatch.

In the middle season, emergence of *B. hypocrita sapporoensis* workers was late in 2012 irrespective of earlier flowering, resulting in low pollinator activity from late June to mid-July. Lower nesting success of queen bees and/or larval growth owing to the early-season mismatch might put off the colony development. Furthermore, relatively cooler July temperature in 2012 (soil temperature was 1.6 °C lower than in 2011) might have caused slower colony development (Yoon et al. 2002). For clarification of the mid-season mismatch mechanism, however, further study of colony growth pattern in alpine environment is necessary.

Earlier completion of the flowering season was the most prominent phenological change in the warm year. The abundance of *B. hypocrita sapporoensis* quickly decreased in mid-August with the decreasing number of flowering species. This may reflect a flexible colony development depending on the availability of floral resources because a positive correlation between flowering phenology and bee abundance was retained even in the warm year. Regulation of life cycles depending on floral resource has been reported in other *Bombus* species (Schmid-Hempel and Durrer 1991). Yearly variation in the frequency of *B. hypocrita sapporoensis* may reflect its adaptive life cycle

against temporal fluctuation of floral resources in the alpine ecosystem. In contrast, abundance of *B. beaticola moshkarareppus* and *B. yezoensis* continuously increased toward late August. In early September, when flowering of most species had finished, these bees (workers, males and queens) were commonly visiting the sparse remaining flowers of *Cirsium amurense* and *Saussurea riederi* var. *yezoensis*, indicating a highly competitive situation. The effect of the late-season mismatch on subsequent bee population dynamics is an important issue for future study.

Several studies predict that phenological mismatch between plants and pollinators may not be common because of similar phenological shifts between them or flexible plant-pollinator interactions owing to generalist pollinators (Hegland et al. 2009; Bartomeus et al. 2011; Rafferty and Ives 2011). Also, in the field studies conducted in subalpine meadows of North America, population dynamics of solitary bees, wasps, and syrphid flies were largely consistent with flowering phenology over years (Forrest and Thomson 2011; Iler et al. 2013). On the other hand, risk of phenological mismatch increases when flowering periods are short (Miller-Rushing et al. 2010; McKinney et al. 2012). Based on the long-term phenological records of flowers and their pollinators, Memmott et al. (2007) simulated the effects of global warming on plant-pollinator interactions, and predicted that phenological shifts would reduce the floral resource available to 17–50% of pollinator species due to reduced overlap between flowers and pollinators. Results of the present study support this prediction. Differences in the phenological responses between plants and pollinators between regions and among pollinator types may reflect the differences in the species-specific life cycles and/or habitat-specific microclimate. In ground-nesting bees, soil temperature at overwintering and nesting habitats may be a crucial factor determining life cycle.

In this study, I investigated the feature of phenological synchrony between typical alpine plant communities and bee activity. To evaluate the mechanism of phenological mismatch, more extensive quantification of flowering phenology

and floral resources in accordance with foraging range of pollinators are preferable (e.g. Aldridge et al. 2011). Furthermore, measurements of pollination success of plants and population dynamics of pollinators are crucial to understand the ecological significance of phenological mismatch (Thomson 2010; Kudo and Ida 2013). Because large efforts are required for these measurements, however, long-term monitoring dependent on volunteer activity may not be sufficient. Data for this study were largely based on the descriptive records of flowering species and bee counts (Kudo and Yokosuka 2012; Kudo and Imoto 2012). Even such simple monitoring can provide insights to evaluate the dynamics of plant-pollinator linkage over years. The results of this study highlight the importance of ground temperature and snowmelt regime as key factors of phenological cues, and species-specific responses of pollinators to weather fluctuation based on the life cycles in an alpine ecosystem under a changing climate.

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References

Aldridge G, Inouye DW, Forrester J, Barr WA, Miller-Rushing J (2011)
Emergence of a mid-season period of low floral resources in a montane

- meadow ecosystem associated with climate change. *J Ecol* 99:905-913
- Alford DV (1969) A study of the hibernation of bumblebees (Hymenoptera: Bombidae) in southern England. *J Anim Ecol* 38:149-170
- Amin MR, Suh SJ, Kwon YJ (2007) Impact of artificial photoperiodism on the colony development of the bumblebee *Bombus terrestris*. *Ecol Sci* 10:315-321
- Bartomeus I, Ascher JS, Wagner D, Danforth BN, Colla S, Kornbluth S, Winfree R. (2011) Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *PNAS* 108:20645-20649
- Beekman M, van Stratum P, Lingeman R (1998) Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). *Entmol Exp Appl* 89:207-214
- Bergman P, Molau U, Holmgren B (1996) Micrometeorological impacts on insect activity and plant reproductive success in an alpine environment, Swedish Lapland. *Arct Alp Res* 28:196-202
- Bingham RA (1998) Efficient pollination of alpine plants. *Nature* 391:238-239
- Diekmann M (1996) Relationship between flowering phenology of perennial herbs and meteorological data in deciduous forests of Sweden. *Can J Bot* 74:528-537
- Ellwood ER, Diez JM, Ibáñez I, Primack RB, Kobori H, Higuchi H, Silander JA (2012) Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming? *Oecologia* 168:1161-1171
- Fitter ML, Fitter RSR (2002) Rapid changes in flowering time in British plants. *Science* 296:1689-1691
- Forrest JRK, Thomson JD (2011) An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecol Monogr* 81:469-491
- Gordo O, Sanz JJ (2003) Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian Peninsula (1952-2004). *Ecol Entmol* 31:261-268

- Hegland SJ, Nielsen A., Lázaro A, Bjerknes A-L, Totland Ø. (2009) How does climate warming affect plant-pollinator interactions? *Ecol Lett* 12:184-195
- Heinrich B (1979) *Bumblebee Economics*. Harvard University Press, Cambridge
- Høye TT, Forchhammer MC (2008) Phenology of high-arctic arthropods: Effects of climate on spatial, seasonal, and inter-annual variation. *Adv Ecol Res* 40:299-324
- Hülber K, Winkler M, Grabherr G (2010) Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. *Funct Ecol* 24:245-252
- Iler, AM, Inouye DW, Høye TT, Miller-Rushing AJ, Burkle LA, Johnston EB (2013) Maintenance of temporal synchrony between syrphid flies and floral resources despite differential phenological responses to climate. *Glob Change Biol* 19:2348-2359
- Inouye DW (2008) Effect of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89:353-362
- Kameyama Y, Kudo G (2009) Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: effects of pollinators and self-incompatibility. *Ann Bot* 103:1385-1394
- Kudo G (1991) Effects of snow-free period on the phenology of alpine plants inhabiting snow patches. *Arc Alp Res* 23:436-443
- Kudo G (2006) Flowering phenologies of animal-pollinated plants: reproductive strategies and agents of selection. In: Harder LD, Barrett SCH (eds) *Ecology and Evolution of Flowers*. Oxford University Press, New York, pp 139-158
- Kudo G, Hirao AS (2006) Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: Implications for global-change impacts. *Popul Ecol* 48:49-58
- Kudo G, Hirao AS, Kawai Y (2011) Pollination efficiency of bumblebee queens

- and workers in the alpine shrub *Rhododendron aureum*. *Int J Plant Sci* 172: 70-77.
- Kudo G, Ida TY (2013) Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology* (in press).
- Kudo G, Imoto T (2012) Monitoring of bumblebee assemblages in the alpine zone of Daisetsuzan National Park. *Jap J Cons Ecol* 17:263-269 (in Japanese with English summary)
- Kudo G, Suzuki S (1999) Flowering phenology of alpine plant communities along a gradient of snowmelt timing. *Polar Biosci* 12:100-113
- Kudo G, Suzuki S (2002) Relationships between flowering phenology and fruit-set of dwarf shrubs in alpine fellfields in northern Japan: a comparison with a subarctic heathland in northern Sweden. *Arc Antarc Alp Res* 34:185-190
- Kudo G, Yokosuka K (2012) Spatiotemporal variations in flowering phenologies of alpine plant communities: long-term volunteer monitoring in an alpine ecosystem. *Jap J Cons Ecol* 17:49-62 (in Japanese with English summary)
- Memmott J, Craze PG, Waser NM, Price MV (2007) Global warming and the disruption of plant-pollinator interactions. *Ecol Lett* 10:710-717
- McKinney AM, Caradonna PJ, Inouye DW, Barr B, Bertelsen CD, Waser NM (2012) Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources. *Ecology* 93:1987-1993
- Miller-Rushing AJ, Høye TT, Inouye DW, Post E (2010) The effects of phenological mismatches on demography. *Phil Trans R Soc B* 365:3177-3186
- Mote PW, Hamlet AF, Clark MP, Lettenmaier DP (2005) Declining mountain snowpack in western North America. *Bull Ame Meteor Soci* 86:39-49
- Parmesan C (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Glob Change Biol* 13:1860-1872

- R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. <http://www.R-project.org>
- Rafferty NE, Ives AR (2011) Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecol Lett* 14:69-74
- Rixen C, Dawes MA, Wipf S, Hagedorn F (2012) Evidence of enhanced freezing damage in treeline plants during six years of CO₂ enrichment and soil warming. *Oikos* 121:1532-1543
- Root TL, Price JF, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming in wild animals and plants. *Nature* 421:57-60
- Schmid-Hempel P, Durrer S (1991) Parasites, floral resources and reproduction in natural populations of bumblebees. *Oikos* 62:343-350
- Thomson JD (2010) Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. *Phil Trans R Soc B* 365:3187-3199
- Wipf S (2010) Phenology, growth, and fecundity of eight subarctic tundra species in response to snowmelt manipulations. *Plant Ecol* 207:53-66
- Yoon HJ, Kim SE, Kim YS (2002) Temperature and humidity favorable for colony development of the indoor-reared bumblebee, *Bombus ignites*. *App Entomol Zool* 37:419-423

Table 1. Monthly air temperature (at 1.5 m height) and soil temperatures (at 10 cm depth) at the fellfield and snowbed sites in 2011 and 2012.

Year	April	May	June	July	August	September
Air temperature (°C)						
2011	-6.2	-0.2	7.3	12.8	12.2	8.0
2012	-4.3	2.4	7.9	11.7	13.4	11.1
Δ_{12-11} (°C)	1.9	2.6	0.6	-1.1	1.2	3.1
Soil temperature (°C)						
Fellfield ¹⁾						
2011	-2.9	0.6	8.1	14.3	13.7	9.7
2012	-4.0	2.8	8.8	12.7	14.2	11.8
Snowbed ²⁾						
2011	-1.5	-0.7	0.0	10.9	12.8	8.8
2012	-1.2	-0.1	2.9	10.8	14.3	12.3

1) Soil thawing date at the fellfield site was 23 May in 2011 and 1 May in 2012.

2) Snow-melting period at the snowbed plot was 10-31 July in 2011 and 25 June-20 July in 2012.

Table 2. Results of GLMs for bee abundance conducted in 2011 and 2012 for individual *Bombus* species. Observation year, date (day of year), cloud cover (0-10) and air temperature during observation are included as explanatory variables. Selected variables based on AIC are shown in each model.

	Coefficient	SE	z value	P value
<i>Bombus hypocrita</i> (overwintered queen)				
Intercept (Year 2011)	2.60	1.31	1.99	0.045
Year 2012	-1.40	0.34	-4.12	<0.001
Day ²	-4.49x10 ⁻⁴	8.17x10 ⁻⁵	-5.52	<0.001
Cloud cover	0.63	0.13	4.69	<0.001
Temperature	0.78	0.21	3.80	<0.001
<i>Bombus hypocrita</i> (current generation)				
Intercept (Year 2011)	-149.0	24.5	-6.07	<0.001
Year 2012	-0.97	0.30	-3.19	0.001
Day ²	-3.09x10 ⁻³	5.07x10 ⁻⁴	-6.10	<0.001
Day	1.38	0.22	6.17	<0.001
<i>Bombus beaticola</i>				
Intercept (Year 2011)	132.8	31.6	-4.20	<0.001
Day ²	-2.47x10 ⁻³	6.23x10 ⁻⁴	-3.96	<0.001
Day	1.16	0.28	4.12	<0.001
<i>Bombus hypnorum</i>				
Intercept (Year 2011)	-191.9	40.4	-4.75	<0.001
Year 2012	-2.26	0.36	-6.37	<0.001
Day ²	-4.24x10 ⁻³	8.83x10 ⁻⁴	-4.81	<0.001
Day	1.89	0.38	4.80	<0.001
Temperature	-0.14	0.08	-1.85	0.063
<i>Bombus yesonensis</i>				
Intercept (Year 2011)	-173.0	44.5	-3.89	<0.001
Day ²	-2.99x10 ⁻³	8.57x10 ⁻⁴	-3.49	<0.001
Day	1.43	0.39	3.66	<0.001
Cloud cover	0.13	0.05	2.57	0.010
Temperature	0.28	0.09	3.19	0.001

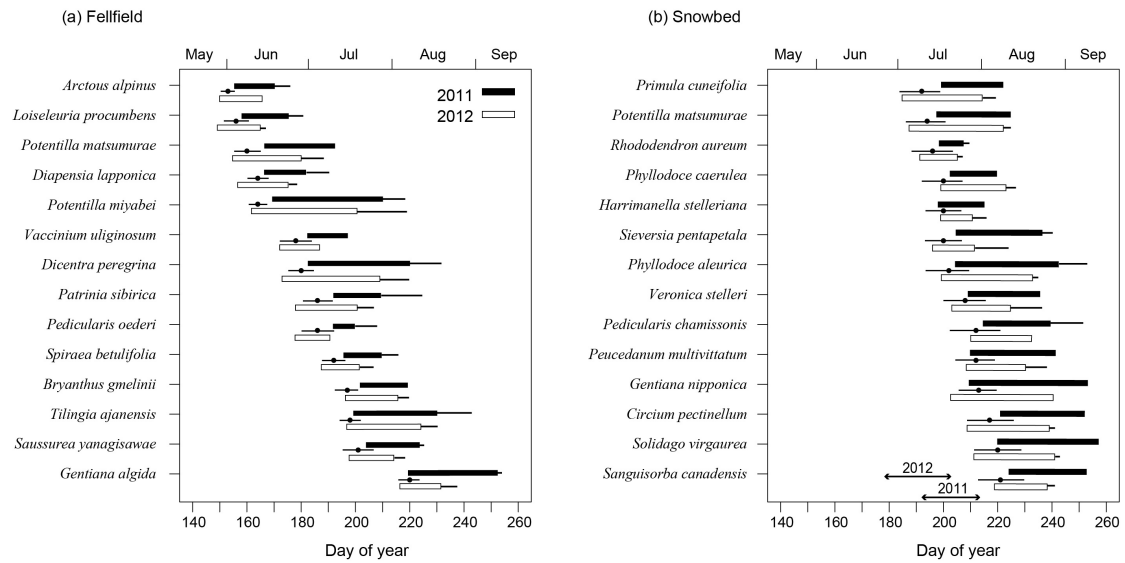


Fig.1 Flowering phenologies of 14 major species inhabiting the fellfield (a) and snowbed plots (b) in 2011 and 2012. Each column indicates major flowering period (stage A to C) and the vertical line indicates the remaining flowers (stage D) in each species. Dots indicate average flowering-onset date of individual species during 2006–2012 with SD. Snow-melting period within a plot is indicated in the snowbed plot.

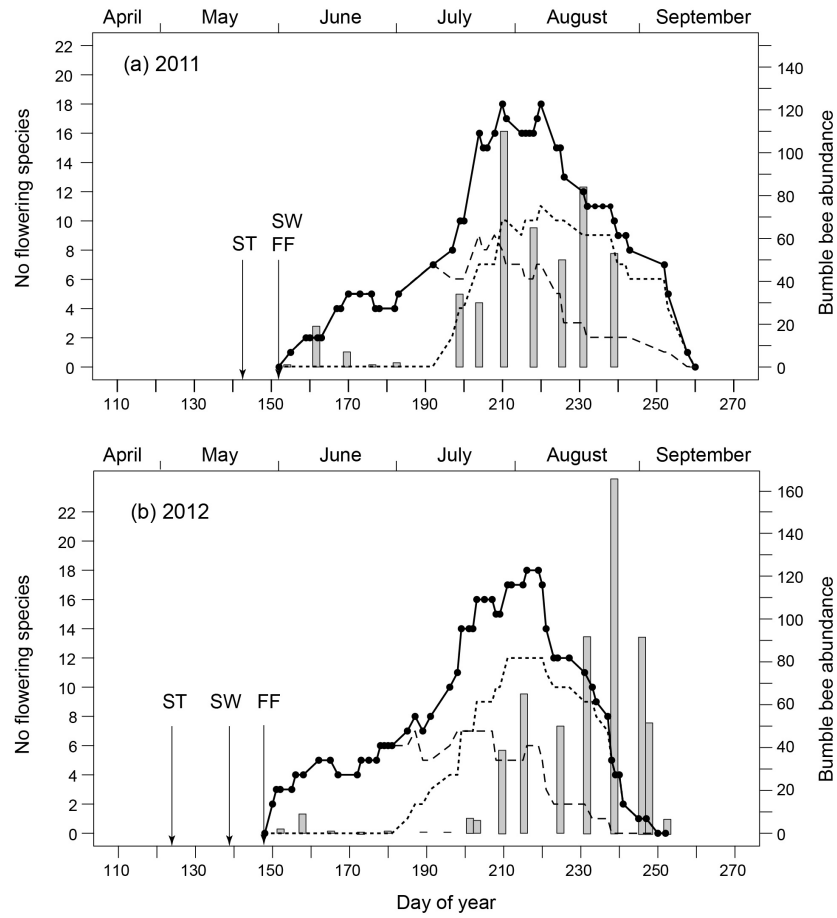


Fig.2 Seasonal transitions of the numbers of overlapping flowering species (solid line: total species, broken line: fellfield plot, dotted line: snowbed plot) and bees observed (histogram) in 2011 and 2012. Total bee number of all *Bombus* species is shown. ST: soil thawing date, SW: soil warming date in which soil temperature attained 6–8 °C, FF: first flowering date, PF: peak flowering period during which >50% of species were opening.

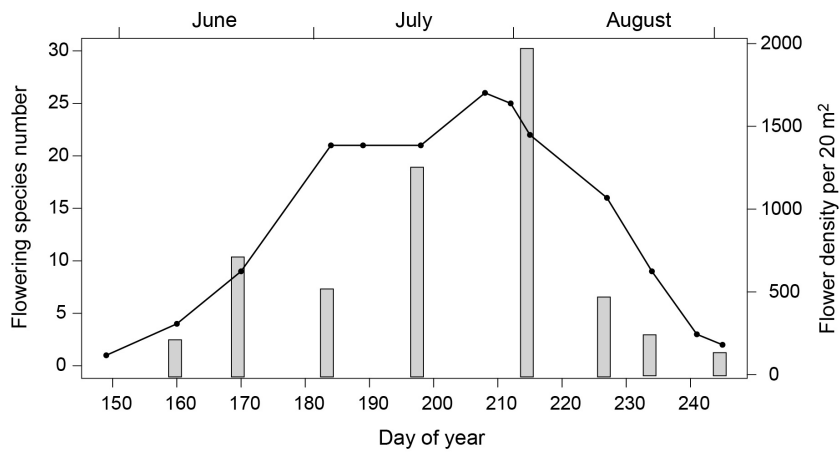


Fig.3 Seasonal changes in the number of overlapping flowering species (line) and flower densities (histogram) observed at regional scale on Mt. Kaun in 2012. Data of five plots (20-m x 20-m in size) arranged along the snowmelt gradient (about 400 m in length) are pooled.

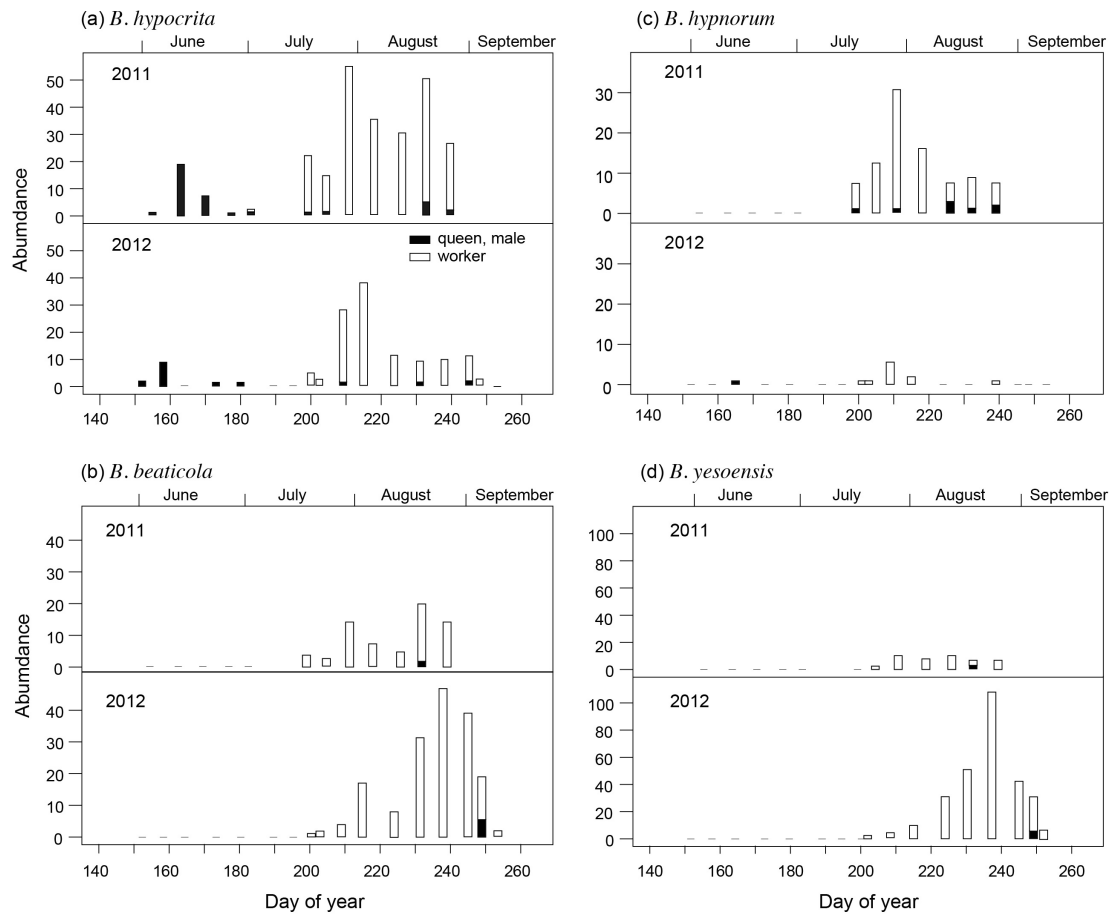


Fig.4 Seasonal trends in bee abundance (the number of observed bees) of four major *Bombus* species observed in each census in 2011 and 2012.

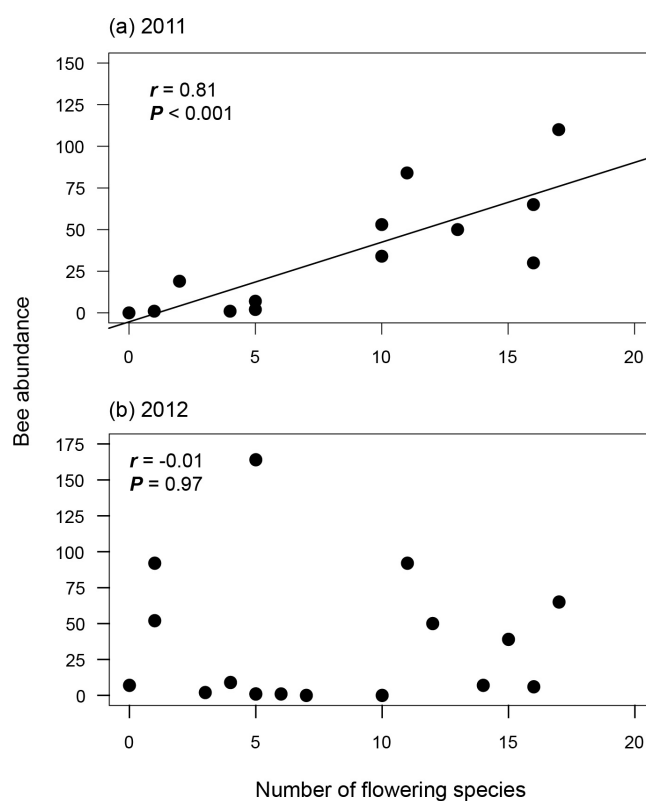


Fig.5 Relationships between the number of flowering species and the number of bee individuals (sum of all species) observed in each census in 2011 (a) and 2012 (b). Pearson's correlation coefficient and significance values are shown.