ORIGINAL PAPER

Genetic differentiation in the timing of budburst in *Fagus crenata* in relation to temperature and photoperiod

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Abstract
Climate change is expected to influence plant productivity particularly through changes in the timing of budburst. Nonetheless, knowledge about the intraspecific variation of the timing of budburst and its relationship with climate is insufficient for most tree species. Based on the common garden experiments of *Fagus crenata*, we investigated the interrelationships between the day of budburst, cumulative degree-days (temperature sum), chilling duration, and photoperiod at the timing of budburst for the trees of different combinations of 11 sites of seed origin and seven experimental sites in Japan. We found that the relationship between the latitude of experimental sites and the timing of budburst differed for the trees of different latitudes of origins. The timing of budburst was earlier for the trees of more northern populations throughout the latitudes of experimental sites. Variation in the timing of budburst among the trees of different seed origins was smaller for more northern experimental sites. Such patterns were caused by directional changes in the relationships between temperature sum, chilling duration, and photoperiod among the trees of different origins: the asymptotes of the curvilinear relationship between chilling duration and temperature sum, chilling duration and photoperiod, and temperature sum and photoperiod, decreased for more northern populations. With the northward expansion of species distribution, the responses of budburst to climate probably changed genetically in such ways in this species. Our results suggest that intraspecific variations in the relationships between the timing of budburst and associated meteorological factors inevitably influence the overall pattern of the timing of budburst at the geographic scale, and the timing of budburst might deviate from predictions when intraspecific variations are not considered.

Keywords Chilling duration · Global warming · Intraspecific variation · Provenance · Spring phenology · Thermal time

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Introduction

In temperate regions, leaf phenology is one of the important factors that affect plant productivity by regulating the annual growth period (Root et al. 2003; Polgar and Primack 2011). Climate change is expected to influence plant productivity particularly through changes in the timing of budburst (Menzel and Fabian 1999; Root et al. 2003; Polgar and Primack 2011). Advances in the timing of budburst in response to global warming have been observed in a number of temperate tree species (Root et al. 2003; Parmesan and Yohe 2003; Parmesan 2006; Menzel et al. 2006). The timing of budburst is expected to be evolutionarily regulated by the strong selection pressures of counteracting directions: early budburst to increase the growth period and late budburst to reduce the risk of frost damage (Hufkens et al. 2012; Vitasse et al. 2014a; b; Lenz et al. 2016). Physiologically, the timing of budburst is determined by complex interactions of multiple

factors, including the chilling duration from autumn to winter, forcing temperature from winter to spring (temperature sum), and photoperiod, and differs greatly across species (Polgar and Primack 2011; Osada 2017; Panchen et al. 2014). As a consequence, the magnitude of changes in the timing of budburst in response to warming differs among species (Körner and Basler 2010). Yet, knowledge about the intraspecific variation of the timing of budburst and its relationship with climate is insufficient for most tree species and needs to be elucidated by a combination of observational and experimental studies (Laube et al. 2014; Clark et al. 2014).

Intraspecific variation in leaf phenology is important basic information for evaluating the meteorological factors that affect the timing of budburst and its response to climate change. Leaf phenology has been intensively studied at the geographical scale for species of wide distributions and has often been related to the temperature of growth habitats (Chmielewski and Rötzer 2001; Ahas et al. 2002; Čufar et al. 2012; Chmura and Rozkowski 2002; Raulier and Bernier 2000; Matsumoto et al. 2003). Some of these studies assumed that the phenological response to climate is similar for different populations of a given species in a range of natural habitats (e.g., Raulier and Bernier 2000). Because intraspecific variation is often found in a variety of plant traits (Albert et al. 2010; Jung et al. 2010; Fajardo and Piper 2011; Osada et al. 2015), such an assumption might not be applicable particularly to the species of wide distributional ranges. For example, the species of higher genetic diversity showed more intraspecific variations in the phenological response to temperature (Doi et al. 2010).

Common garden experiments and reciprocal transplanting are effective methods for evaluating the relative importance of the genetic and environmental control of leaf phenology (Zohner and Renner 2014). The genetic diversity of leaf phenology has been detected by common garden experiments in various temperate deciduous tree species, such as *Populus* spp. (Pellis et al. 2004), *Betula pendula* (Rousi and Pusenius 2005), *Ulmus minor* (Ghelardini et al. 2006), *Quercus petraea* (Alberto et al. 2011; Vitasse et al. 2010), and *Fagus sylvatica* (Vitasse et al. 2010; Robson et al. 2013; Schueler and Liesebach 2015; Kramer et al. 2017; Von Wuehlisch et al. 1995) in Europe; *Populus deltoides* subsp. *monilifera*, *Tamarix ramosissima*, *Tamarix chinensis* (Friedman et al. 2011), and *Populus balsamifera* in North America (Keller et al. 2011) and *Nothofagus pumilio* in South America (Premoli et al. 2007). For example, the timing of budburst and the required temperature sum to budburst were directly related to the latitude and altitude of origins of the clones in *U. minor*, and the clones from more northern latitudes and higher altitudes required a longer chilling duration for dormancy release (Ghelardini et al. 2006). Most of these studies were conducted in the regions of dry summer (i.e., the oceanic climate typical to west coasts), and comparable

data are rather limited in the regions of wet summer (i.e., the temperate humid climate).

Fagus crenata Blume is a dominant species in cool-temperate forests in Japan (Hiura 1995; Homma 1997; Matsui et al. 2004; Shimano 2002). This species is distributed in a wide range of latitudes (about 32–43° N), and genetic differentiation is considerable among the tree populations of different regions (Hiraoka and Tomaru 2009; Tomaru et al. 1997; Fujii et al. 2002). In accordance with this, intraspecific variation is found in leaf and shoot traits (Hagiwara 1977; Hiura 1998; Osada et al. 2015). So far, intensive studies of leaf phenology have been conducted in a closely related species *F. sylvatica* in Europe. Murray et al. (1989) showed that an increased duration of chilling temperatures led to a decrease in the heating requirement for budburst in most deciduous broad-leaved species but not in *F. sylvatica* in Europe. They suggested that *F. sylvatica* has a very high chilling requirement to fully release bud dormancy, resulting in a negative linear relationship between the chilling duration and temperature sum to budburst, in contrast with an exponentially decreasing relationship in other species (Murray et al. 1989; Vitasse and Basler 2013). In addition, photoperiod is considered important in determining the timing of budburst of *F. sylvatica* (Kramer 1994; Basler and Körner 2012), and photoperiod appears to compensate for the low temperature sum, resulting in little variation in the timing of budburst across the years (Caffarra and Donnelly 2011; Caffarra et al. 2011; Vitasse and Basler 2013). It is noteworthy that genetic distance is the least between *F. crenata* and *F. sylvatica* in comparison with other *Fagus* species (Denk et al. 2005). Information on the relationships between the chilling duration, temperature sum (cumulative degree-days), and photoperiod in *F. crenata* at the geographic scale would thus help us to understand the effect of climate change on *Fagus* species in the cool-temperate forests of Europe and Asia.

We complied the data of the day of budburst for the trees of *F. crenata* of common garden experiments in Japan (Table 1). We addressed the following questions: (1) Does the relationship between the latitude of experimental sites and the timing of budburst differ among the *F. crenata* trees of different latitudes of origins? (2) Do the relationships between the timing of budburst, temperature sum, chilling duration, and photoperiod differ among the *F. crenata* of different latitudes of origins? (3) Does photoperiod regulate the timing of budburst despite smaller values of chilling duration in the *F. creata* of southern sites, resulting in a small variation in the timing of budburst? (4) How do the differences among the trees of different origins affect overall patterns of the timing of budburst at the geographic scale in *F. crenata*? Comparison of our results with the studies of *F. sylvatica* will complement the knowledge on the phenological responses to climate in *Fagus* species.

Table 1 Information about the sites of common garden experiments of *Fagus crenata*

Site code	Site	Latitude (°N)	Longitude (°E)	Altitude (m)	Number of seed origin	Years
Izu[a]	Arboricultural Research Institute, Tokyo University	34.6	138.9	250	11	1994–2007
Tottori[b]	Hiruzen Experimental Forest, Tottori University	35.3	133.6	510	10	1994, 2005
NiigataU[c]	Experimental Forest, Niigata University	37.9	139.0	40	6	1994–1995
NiigataF[d]	Niigata Forest Research Institute	38.3	139.5	30	10	1998–2004
Iwate[e]	Gomyojin Experimental Forest, Iwate University	39.4	140.6	240	7	1994, 1996
Tomakomai[f]	Tomakomai Experimental Forest, Hokkaido University	42.7	141.6	220	9	2014–2015
Furano[g]	Hokkaido Forest, Tokyo University	43.2	142.4	80	11	1992–1995

[a] Kaji and Nishiya (1996) and Murase et al. (unpublished data)

[b] Nishiyama and Yamamoto (2007)

[c] Nakata and Nakayama (1995)

[d] Nunokawa and Tsukahara (2005) and Nunokawa and Tsukahara (unpublished data)

[e] Mizuguchi et al. (1997)

[f] Osada (unpublished data)

[g] Kurahashi (1996)

Materials and methods

Common garden experiments

The seeds of *F. crenata* were collected from 19 provenances in Japan during 1986–1988 and were grown in the experimental garden of the Hokkaido Experimental Forest, University of Tokyo (43.2° N, 142.4° E) (Watanabe and Shibano 1996). The seedlings were then distributed to 13 experimental sites in Japan during 1990–1991 and were grown in each of these sites. The phenology of leaf emergence was surveyed for seven of these sites (Watanabe and Shibano 1996). The interval of phenology census was about 1 week. The protocol of phenology census from budburst to leaf maturation was the same for all but one site (Tomakomai; N. Osada, unpublishded data), and therefore we focused only on the day of budburst. In all studies, the day of budburst was defined as the day when bud scales start to open and leaf primordia can be identified for more than half of the crown within a tree. Overall, we complied the phenology data of the different combinations of 11 sites of seed origin and seven experimental sites (Tables 1 and 2). The years and durations of phenology census differed across the sites: census year varied from 1992 to 2015, and duration from two to 14 years (Table 1).

Meteorological factors

We used temperature data from the weather stations of the Japan Meteorological Agency (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). The temperature was estimated from that at the weather station near the experimental site and it was corrected by the difference in altitude with a lapse rate of 0.55 °C/100 m. The daily mean temperature (T_{mean}) for each year of phenology census was calculated for each experimental site. The photoperiod was calculated from the latitude, longitude, and altitude of each experimental site (Nagasawa 1999).

Data analysis

Planted trees grew from seedlings into tall trees during 1992–2015 (about 6–8 m high in 2015 at the Tomakomai site). Because the phenology of leaf emergence often changes ontogenetically (Seiwa 1999; Augspurger and Bartlett 2003; Vitasse 2013), phenological patterns might

Table 2 Information about the sites of seed origin (Watanabe and Shibano 1996)

Seed origin	Latitude (°N)	Longitude (°E)
Yatsushiro	32.5	Unknown
Yabe	32.6	131.2
Amagi	34.8	Unknown
Numazu	35.1	Unknown
Ashiu	35.4	135.8
Yamanashi	35.5	138.8
Fukui	36	Unknown
Iwate	38.5	Unknown
Kikonai	41.6	140.3
Ohirayama	42.6	140.2
Siraikawa	42.8	140.3

not be directly comparable across the experimental sites of different census years. Thus, we preliminarily examined whether the temporal trends in the timing of budburst were apparent for the two experimental sites in which the census period was 7 and 14 years, respectively. According to this analysis, directional trends were not found in both sites (Fig. 1): the effects of year, mean temperature from January to March, and their interaction were insignificant for all sites of seed origin in two-way ANOVA ($P > 0.05$). The data of phenological census for all years were therefore used for the following analyses.

The cumulative degree-days of > 5 °C in mean daily temperature until the timing of budburst (temperature sum) were calculated from January 1 and from February 1 for each tree. The temperature sum from January 1 has

often been used for the phenology of various species including *F. sylvatica* (Murray et al. 1989; Vitasse and Basler 2013), and thus is directly comparable with other species. In most regions of Japan, the daily temperature drops to a minimum at the end of January (http://www.data.jma.go.jp/gmd/risk/obsdl/index.php). This means that the influence of the day of declining temperature was inevitably included in the temperature sum from January 1 but not from February 1 in the regions of > 5 °C in mean daily temperature. In our case, such a pattern was found in one experimental site (Izu; Appendix A in the Electronic supplementary material). However, the overall patterns were similar between the temperature sum from January and from February in all analyses, and we show the results of the former case. In addition, the numbers of chilling
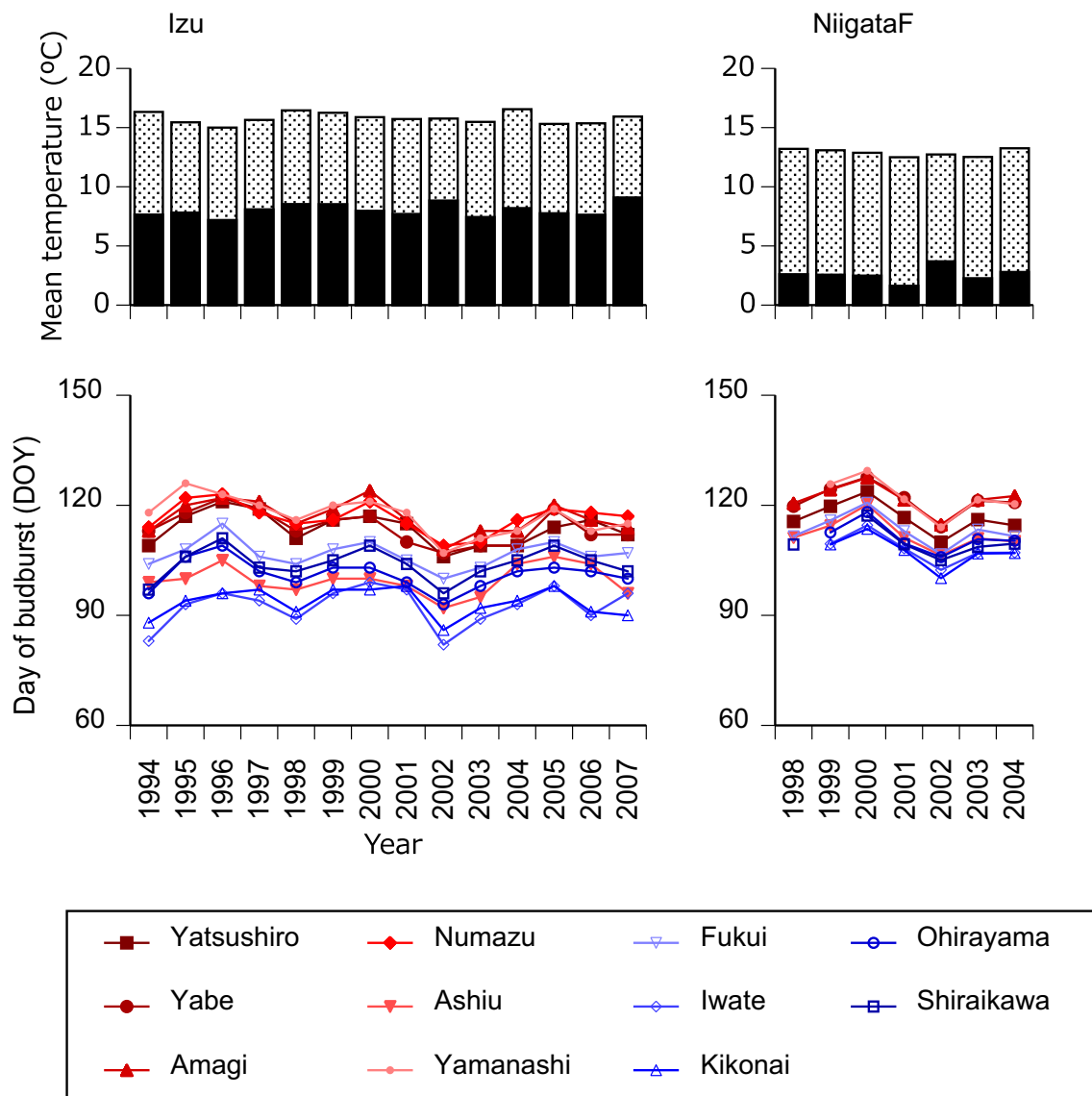

Fig. 1 Long-term patterns of mean annual temperature (dotted bars), mean temperature from January to March (black bars), and the day of budburst of *Fagus crenata* in two experimental sites. Different symbols indicate the trees of different origins (see Table 2 for details)

days of < 5 °C from November 1 to the day of budburst (chilling duration) were calculated as in previous studies (Murray et al. 1989; Vitasse and Basler 2013). Although difference in the base temperatures of temperature sum and chilling duration might affect the results, we simply used the temperature of 5 °C in this study for ease of comparison with the results of previous studies of *F. sylvatica* (Murray et al. 1989; Vitasse and Basler 2013).

Because mean annual temperature of the experimental sites was strongly correlated with latitude ($r = -0.88$, $P = 0.0084$) and the altitude was not recorded in some sites of seed origins (Table 2), we investigated the relationships between latitude and phenological traits. Generalized linear models were applied to investigate the effects of latitudes of experimental sites and seed origins on the day of budburst, temperature sum, and photoperiod at the day of budburst, using gamma distributions and a log link function. Model selection was conducted for various combinations of the latitudes of experimental sites and seed origins by using the Akaike's Information Criterion (AIC).

Previous studies have categorized the relationship between chilling duration and temperature sum into several patterns based on the degree of curvature (Vitasse and Basler 2013; Murray et al. 1989); i.e., differences in the importance of the chilling requirement to release bud dormancy in relation to the temperature sum to budburst. However, the chilling duration and temperature sum are expected to be autocorrelated because longer chilling duration inevitably results in a smaller temperature sum unless the temperature from November to December has a profound effect. Nonetheless, different patterns in the relationship between chilling duration and temperature sum reflect the species characteristics of the phenological response to temperature. We therefore investigated this relationship and compared it with previous studies of *F. sylvatica*. The curvilinear relationship was regressed by the least squares method as the following three-parameter exponential curve:

$$s_t = a_1 + b_1{}^* \exp\left(c_1{}^* d_c\right) \quad (1)$$

where s_t, d_c, a_1, b_1, and c_1 indicate the temperature sum, chilling duration, asymptote, scale, and degree of concaveness, respectively (Fig. 2). Large (but negative) values of c_1 indicate a less concave (approaching a linear) relationship between chilling duration and temperature sum, which was found in *F. sylvatica* (Vitasse and Basler 2013; Murray et al. 1989). Similarly, we investigated how chilling duration and temperature sum influenced photoperiod at the day of budburst (p_p) as follows:

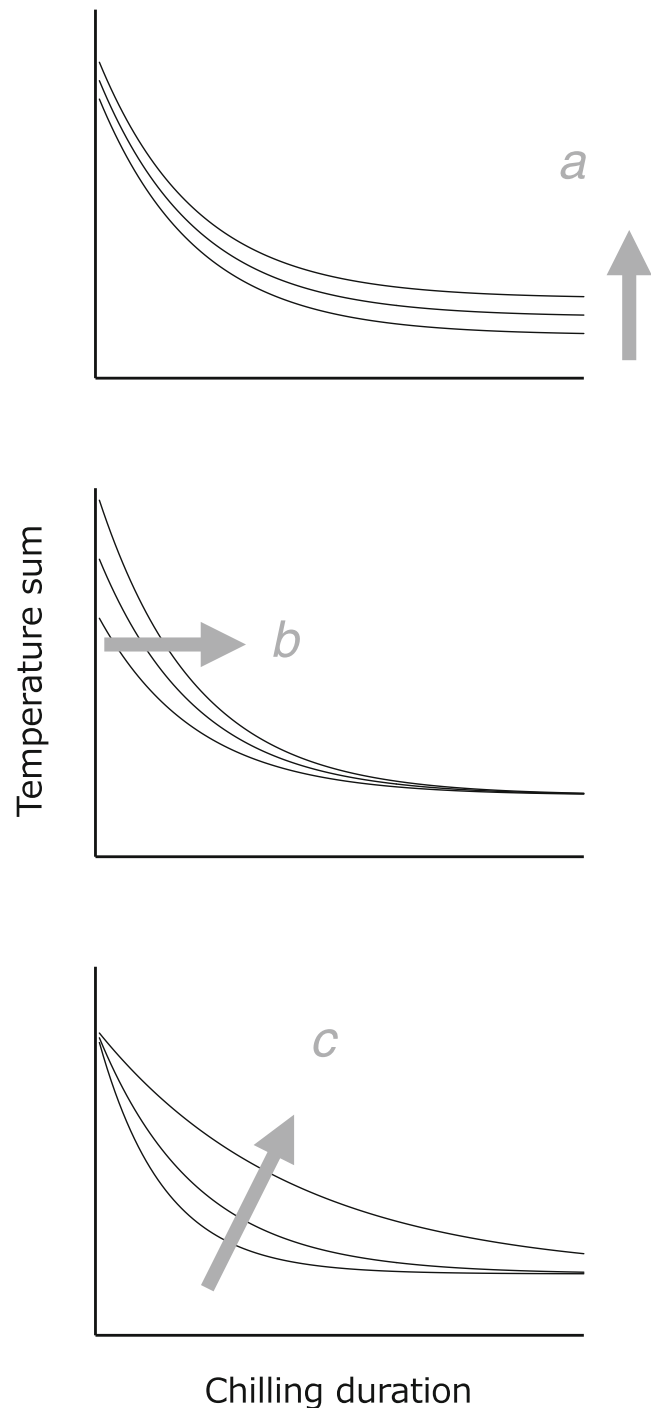$$p_p = a_2 + b_2{}^* \exp\left(c_2{}^* d_c\right) \quad (2)$$

Fig. 2 Changes in the relationship between chilling duration and temperature sum in Eq. (1) in response to increases in the parameters a_1, b_1, and c_1

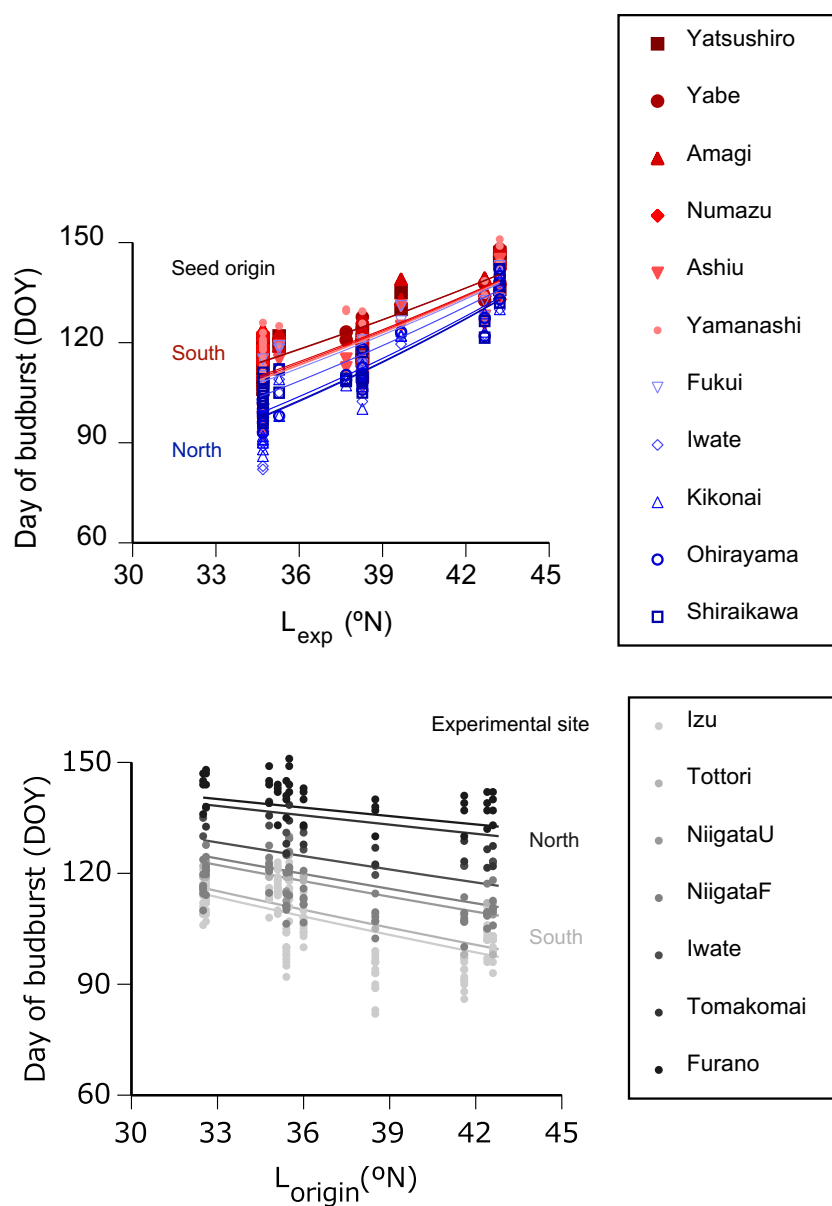$$p_p = a_3 + b_3{}^* \exp\left(c_3{}^* s_t\right) \quad (3)$$

Based on these analyses, we investigated the relative importance of these parameters on the overall pattern of the day of budburst in *F. crenata* at the geographic scale.

Results

Differences in the timing of budburst

Overall, budburst occurred later for the *F. crenata* trees of more northern sites (Fig. 3). The model that included the latitudes of experimental sites and seed origins and their interaction was selected based on AIC (Table 3). Budburst occurred later for the trees of more northern sites when the trees of same origins were compared, whereas it occurred later for the trees of more southern populations at a given experimental site (Fig. 3). The interaction term indicates that differences in the day of budburst between the trees of different origins was greater in southern than in northern experimental sites (Table 3; Fig. 3).

Differences in temperature sum, chilling duration, and photoperiod among trees

The temperature sum was greater for the trees of more southern sites and for those of more southern populations (Fig. 4; Table 3). The latter pattern was obtained simply because budburst occurred later for the trees of more southern populations at the same experimental site (Fig. 3). Although the model that included the latitudes of experimental sites and seed origins but not their interaction was selected (Table 3), the log link function indicated that the temperature sum was more variable between the trees of different populations for more southern sites (Fig. 4).

Chilling duration was strongly correlated with the latitude of experimental sites ($r = 0.90$, $n = 33$, $P < 0.001$). The

Fig. 3 Relationships between the latitude of experimental sites (L_{exp}) and the day of budburst for the trees of different origins and between the latitude of seed origins (L_{origin}) and the day of budburst for the trees of different experimental sites. All combinations of the sites of experiments and seed origins and observation years are shown. Regression curves are shown for the trees of each origin and each experimental site based on the results of generalized linear models (see "Materials and methods"). See Tables 1 and 2 for information about the sites of seed origin and experiment

Table 3 Parameters and Akaike's Information Criterion (AIC) of the models of different combinations of the latitudes of experimental sites, latitudes of seed origin, and their interaction on the day of budburst, temperature sum, and photoperiod among the trees of *Fagus crenata*

Model	Intercept		Latitudes of experimental sites (A)		Latitudes of seed origin (B)		$A * B$		AIC
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	
The day of budburst									
M1	*5.75*	*0.43*	*− 0.014*	*0.012*	*− 0.057*	*0.012*	*0.0012*	*0.0003*	*2237*
M2	4.11	0.06	0.03	0.001	− 0.013	0.001			2249.4
M3	3.66	0.05	0.029	0.001					2375.8
M4	5.18	0.07			− 0.012	0.002			2626.8
M5	4.75	0.01							2664.9
Temperature sum									
M1	10.87	2.2	− 0.097	0.059	0.005	0.059	− 0.0014	0.0016	3815.6
M2	*12.85*	*0.28*	*− 0.149*	*0.006*	*− 0.048*	*0.005*			*3814.4*
M3	11.1	0.23	− 0.15	0.006					3882.2
M4	7.38	0.32			− 0.048	0.008			4156.5
M5	5.62	0.03							4182.6
Photoperiod									
M1	*2.65*	*0.14*	*0.003*	*0.004*	*− 0.014*	*0.004*	*0.0003*	*0.0001*	*81.438*
M2	2.25	0.02	0.013	0	− 0.004	0			88.352
M3	2.12	0.02	0.013	0					202.67
M4	2.74	0.03			− 0.003	0.001			651.38
M5	2.61	0							669.92

The models with the lowest AIC values are set in italics

relationship between chilling duration and the latitude of experimental sites was the same for the trees of different seed origins, because the trees of the same experimental site experienced the same climate, and temperature did not decline to < 5 °C after the day of budburst of the earliest trees in all sites.

The photoperiod at the day of budburst was longer for the trees of more northern sites (Fig. 4). The model that included the interaction between the latitudes of experimental sites and seed origins was selected (Table 3); i.e., photoperiod was longer for the trees of more northern sites and for those of more southern populations, and the difference in photoperiod was greater for the trees of more southern sites (Table 3; Fig. 4). At a given site, photoperiod was longer for the trees of more southern populations (Fig. 4), again because budburst occurred later for the trees of more southern populations at the same experimental site (Fig. 3). Note that trees must respond not to the photoperiod at the day of budburst but to the photoperiod at several days to weeks before budburst. Based on the seasonal changes in photoperiod, patterns were similar even when the trees of different seed origins responded similarly to the photoperiod of the same preceding days (Appendix B in the Electronic supplementary material).

Relationships between temperature sum, chilling duration, and photoperiod

The patterns of the relationships between chilling duration and temperature sum, chilling duration and photoperiod, and temperature sum and photoperiod were similar among the trees of different origins except for the relationship between chilling duration and photoperiod at one site (Numazu; Fig. 5). The parameters of exponential curves of this exceptional site were dispersed from those of the other sites (Fig. 6) probably because of the lack of midpoints in Fig. 5 (80–120 in the range of chilling duration), and therefore we excluded the data of Numazu from the following analyses.

The relationship between chilling duration and temperature sum was negative exponential for the trees of the same origin (Fig. 5): temperature sum was smaller for the trees that experienced more chilling, but temperature sum gradually approached asymptotic values with increasing chilling duration. The parameter a_1 of Eq. (1) was smaller for more northern populations, whereas the relationship between the latitude of seed origins and b_1 and that between the latitude of seed origins and c_1 were marginal (Fig. 6). Thus, temperature sum was smaller for the trees of more northern populations at a given chilling duration (Figs. 5 and 6).
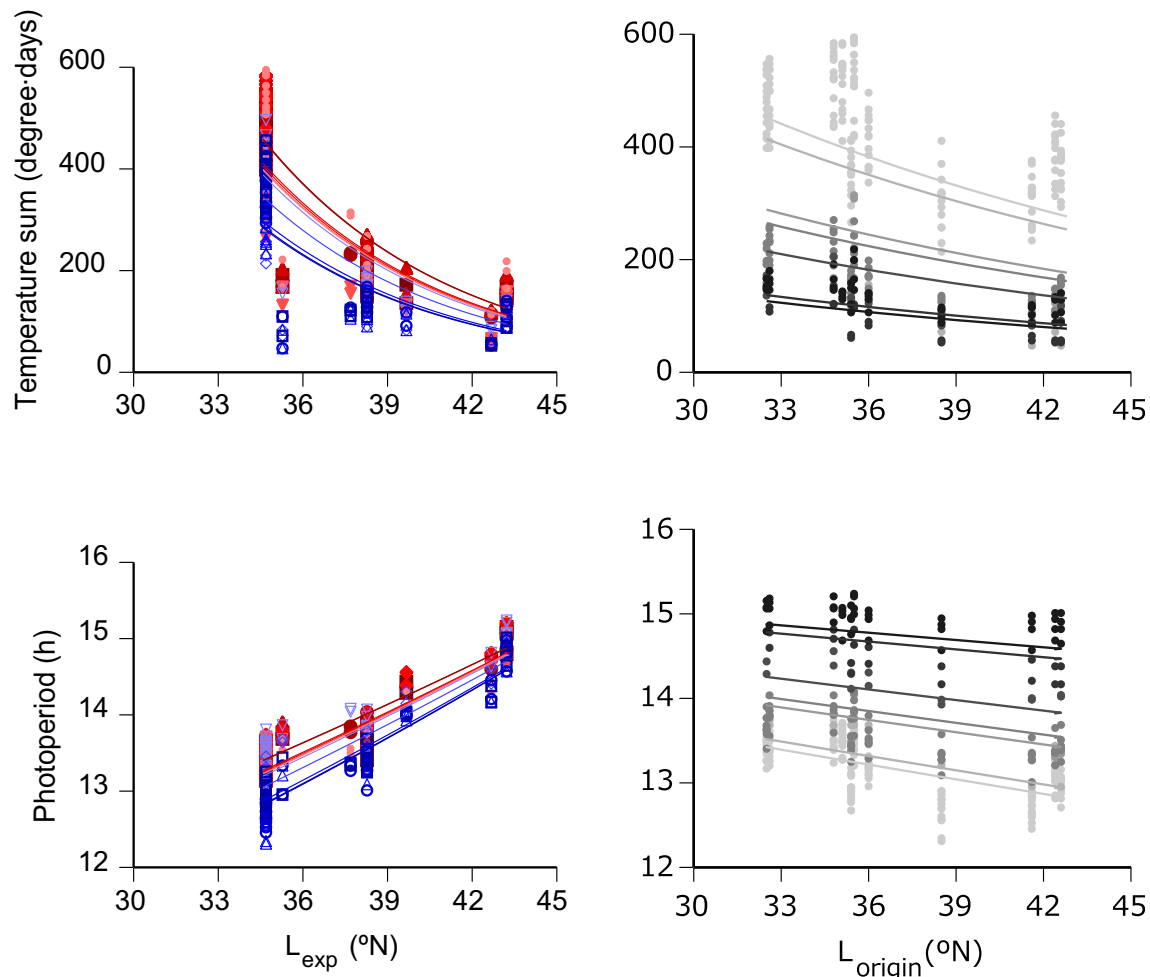
Fig. 4 Relationships between the latitude of experimental sites (L_{exp}) and the temperature sum (cumulative degree-days of > 5 °C from January 1), between L_{exp} and the photoperiod at the day of budburst, between the latitude of seed origins (L_{origin}) and the temperature sum, and between L_{origin} and photoperiod. All combinations of the sites of experiments and seed origins and observation years are shown. Regression curves are shown for the trees of each origin and each experimental site based on the results of generalized linear models (see "Materials and methods"). See Tables 1 and 2 for information on the sites of seed origin and experiment and Fig. 3 for an explanation of the symbols

The relationship between chilling duration and photoperiod was positive exponential for the trees of the same origin (Fig. 5): photoperiod was shorter for the trees that experienced less chilling, but it gradually approached asymptotic values with decreasing chilling duration for the trees of the same origin. The parameter a_2 was marginally correlated with the latitude of seed origins and decreased with increasing the latitude of seed origins, whereas the parameters b_2 and c_2 were not correlated with the latitude of seed origins (Fig. 6).
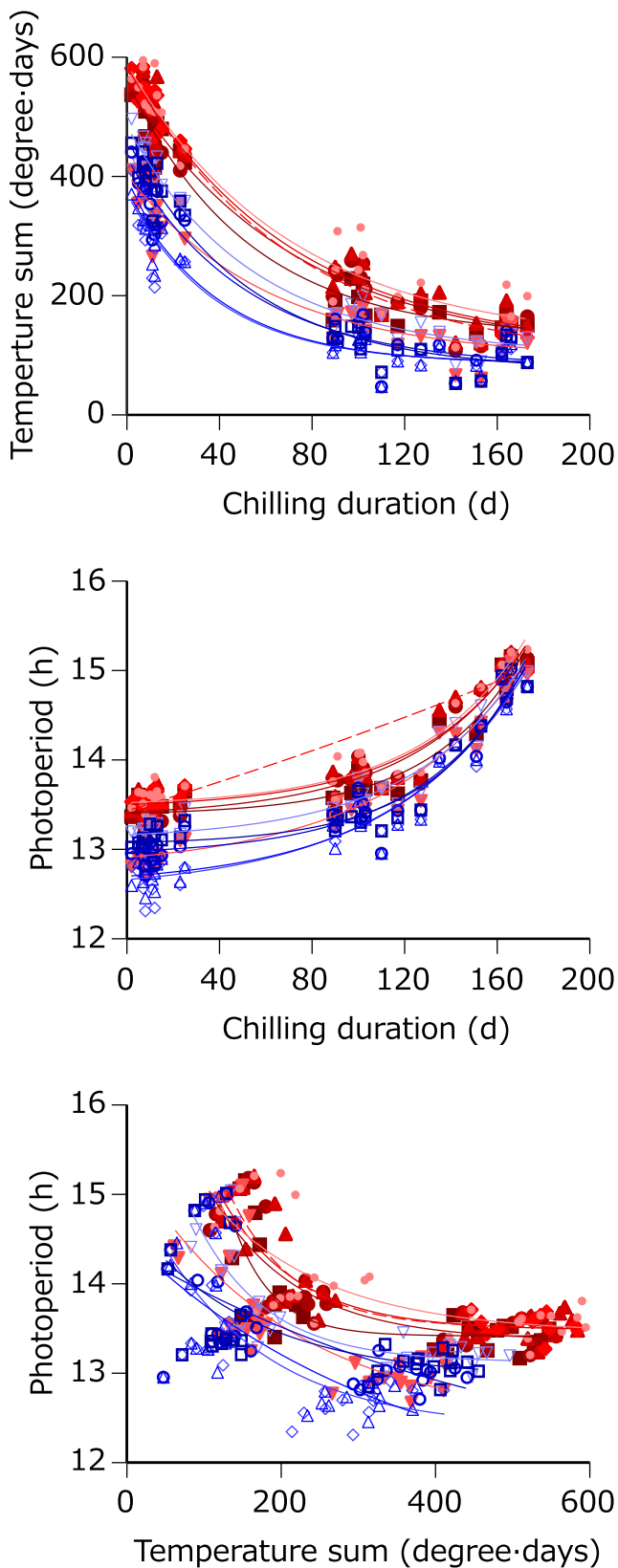
The relationship between temperature sum and photoperiod was negative exponential for the trees of the same origin (Fig. 5): photoperiod was smaller for the trees of larger temperature sum for the trees of the same origin. The parameter a_3 decreased and c_3 increased with the latitude of seed origins, although the variance was large in some points (Fig. 6). Thus, photoperiod was shorter for the trees of more northern populations at a given temperature sum, and photoperiod

approached a minimum at larger temperature sum in the trees of more southern populations.

Overall relationships among the daily changes in temperature sum and photoperiod and the day of budburst are shown in Fig. 7 for the trees of the most northern (Shiraikawa) and the most southern populations (Yatsushiro). Although later budburst for the trees of more northern sites was associated with longer photoperiod and smaller temperature sum, relative importance of photoperiod and temperature sum was not constant but the increase in photoperiod became greater influence for the trees of more northern sites (Fig. 7).

Discussion

Based on the common garden experiments of *F. crenata*, our predictions were partly supported: (1) The relationship between the latitude of experimental sites and the day of

Fig. 5 Relationships between the chilling duration (days of < 5 °C from November 1 to the day of budburst) and the temperature sum (cumulative degree-days of > 5 °C from January 1), chilling duration and photoperiod at the day of budburst, and the temperature sum and photoperiod. All combinations of the sites of experiments and seed origins and observation years are shown. Broken lines indicate the exceptional relationships in Numazu (see "Results"). See Fig. 3 for an explanation of the symbols

at the day of budburst differed between the trees of different latitudes of seed origins (Figs. 4, 5, 6, and 7). (3) Even for the trees of the same origin, the day of budburst varied greatly between the trees of different latitudes of experimental sites (Fig. 3), and the photoperiod at the timing of budburst was much longer for the trees of more northern sites (Figs. 4, 5, 6, and 7). Thus, the regulation of the timing of budburst by photoperiod would not be, if exists, simple. (4) The timing of budburst was earlier for the trees of more northern populations throughout the latitudes of experimental sites, and variation in the timing of budburst among the trees of different seed origins was smaller for more northern experimental sites (Fig. 3). Such patterns were caused by the directional changes in temperature sum, chilling duration, and photoperiod among the trees of different origins: the asymptotes of the curvilinear relationship (*a*) was smaller for the trees of more northern populations in the relationships between temperature sum, chilling duration, and photoperiod (Figs. 5 and 6). Thus, *a* might be the key parameter that regulates the timing of budburst among the trees of different latitudes of seed origins.

Geographic variation in the timing of budburst

Geographic trends in the timing of budburst have been variable and inconsistent across temperate deciduous species in common garden experiments (Friedman et al. 2011; Ghelardini et al. 2006; Whiteley et al. 2003; Hall et al. 2007). For example, the timing of budburst was earlier for the trees of more northern populations at a given experimental site in *F. crenata* (Fig. 3), but the opposite was true in *U. minor* (Ghelardini et al. 2006) and *F. sylvatica* (Robson et al. 2013), and there was no latitudinal trend in the timing of budburst in *Populus tremula* (Hall et al. 2007). Differences in the geographic trends in the timing of budburst have been explained by the interactions between chilling and thermal time requirements for budburst at colder or warmer sites than at the site of origin (Ghelardini et al. 2006). However, our results did not hold for this explanation, because the same trends were observed for the experimental sites of various latitudes in *F. crenata*, although differences in the timing of budburst became smaller with increasing latitude (Fig. 3). We speculate that differences in species and climate are important determinants of geographic variations of the timing of budburst. In
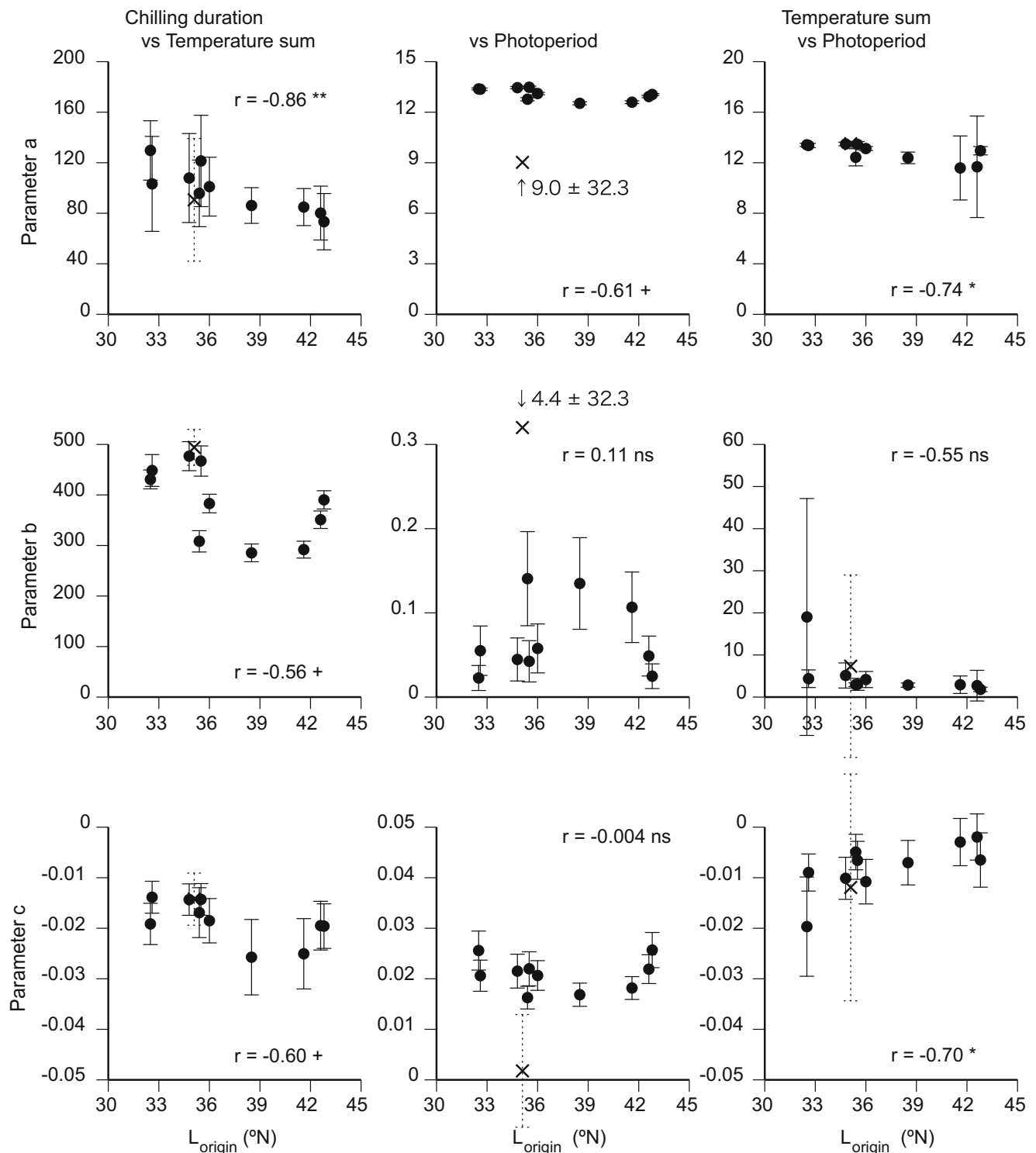
budburst differed between the trees of different latitudes of origins (Fig. 3). (2) The relationships between the day of budburst, temperature sum, chilling duration, and photoperiod

Fig. 6 Parameters of the regression curves of Fig. 5 in relation to the latitude of seed origins (L_{origin}). Crosses with dotted error bars (bars are omitted, but values of mean ± SE are shown in cases of large errors) indicate the exceptional relationships in Numazu (see "Results"; Fig. 5). Correlation coefficients are shown with significance values for the ten sites (**$P < 0.01$; *$P < 0.05$; +$P < 0.10$; ns$P > 0.10$)

particular, avoidance of late frosts and exploitation of soil water before the onset of summer drought are considered important in the determination of the timing of budburst for the trees of the west coast oceanic climate in Europe (Robson et al. 2013), whereas the latter consideration may not be applicable for the trees of the temperate humid climate in Japan due to its wet summer. Although causal relationship is not clear, differences in the geographic trends of the timing of budburst
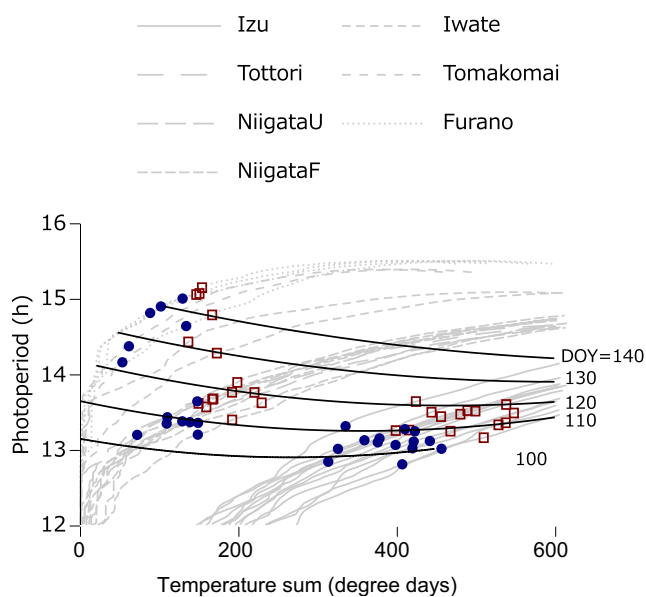
Fig. 7 Overall relationships among the daily changes in temperature sum and photoperiod of all experimental sites and the day of budburst for the trees of the most northern (Shiraikawa, closed circles) and the most southern populations (Yatsushiro, open squares)

between *F. crenata* and *F. sylvatica* might in part be related to such climatic differences.

The timing of budburst was earlier for the trees of more northern populations throughout the latitudes of experimental sites, and variation in the timing of budburst among the trees of different seed origins was smaller for more northern experimental sites (Fig. 3). Based on the data of long-term geographic studies of phenology for various plant species, the species of higher genetic diversity showed more intraspecific variations of the phenological response to temperature (Doi et al. 2010). Our results suggest that genetic differentiation may not necessarily increase such variations (Fig. 3). Genetic variations in phenological response must be clarified for a number of species to understand why different trends arise in different species. Such differences in genetic variation in the timing of budburst among species are particularly important because it will cause species differences in the phenological response to global warming.

Meteorological factors affecting the timing of budburst

The relationships between temperature sum, chilling duration, and photoperiod were differentiated among the trees of different origins with distinct latitudinal patterns in *F. crenata*: the asymptotes a_1, a_2, and a_3 of curvilinear relationships (Eqs. 1–3) were smaller for the trees of more northern populations, whereas most other parameters did not show clear patterns (Figs. 5 and 6). Thus, the trees of more northern populations began budburst at lower temperature sum and shorter

photoperiod at a given chilling duration and at shorter photoperiod at a given temperature sum. Interrelationships between temperature sum, chilling duration, and photoperiod are notable among the trees of different latitudes of seed origins (Fig. 5). With the northward expansion of the species distribution, the responses of budburst to climate were probably caused by such genetic changes in this species.

Because the altitude of some sites of seed origin was not recorded, the effect of temperature could not be determined but the effect of latitude was investigated in this study. The altitude of the distributional sites of *F. crenata* tends to be lower in more northern sites (Hiura 1995), suggesting that inclusion of information on altitude and temperature might improve the weak relationships between the meteorological factors (Fig. 6). Nevertheless, this species is considered to have migrated from the south to the north after the last glacial maximum (Tomaru et al. 1997; Hiraoka and Tomaru 2009), and therefore latitudinal changes in phenological response to meteorological factors would reflect the changes associated with migration.

The negative exponential curve explained well the relationship between chilling duration and temperature sum for the trees of the same origin, indicating that temperature sum approached constant minimum values with increasing chilling duration. This is quite in contrast to the results of *F. sylvatica*, which showed a negative linear relationship between them, probably because *F. sylvatica* requires a very long chilling duration to fully release bud dormancy (Murray et al. 1989; Vitasse and Basler 2013). The ranges of chilling duration and temperature sum were similar between our study and the studies of *F. sylvatica* (Murray et al. 1989; Vitasse and Basler 2013). Difference in the relationship between chilling duration and temperature sum might be caused simply by the difference in species, but our results also indicate that genetic differentiation between populations of different regions might obscure the relationship, i.e., the trees of smaller chilling duration (warmer regions) have larger temperature sum, and those of larger chilling duration (colder regions) have smaller temperature sum in their original habitats. Thus, the relationship between chilling duration and temperature sum becomes more linear when only the trees of original habitats were compared (Fig. 5). Variations in the relationship between chilling duration and temperature sum among species (Murray et al. 1989) might be partly caused by such genetic differences, and care must be taken in considering the relationship between chilling duration and temperature sum particularly for the genetically differentiated species of wide distributional ranges. It is important to note that a linear relationship between chilling duration and temperature sum was detected for *F. sylvatica* by using the data of 10 populations of variable altitudes of the Pyrénées mountains in Southern France (Vitasse and Basler 2013; Vitasse et al. 2009); genetic differentiation might be smaller in the populations of different altitudes than of

latitudes. Further studies are needed to evaluate the genetic effect in the relationship between chilling duration and temperature sum.

Photoperiod appears to compensate for a low forcing temperature, resulting in little variation in the timing of budburst across the years in *F. sylvatica* (Caffarra and Donnelly 2011; Caffarra et al. 2011; Vitasse and Basler 2013). Based on this result, Vitasse and Basler (2013) proposed two alternative models of the effect of photoperiod in *F. sylvatica*. The first is a fixed photoperiod model that requires a fixed photoperiod threshold to release dormancy. This model was not applicable to *F. crenata* because there was a large variation in photoperiod among the trees of the same origin (Figs. 5 and 7), and even when the seasonal changes in photoperiod were considered, the photoperiod during the days preceding the timing of budburst was longer for the trees of more northern sites among the trees of the same origin (Fig. 7; Appendix B in the Electronic supplementary material). The other model considers relative changes in the importance of temperature and photoperiod: the temperature requirements reduce with an increase in photoperiod. This model might be applicable to our results because a small temperature sum was associated with a long photoperiod for the trees of the same origin (Figs. 5 and 7). In particular, relative effect of temperature sum and photoperiod was not constant, and the increase in photoperiod had much greater influence at smaller temperature sum for the trees of the same origin (Fig. 5). Furthermore, such effect changed genetically with increasing the latitude of seed origins, particularly by decreasing the value of parameter *a* (Figs. 5 and 6).

Note that a difference in the temporal changes in photoperiod and temperature sum would be of particular importance when the relative effects of these meteorological factors are considered for the trees of the same origin: a large temporal increase in temperature sum may obscure or mask the importance of photoperiod, which increases at a much lower rate (Fig. 7; Appendix B in the Electronic supplementary material). Even so, the difference of photoperiod between the trees of early and late budburst was 1.80–2.64 h for the trees of the same origin (Figs. 4 and 7), and such a change in photoperiod required more than 1 month at a given experimental site (Fig. 7; Appendix B in the Electronic supplementary material). Thus, interrelationships of temperature and photoperiod are obvious in *F. crenata*, and the underlying physiological mechanisms must be clarified in future studies by controlling temperature and photoperiod as well as by developing the phenological process-based models that incorporate the interactive effects of chilling and photoperiod (Gauzere et al. 2017). In addition, recent studies have focused on the molecular basis of physiological control in phenology (Singh et al. 2017) and investigated the genetic variations in bud flush and bud set and the associated genes in *P. balsamifera* (Olson et al. 2013). Similar studies in *F. crenata* will be noteworthy for

comparing the results with *P. balsamifera* as well as with *F. sylvatica* and other *Fagus* species.

In summary, our results clearly showed that the relationships between temperature sum, chilling duration, and photoperiod differed in a systematic manner among the trees of different latitude of seed origins (Figs. 4, 5, and 6), and as a consequence, the timing of budburst was differentiated among the trees of different origins in *F. crenata* (Fig. 3). Thus, if genetic differentiation cannot keep up with climate change, the timing of budburst of *F. crenata* might become much later by the decrease in chilling duration for the warmer regions (Fig. 5), where photoperiodic control might be weaker (Figs. 5 and 7). Moreover, genetic differentiation might further delay the timing of budburst by increasing the parameter *a* (Figs. 4, 5, 6, and 7). Interrelationships between the timing of budburst and associated meteorological factors must be clarified for other temperate deciduous species, because they inevitably influence the overall pattern of the timing of budburst at the geographic scale, and the day of budburst might deviate from predictions when intraspecific variations are not considered.

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