

Early onset of spring increases the phenological mismatch between plants and pollinators

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Abstract. Climate warming accelerates the timing of flowering and insect pollinator emergence, especially in spring. If these phenological shifts progress independently between species, features of plant–pollinator mutualisms may be modified. However, evidence of phenological mismatch in pollination systems is limited. We investigated the phenologies of a spring ephemeral, *Corydalis ambigua*, and its pollinators (bumble bees), and seed-set success over 10–14 years in three populations. Although both flowering onset and first detection of overwintered queen bees in the *C. ambigua* populations were closely related to snowmelt time and/or spring temperature, flowering tended to be ahead of first pollinator detection when spring came early, resulting in lower seed production owing to low pollination service. Relationships between flowering onset time, phenological mismatch, and seed-set success strongly suggest that phenological mismatch is a major limiting factor for reproduction of spring ephemerals. This report demonstrates the mechanism of phenological mismatch and its ecological impact on plant–pollinator interactions based on long-term monitoring. Frequent occurrence of mismatch can decrease seed production and may affect the population dynamics of spring ephemerals.

Key words: *Bombus* spp.; bumble bee; climate change; *Corydalis ambigua*; flowering phenology; hibernation; Hokkaido, Japan; phenological mismatch; pollinator activity; snowmelt time; spring ephemerals.

INTRODUCTION

Phenological shifts caused by climate change have been reported from many natural ecosystems during recent decades especially in high-latitude and high-altitude regions and in spring (Menzel and Fabian 1999, Fitter and Fitter 2002, Root et al. 2003, Gordo and Sanz 2005). Phenological modifications within and between communities should influence the biological interactions between plants and animals that include antagonistic and mutualistic relationships. Ecological significance of the phenological mismatch has been reported mainly in plant–herbivore interactions, such as timings of leaf emergence and appearance of insects in spring (e.g., Dewar and Watt 1992, Visser and Holleman 2001). As a mutualistic relationship between plants and animals, furthermore, the impact of the phenological mismatch between flowers and pollinators has been noticed in recent studies (Memmott et al. 2007, Hegland et al. 2009, Bartomeus et al. 2011, McKinney et al. 2012).

Although long-term studies on phenological interactions between plants and pollinators are very limited, there is a perspective that the phenological mismatch in the pollination process may be uncommon in natural ecosystems (e.g., Rafferty and Ives 2011). This is because

phenological shifts may progress at similar rates between plants and pollinators during the course of climate change (Hegland et al. 2009, Bartomeus et al. 2011) and/or plant–pollinator mutualisms can be restored even when heterogeneous phenological shifts occur among plant and pollinator species if they are generalists (Alarcón et al. 2008, Hegland et al. 2009). However, phenological mismatch tends to be serious under some specific situations. First, when either plant or pollinator species is a specialist in the pollination system, a phenological shift should decrease the fitness of the specialist (Memmott et al. 2007). Second, phenological shifts are especially large at the beginning of the growth season, i.e., spring (Fitter and Fitter 2002), thus phenological mismatch may be prominent in this period (Thomson 2010, McKinney et al. 2012). Finally, phenological mismatch is most probable when the available period of the resource (e.g., flowering period for specific pollinators) is short (Miller-Rushing et al. 2010, McKinney et al. 2012). These situations may occur between spring ephemerals and pollinators in the northern forest ecosystem (Kudo et al. 2008).

Spring ephemerals inhabiting deciduous forests commonly have a short life cycle restricted within a cool but bright period from early spring to early summer before canopy closure (Motten 1986). The flowering of spring ephemeral species progresses intensively during the early short period of this phenological window. Recently, Kudo et al. (2004) and Thomson (2010) reported that

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TABLE 1. Site characteristics: elevation, snow conditions, flowering time, and major pollinators in three natural deciduous forests in Hokkaido, Japan.

Site	Elevation	Snow depth in mid-winter	Snowmelt time	Flowering onset	Major pollinator†
NFP	lowland, 75 m	snowy, 80–140 cm	intermediate, mid-April	early, late April	<i>Bombus hypocrita</i>
TOEF	lowland, 90 m	less snowy, 20–50 cm	early early April	early, late April	<i>Bombus hypocrita</i>
JOZ	montane, 360 m	snowy, 90–180 cm	late, early May	late, early May	<i>Bombus hypocrita</i> , <i>Bombus hypnorum</i>

Note: See Appendix A for details of snowmelt time and flowering-onset time.

† Short-tongued *B. hypocrita* is an illegitimate pollinator, while long-tongued *B. hypnorum* is a legitimate pollinator of *Corydalis ambigua* (Papaveraceae).

the pollen limitation of bee-pollinated spring ephemerals, which are predominantly visited by overwintered queens of bumble bees, increased when flowering occurred earlier. These studies suggest the existence of phenological mismatch indirectly but the responses of plants and pollinators to warm springs have not been quantified in previous studies. In the present study, we analyze the between-year variations in flowering onset, pollinator detection, and pollination success of a spring ephemeral species, *Corydalis ambigua*, which is predominantly visited by bumble bees, based on 10–14 years' monitoring in three populations in northern Japan. We aim to clarify (1) the mechanism of phenological mismatch under fluctuating spring environments, (2) the ecological significance of phenological mismatch in the plant–pollinator interaction, and (3) provide perspectives on the dynamics of phenological mismatch during warming climate in a northern forest ecosystem.

MATERIALS AND METHODS

Site description

This study was conducted in three natural deciduous forests in Hokkaido, northern Japan (Table 1). The main site was in the Nopporo Forest Park (NFP; 43°02' N, 141°31' E, 75 m elevation), a lowland forest 13 km east of Sapporo, where monitoring has been conducted for 14 years since 1999. The second site was in the Tomakomai Experimental Forest of Hokkaido University (TOEF; 42°42' N, 141°31' E, 90 m elevation), a lowland forest 55 km south of Sapporo, where monitoring was conducted from 1999 to 2008. The third site was in a montane forest in Jozankei (JOZ; 42°59' N, 141°06' E, 360 m elevation), 33 km northwest of Sapporo, where monitoring has been conducted for 14 years since 1999. NFP and JOZ belong to the Japan Sea coastal climate with heavy snow, while TOEF belongs to the Pacific Ocean side climate with little snow. Snow usually disappears in mid-April in NFP, early April in TOEF, and early May in JOZ. Frozen soil is commonly formed throughout the winter and early spring in TOEF, while formation of frozen soil is occasional in NFP and JOZ owing to insulation by thick snow cover. Spring air temperatures (average of last 30 years; Japan Meteorological Agency, Tokyo, Japan) are 0.6°C in March, 7.1°C in April, and 12.4°C in May in Sapporo (closest weather station to NFP), 0.1°C, 5.1°C, and 9.5°C

in Tomakomai (closest weather station to TOEF), and –1.5°C, 5.0°C, and 10.3°C in JOZ (estimation from the lapse rate of –0.6°C/100 m elevation).

Plant species and pollinators

Corydalis ambigua (Papaveraceae) is a perennial herbaceous species inhabiting the floor of deciduous forests in northern Japan (see Plate 1). It has a typical life cycle of spring ephemerals. Flowering occurs 10–14 days after snowmelt (mid-April to mid-May; Table 1; Appendix A); the major flowering period lasts 10 days within populations; and seeds are dispersed about 10–14 days after flowering. Aboveground parts commonly die after seed dispersal by the time of canopy closure in early summer. Thus, the annual growth period is only one month. This species is self-incompatible and predominantly pollinated by overwintered bumble bee queens (*Bombus* spp.; see Plate 1) that visit plants to feed on nectar before nesting (Higashi et al. 1988, Kudo and Kasagi 2004). A defoliation experiment revealed that current photosynthetic products did not contribute to fruit development, suggesting that carbon storage in underground tissue is used for seed production (Kudo and Ida 2010). It means that resource limitation is not a primary factor determining annual seed production in this species (Kudo et al. 2004).

Lowland *C. ambigua* populations (NFP and TOEF) are commonly visited by a short-tongued bee species, *Bombus hypocrita sapporoensis* (hereafter referred to as *B. hypocrita*), which is the earliest species to emerge from hibernation. They usually rob nectar by perforating spurs of flowers and seldom visit legitimately but are an available pollinator owing to accidental pollen removal and deposition during nectar robbing (Higashi et al. 1988). Therefore, *B. hypocrita* is recognized as a robber-like pollinator of *C. ambigua*. Our previous study revealed that *C. ambigua* in lowland populations highly depends on *B. hypocrita* visits for seed production (Kudo and Kasagi 2004). In contrast, the montane population (JOZ) is frequently visited by both *B. hypocrita* and a long-tongued species, *B. hypnorum koropokkrus* (here after referred to as *B. hypnorum*) that behaves as a legitimate pollinator. Although *B. hypocrita* is the most common pollinator of *C. ambigua*, pollination efficiency with *B. hypocrita* is lower than that of *B. hypnorum* (Kudo and Kasagi 2004). Other bumble

bee species, *B. ardens sakagami* and *B. diversus tersatus* also visit *C. ambigua* flowers legitimately in every site but less frequently (Kudo and Kasagi 2004). Our previous observations (Kudo and Kasagi 2004; G. Kudo, unpublished data) revealed that *B. hypocrita* achieved 90% and 86% of flower visits to *C. ambigua* and the remaining 10% and 14% were queens of *B. ardens* in NFP and TOEF, respectively. In contrast, 40% of visitors were *B. hypnorum*, 58% were *B. hypocrita*, and only 2% were other *Bombus* species in JOZ.

Monitoring of first flowering, initial bumble bee activity, and seed production

In each population, we set a monitoring plot of about 50 × 50 m in the forest, and recorded the first flowering day of *C. ambigua* (hereafter, flowering onset day) and the first detection day of bumble bees every year after snowmelt in early spring. There is a controversy on the use of first flowering day as an index of phenological shift (Miller-Rushing et al. 2008). Because the flowering of *C. ambigua* populations progresses rapidly and it usually takes less than five days from first flowering to peak flowering, use of first flowering day is a practical metric. Initial bumble bee activity was observed around the plots based on observation of flower visiting or flying queen bees or first detection of nectar robbing scars on *C. ambigua* flowers made by *B. hypocrita*. Note that the bee detection day in the plot might not exactly reflect the emergence day from hibernation. Because *C. ambigua* is the earliest major nectar resource for overwintered bees, first detection of nectar robbing scars may reflect the time of emergence from hibernation when flowering occurred ahead of bee emergence. However, detection of bees before flowering highly depends on the frequency of observation. Therefore, our measurement of initial bee activity on the plot might contain some bias when bees emerged before flowering.

Phenological mismatch is defined as the delay in initial bee activity from flowering onset in this study. Therefore, negative phenological mismatch values indicate bee detection before flowering onset. Furthermore, snowmelt day around the plot was also recorded every year in NFP and TOEF. However, determination of snowmelt day in JOZ was difficult because of the heterogeneous snow-melting pattern owing to the undulating slopes. Thus, we expressed the snowmelt time in JOZ at 10-day intervals for analysis, i.e., 5th (early), 15th (middle), or 25th (late) of each month (see Appendix A).

Seed-set success under natural pollination was measured for 40–60 arbitrarily selected plants producing a single inflorescence in each plot in every year. In the flowering season, we recorded number of flowers per inflorescence, marked the inflorescences with numbering tags, and exposed them to open pollination. Just before seed dispersal, we harvested all infructescences separately and counted the number of fruits. At the time of fruit harvesting, infructescences that were mechanically dam-

aged or browsed by herbivores or that had dispersed were excluded, resulting in the number of plants harvested ranging from 21 to 60 plants per site-year. In the laboratory, we opened all fruits carefully and then counted the number of mature seeds and ovules produced in each fruit. Seed-set success per plant was calculated as a ratio of seed number to original ovule number produced in each inflorescence.

To evaluate the potential seed production without pollen limitation, we conducted a hand-pollination experiment in each population. Nineteen to 50 plants were arbitrarily selected and marked with numbered tags. All flowers in each plant were hand-pollinated with pollen of a separate donor located >5 m away to avoid negative effects of inbreeding depression. Seed-set success per plant by hand-pollination was obtained as mentioned before. The hand-pollination experiment was conducted three or four times during the observation period (1999–2012; see Appendix A).

Statistical analysis

Phenological mismatch was analyzed using a generalized linear model (GLM) postulating normal error distribution with log(flowering onset day) (day of the year, 1 January is day 1) and site (NFP, TOEF, JOZ) as explanatory factors. Effects of environmental factors (snowmelt time and spring temperature) on phenologies of flowering onset and initial bee activity were analyzed for every site with GLM postulating lognormal error distribution, in which type of events (flowering onset or first bee detection), site, log(snowmelt day), April mean air temperature, and their interactions were included as explanatory factors. GLM analyses were performed with SAS 9.3, using the genmod/glimmix procedure (*F* tests, with [possibly fractional] degrees of freedom calculated according to Kenward and Roger [1997]). Overdispersion was corrected by overdispersion parameter with deviance according to McCullagh and Nelder (1989).

Seed-set success (seed/ovule ratio) under natural pollination was compared among sites over years from two perspectives. First, temporal fluctuation pattern of seed-set success over years was analyzed at each site with an auto-regressive model using R version 2.12.1 (using the stats package, AR function based on Yule-Walker method; R Development Core Team 2011). In this analysis, differences in seed-set success (after log-transformation) between the current and previous years were used for the calculation of auto-regression, and the best model was selected based on the Akaike's information criterion (AIC). Analyses were conducted during 2006–2012 in NFP (due to a lack of data for 2004), 2000–2008 in TOEF, and 2000–2012 in JOZ. Second, to assess the phenological responses, seed-set success was analyzed with GLMs postulating binomial error distribution (logit-link) in which site, year, flowering onset, or phenological mismatch, and their interactions were included as explanatory factors. To facilitate interpretation, we illustrated the relationships of dependent

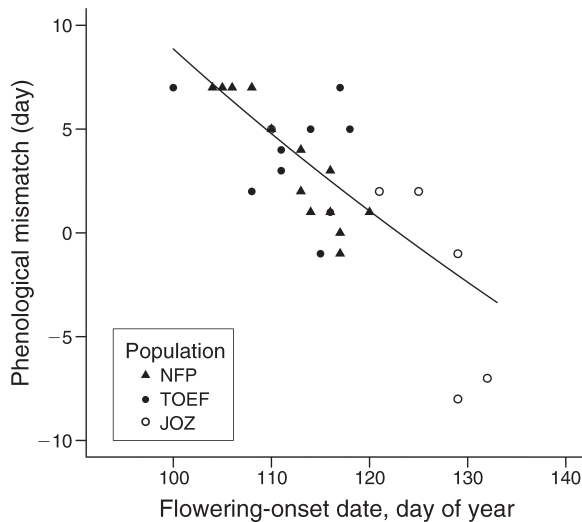


FIG. 1. Relationship between phenological mismatch between plants and pollinators (flowering-onset date and bee detection date) and flowering-onset date in three populations of *Corydalis ambigua* (Papaveraceae) in three natural deciduous forests in Hokkaido, Japan. The extent of phenological mismatch is simply determined by the time of flowering irrespective of populations.

variables to specific categorical independent variables with least-squares means, which represented average responses as though the measured effects of other independent variables considered in an analysis (e.g., covariates) were held constant (Milliken and Johnson 1984).

Seed-set success of hand-pollinated plants was compared with that of naturally pollinated plants in the same years to evaluate pollen limitation. Generalized linear mixed-model (GLMM) postulating binomial error distribution (logit-link) was used for the analysis in which pollination treatment (natural vs. hand pollination) was set as an explanatory factor and year as a random factor in each site (using the glmmML library; R Development Core Team 2011).

RESULTS

Phenologies of flowers and pollinators

Mean values of the first flowering dates of *C. ambigua* were 23 April (range 14–30 April) in NFP, 22 April (10–28 April) in TOEF, and 6 May (20 April–13 May) in JOZ during the observation periods. Mean values of the first detection dates of queen bees were 26 April (21 April–1 May) in NFP, 26 April (17 April–4 May with one year missing data) in TOEF, and 6 May (25 April–8 May with six years missing data) in JOZ (Appendix A).

Phenological mismatch was negatively correlated with flowering onset (Fig. 1; partial regression coefficient of $\log(\text{flowering onset})$, $b \pm \text{SE} = -42.8 \pm 7.84$). This trend was consistent among sites (site, $F_{2,24} = 3.04$, $P = 0.07$; $\log(\text{flowering onset})$, $F_{1,24} = 29.83$, $P < 0.0001$; site \times $\log(\text{flowering onset})$, $F_{2,24} = 3.04$, $P = 0.07$). Therefore,

first bee detection did not follow the flowering onset time of *C. ambigua* when flowering occurred early in the spring in every site.

Generally, spring phenologies were positively correlated with snowmelt time and negatively with April temperature, but the effects of snowmelt time and temperature differed among sites (Table 2, Fig. 2). In NFP (Fig. 2a), the sensitivity to snowmelt time was significantly stronger for flowering onset (partial regression coefficient of site \times event \times $\log(\text{snowmelt day})$; 0.84 ± 0.12) than for first bee detection (0.43 ± 0.12 ; $t_{53} = 3.07$, $P = 0.0034$). Therefore, the phenological mismatch between plants and pollinators was accentuated as snow melted earlier. In contrast, April temperature affected neither flowering onset nor bee detection in NFP (Fig. 2b; overall partial regression coefficient of site \times April temperature; 0.0001 ± 0.00429 ; $t_{53} = 0.03$, $P = 0.98$).

In TOEF, snowmelt time did not affect either response (Fig. 2c; flowering onset, 0.064 ± 0.117 , $t_{53} = 0.55$, $P = 0.59$; bee detection, 0.17 ± 0.12 , $t_{53} = 1.44$, $P = 0.16$), while April temperature negatively influenced the flowering onset and bee detection equally (Fig. 2d; overall partial regression coefficient, -0.054 ± 0.0087 , $t_{53} = -6.27$, $P < 0.0001$). These results suggest that the extent of phenological mismatch was constant regardless of snowmelt date.

In JOZ (Fig. 2e), snowmelt time affected flowering onset and bee detection differently ($t_{53} = 3.66$, $P = 0.0006$). The sensitivity to snowmelt time was stronger for flowering onset (partial regression coefficient of site \times event \times $\log(\text{snowmelt day})$, 0.68 ± 0.11) than for bee detection (0.22 ± 0.12), suggesting the increase in phenological mismatch with earlier snowmelt similar to NFP. Furthermore, April temperature negatively influenced the flowering onset and bee detection (Fig. 2f; overall partial regression coefficient, -0.015 ± 0.0053 , $t_{53} = -2.91$, $P = 0.0052$).

Variation in seed-set success

Seed-set success under natural pollination varied highly from year to year within populations, in the ranges of 14–66% in NFP, 4–78% in TOEF, and 20–75% in JOZ (Appendix A). In contrast, seed-set success

TABLE 2. Results of GLM for phenological events (flowering-onset and first bee detection dates) in which site (NFP, TOEF, JOZ), snowmelt time, April temperature, and their interactions are included as explanatory variables.

Effect	<i>F</i>	df	<i>P</i>
Site	8.48	2, 53	0.0006
Event (flowering onset vs. bee detection)	11.46	1, 53	0.0013
$\log(\text{snowmelt day})$	50.39	1, 53	<0.0001
April temperature	40.36	1, 53	<0.0001
Event \times site	5.13	2, 53	0.0092
Site \times $\log(\text{snowmelt day})$	7.56	2, 53	0.0013
Event \times $\log(\text{snowmelt day})$	10.80	1, 53	0.0018
Site \times April temperature	16.42	2, 53	<0.0001
Site \times event \times $\log(\text{snowmelt day})$	5.10	2, 53	0.0094

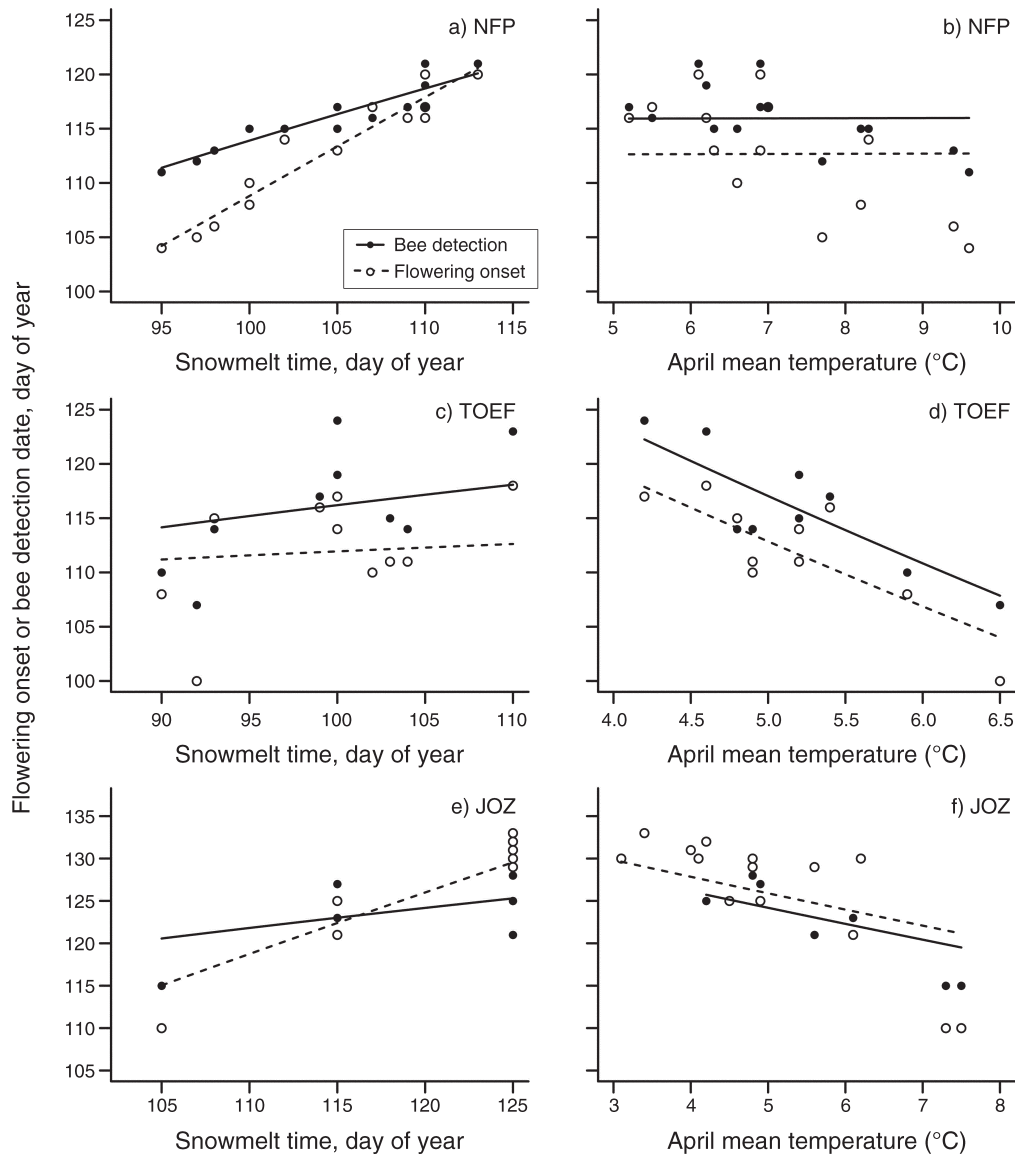


FIG. 2. Relationships between phenological events in spring (flowering onset of *Corydalis ambigua* or first detection of bumble bees) and snowmelt time and between phenological events and April temperature in (a, b) Nopporo Forest Park (NFP), (c, d) Tomakomai Experimental Forest (TOEF), and (e, f) Jozankei (JOZ). Refer to Table 2 for statistical results.

by hand-pollination was stably high throughout the years; 69–80% in NFP, 67–74% in TOEF, 77–85% in JOZ although the experimental periods were limited and fragmented among sites (Appendix A). The GLMM analysis revealed that hand-pollinated plants had significantly higher seed-set success than naturally pollinated plants in every site ($z = 25.60$, $P < 0.0001$ for NFP; $z = 36.72$, $P < 0.0001$ for TOEF; $z = 12.35$, $P < 0.0001$ for JOZ), indicating that seed production of *C. ambigua* was restricted by pollen limitation in every population. An auto-regressive analysis revealed that seed-set success of the current year (Y_n) was negatively correlated with the seed-set of the past year (Y_{n-1}) in

every population, i.e., order = 1 year, indicating alternate-year oscillation in seed-set success (Fig. 3). Interestingly, seed-set patterns were clearly synchronized among populations.

In the GLM analysis of seed-set success considering flowering-onset date, seed-set success significantly differed among years (Table 3), indicating that year-specific factors other than flowering-onset date also could influence seed-set success. After consideration of yearly variation, seed-set success significantly increased with a delay in flowering irrespective of site differences (Fig. 4a; partial regression coefficient, 9.07 ± 1.80). Thus, relatively higher seed-set success in JOZ in

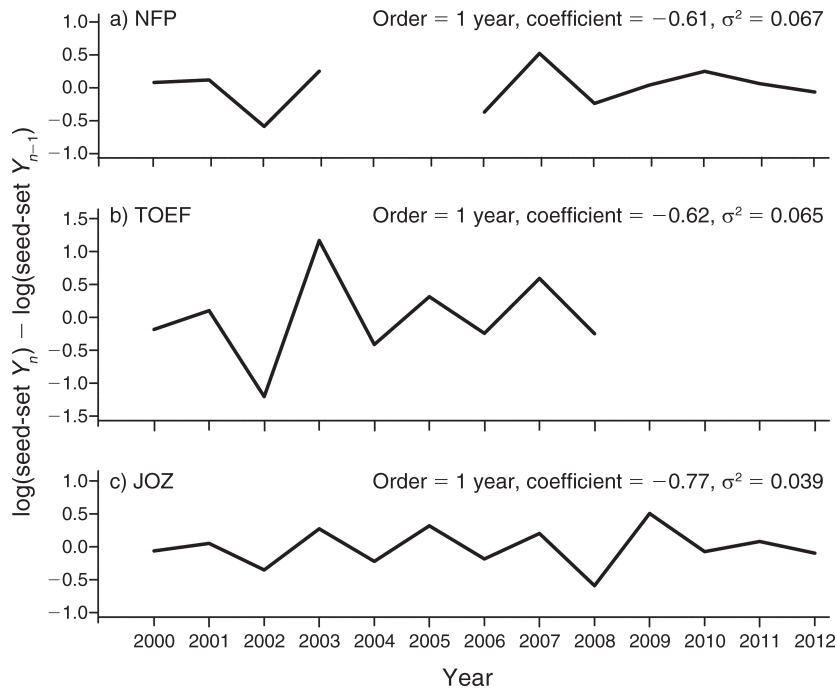


FIG. 3. Between-year differences in log-transformed seed-set success of *Corydalis ambigua* in three populations. Auto-regressive analyses were performed during (a) 2006–2012 in NFP, (b) 2000–2008 in TOEF, and (c) 2000–2012 in JOZ; and selected results (order of effective time scale, coefficient, and variance) are indicated in each population.

comparison with NFP and TOEF was simply caused by the later flowering period owing to later snowmelt time in the montane site.

In the GLM analysis of seed-set success considering phenological mismatch, seed-set success decreased significantly with increasing phenological mismatch (Fig. 4b), and this trend differed among sites (significant site \times mismatch interaction) after consideration of yearly variation (Table 3). Partial regression coefficients of site \times mismatch interaction varied among sites (Fig. 4b; TOEF, -0.160 ± 0.042 , $G_1 = 14.91$, $P < 0.0001$; NFP, -0.115 ± 0.029 , $G_1 = 116.19$, $P < 0.0001$; JOZ, -0.042 ± 0.023 , $G_1 = 3.53$, $P > 0.05$). Thus, the sensitivity of seed-set success to phenological mismatch decreased in the order TOEF, NFP, and JOZ. Adjusted mean seed-set success (after consideration of phenological mismatch effect) was marginally lower in NFP (0.41) than TOEF (0.50) and JOZ (0.50).

DISCUSSION

The present study revealed that the sensitivity to earlier onset of spring is different between *C. ambigua* and bumble bees, which causes phenological mismatch between them, and the phenological mismatch decreases seed-set success. This clear relationship might arise because *C. ambigua* completely depends on overwintered queen bees and because the phenological window of this plant–pollinator interaction is restricted to a short period in early spring.

Mechanism of phenological mismatch

Phenological responses to environmental factors differed between NFP (snowmelt time) and TOEF (spring temperature), and intermediate in JOZ (both of snowmelt time and temperature). In snowy NFP and JOZ, snowmelt time was a primary determinant of spring phenologies especially for plants. Because annual shoots of *C. ambigua* usually emerge under snow and start to grow soon after snow disappearance in NFP and JOZ, flowering onset highly depends on the snowmelt regime of individual years (Kudo et al. 2004), as reported in other spring ephemerals (e.g., Thomson

TABLE 3. Results of GLMs for seed-set success in which flowering-onset date or phenological mismatch, year, site (NFP, TOEF, JOZ), and their interactions are included as explanatory variables.

Effect	F	df	P
Phenological shift (flowering onset) model			
Year	20.07	13, 1487	<0.0001
Site	2.08	2, 1487	0.1250
log(flowering onset)	25.95	1, 1487	<0.0001
Site \times log(flowering onset)	2.10	2, 1487	0.1223
Phenological mismatch model			
Year	10.77	12, 1142	<0.0001
Site	2.81	2, 1142	0.0605
Mismatch	29.08	1, 1142	<0.0001
Site \times mismatch	5.03	2, 1142	0.0067

Note: Effects of flowering onset and phenological mismatch between flowering and pollinator activity are analyzed separately because of a strong correlation between them (see Fig. 1).

2010), provided cool temperature during early spring does not restrict growth initiation (Badri et al. 2007). In the montane site (JOZ), however, not only snowmelt time but also April temperature affected the flowering onset and bee detection times. Because air temperature in JOZ was 2.1°C lower than that in NFP, cool temperature might restrict phenological progress in early snowmelt spring. In less-snowy TOEF, on the other hand, April temperature strongly influenced flowering onset. Snowmelt in TOEF occurred 4.2 days earlier than in NFP on average, and April temperature in TOEF (5.1°C) was 2.0°C lower than that in NFP. Threshold temperature for growth initiation of understory herbs is supposed to be around a daily mean temperature of 5°C (Diekmann 1996). Thus, weather conditions soon after snowmelt in TOEF might be too cool for growth initiation. Actually, annual shoots of *C. ambigua* usually have not emerged when snow disappears in TOEF (G. Kudo, *personal observation*), probably caused by the freezing soil temperature under thin snow cover.

The emergence of queen bees, which commonly hibernate in the ground, may be related to the increase in soil temperature. Alford (1969) reported that bumble bees tended to hibernate at a depth of 5–8 cm in the ground, and emerge from hibernation when the soil temperature rose to 5–9°C in Britain. At the NFP site, the first detection of queen bees corresponded to the time when soil temperature at a depth of 5 cm reached 7°C in 2012 (G. Kudo, *unpublished data*). Soil under thick snow cover usually remains at 0–1°C throughout the winter owing to insulation by the snow. Soil temperature tends to increase more slowly when snow melts earlier in the season, probably owing to exposure to cool weather in early spring. Therefore, an increase in phenological mismatch with acceleration of snowmelt time in NFP may reflect the pattern of soil temperature increase dependent on the release timing of snow cover.

Similar to flowering phenology, first bee detection in TOEF was determined by April temperature independent of snowmelt time. This indicates that spring soil temperature is simply influenced by air temperature when insulation by snow is not effective. However, phenological mismatch was largest in the unusually warm spring in 2002. We speculated that the combination of warm April temperature and late soil thawing accentuated the degree of mismatch. The development of frozen soil usually reaches a maximum in mid-March in TOEF, and its depth is influenced by winter temperature and snow depth (Kinoshita et al. 1978). Therefore, it is difficult to accurately predict the occurrence of phenological mismatch from spring climate alone. As mentioned before, we did not measure exact date of bee emergence from hibernation in this study but recorded the date of first detection in the plots. Therefore, our calculation of phenological mismatch might be underestimated when bee emergence from hibernation occurred earlier than the flowering onset

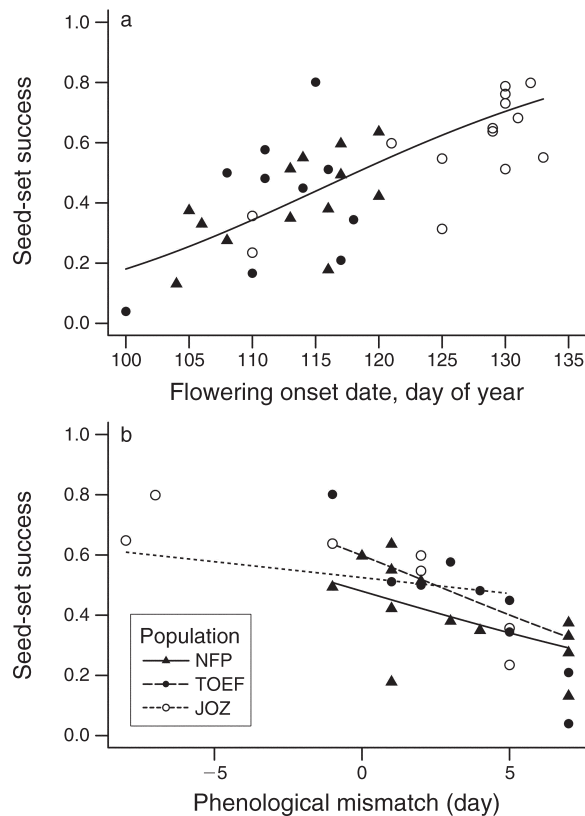


FIG. 4. (a) Relationships between seed-set success (seed/ovule ratio) and flowering-onset date, and (b) relationships between seed-set success and phenological mismatch across three populations of *Corydalis ambigua*. The effect of flowering time on seed-set success is consistent among populations, whereas the effect of phenological mismatch on seed-set success varies between populations. Refer to Table 3 for statistical results.

although the situation was not common in NFP and TOEF (Appendix A). To clarify the responses of bumble bees to spring climate, more extensive monitoring and experimental study on the physiological hibernation control are needed.

There are contrasting viewpoints on the phenological responses to climate change between plants and insects. In the comparisons of many species over geographical scale, the sensitivity of phenology to temperature was higher in insects than in plants (Gordo and Sanz 2005, Parmesan 2007). At the regional scale, in contrast, phenological responses to warm spring climate were more prominent in plants than insects because the threshold temperature for growth initiation or termination from hibernation was higher in insects (Forrest and Thomson 2011). Our study supports the latter case, although Forrest and Thomson (2011) measured emergence time of solitary bees and wasps that overwinter aboveground. Thus, the spring phenology of these insects should be influenced by air temperature. Generally, spring air temperatures are good correlates of the



PLATE 1. Overwintered queen bee (*Bombus hypocrita sapporoensis*) visiting *Corydalis ambigua* flowers. Photo credit: G. Kudo.

first emergence of insects such as honey bees and butterflies (Roy and Sparks 2000, Gordo and Sanz 2006, Bartomeus et al. 2011). In bumble bees, however, emergence time may be determined by the thermal conditions in the ground, which are influenced by the interactions between snow depth, winter temperature, and spring temperature.

Factors affecting seed-set success

The present study suggested that seed-set success of *C. ambigua* was strongly restricted by the lack of pollinators caused by phenological segregation between plants and pollinators. Although the extent of pollen limitation was insufficiently quantified in this study (only 3–4 years of measurements), our limited experiment revealed that hand-pollinated plants could attain stably high seed production even in early flowering years such as 2002 (Appendix A). It suggests that pollen limitation is the most important factor determining seed-set success of *C. ambigua*. To the best of our knowledge, this is the first report demonstrating the ecological significance of phenological mismatch in a pollination system based on the long-term monitoring of reproductive success.

The increase in frost damage with the acceleration of flowering time may be an important factor affecting seed-set success for early flowering plants (e.g., Inouye 2000). However, failure of seed production due to frost

damage was not observed in any population even in early flowering years, indicating that spring frost does not influence the seed production of *C. ambigua* at least in these sites. Furthermore, our results also indicate that factors other than phenological mismatch affect seed production as well. First, abundance of queen bees is an important factor affecting visitation frequency to individual flowers. A recent study conducted in TOEF demonstrated that canopy floral resources clearly fluctuated in alternate years and affected the population dynamics of bumble bees (Inari et al. 2012). Many new queens were produced in the years of mass floral production by canopy trees, which resulted in quantitative pollination service to spring ephemerals next spring, but colony development and new queen production were restricted owing to poor canopy floral resources in the next summer, resulting in low pollination service the following spring. As a result, seed-set success of *C. ambigua* fluctuated in alternate years in response to fluctuations in canopy flower production (Inari et al. 2012). Synchronized alternate-year oscillation in seed-set success among populations may reflect the synchronized canopy flower production among species within and between deciduous forests (Shibata et al. 2002).

Second, differences in the pollination efficiency and life cycle between bumble bee species should affect seed-set success among sites and years. The proportion of

long-tongued bees (*B. hypnorum* or *B. ardens*) was highest in JOZ and lowest in NFP. These long-tongued species have higher pollination efficiencies for *C. ambigua* than short-tongued species (*B. hypocrita*) owing to legitimate flower use (Kudo and Kasagi 2004). In the lowland populations, *B. hypocrita* is the earliest species, while long-tongued species (*B. ardens* and *B. diversus*) commonly appear later than the peak flowering of *C. ambigua* (Inari et al. 2012). In the montane population, however, another long-tongued species, *B. hypnorum*, is commonly active at almost the same time as *B. hypocrita* (Kudo and Kasagi 2004). Therefore, frequent visits of long-tongued species might mitigate the negative effect of phenological mismatch in JOZ.

Implications for the impact of global change

The results of our study revealed that modifications of snow regime (depth and duration) are important drivers of phenological mismatch in northern ecosystems. During the last 50 years, spring temperatures in Sapporo have steadily increased, especially in March (0.33°C per decade in March and 0.24°C per decade in April; Japan Meteorological Agency; Appendix B). Although snowmelt day is negatively correlated with March temperature (Spearman's correlation test, $r = -0.58$, $t_{49} = -4.91$, $P < 0.0001$; Appendix B), there is no evidence that snowmelt date has been accelerated during the last 50 years ($r = 0.091$, $t_{49} = 0.63$, $P = 0.53$; Appendix B) probably as a result of large fluctuations from year to year. Therefore, directional changes in phenological mismatch between spring ephemerals and pollinator activity are not evident at the present time. However, disturbance of plant–pollinator interactions may arise from extreme weather events driven by climate change (e.g., Harrison 2000). During the 14-yr monitoring period, we detected especially large decreases in seed production in 2002 and 2008 when snow disappeared extremely early in the season. Thus, the frequency of unusual events could cause serious ecological impacts in plant–pollinator interactions.

For further understanding of the ecological significance of phenological mismatch, the impact on population dynamics should be evaluated (Miller-Rushing et al. 2010). Unexpected fluctuations in seed production may influence the population dynamics of plants, especially for short-lived species (Miller-Rushing et al. 2011). *C. ambigua* is a relatively short-lived perennial species, and the variation in seed production strongly influenced the population dynamics from year to year (G. Kudo, unpublished data). Therefore, more frequent warm springs with little snow may increase phenological mismatch, resulting in population decline among spring ephemerals.

On the other hand, the late emergence of bumble bees after anthesis of spring ephemerals will cause little impact on the population dynamics of bees because they can use many other floral resources that continuously appear in both understory and canopy layers within

forests (Kudo et al. 2008, Inari et al. 2012). Therefore, the ecological impact of phenological mismatch is asymmetric and highly dependent on the life history of individual species. This suggests that meta-analyses of phenological events of many plants and animals may dilute the ecological significance of phenological mismatch if the asymmetric structures of interactions between species are not taken into account.

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SUPPLEMENTAL MATERIAL

Appendix A

Data set of the long-term analyses of snowmelt time, flowering-onset date, and seed-set success of *Corydalis ambigua*, and first detection date of *Bombus* queens at each site over 10–14 years ([Ecological Archives E094-213-A1](#)).

Appendix B

Climate conditions in Sapporo during the last 50 years: transitions of monthly mean temperatures in March and April, relationship between March temperature and snowmelt date, and snowmelt date ([Ecological Archives E094-213-A2](#)).