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The sensitivity of ginkgo leaf unfolding to the temperature and photoperiod decreases with increasing elevation

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Any figure does not need to used color in print.

Abstract

Climate change substantially affects plant phenology, resulting in earlier vegetation onset across temperate and boreal regions. Phenological shifts caused by warming may alter species interactions across trophic levels because of species-specific responses, and influence the reproductive success of dioecious species if the phenological sensitivity to warming (S_T) differs between genders. We used twigs collected from male and female gingko trees at three elevations on Tianmu Mountain in eastern China. The twigs were cultivated in climate chambers to determine the effects of three temperatures (10, 15, and 20 °C) and two photoperiods (8 and 16 h). We observed slightly earlier leaf unfolding dates in male twigs (1 day), and a higher heat requirement (growing degree hours) for leaf unfolding in female (14,334 ± 588 °C) compared to male twigs (13,874 ± 551 °C). Similar responses to temperature (S_T = 3.7 days $°C^{-1}$), photoperiod and elevation were observed across genders. The long photoperiod treatment shortened the time to leaf unfolding by 9.1 days, but temperature and photoperiod effects on leaf unfolding differed significantly depending on the elevation of the donor trees. Specifically, S_T was higher (4.17 days $°C^{-1}$) and the photoperiod effect on S_T was larger (decreased by 1.15 days $°C^{-1}$) at the lowest elevation than at the higher elevations (S_T = 3.26 days $°C^{-1}$; decreased by 0.48 days $°C^{-1}$). This may be related to environment-induced local adaptations and self-protection mechanisms of trees at high elevations to avoid frost damage. Our results indicate that the photoperiod and genetic adaptations to local environments influenced the warming-induced phenological responses in ginkgo, but these responses were generally similar between the genders. For a given species, individuals in different climates may exhibit different phenological responses to higher temperatures, with individuals in warmer regions likely becoming increasingly limited by the photoperiod as the climate warms further.

Keywords: climate change, temperature sensitivity, photoperiod, elevation, twig cutting experiment, gymnosperm

1. Introduction

The timing of vegetation onset is a crucial regulatory factor affecting the terrestrial carbon sequestration in temperate and boreal regions (Keeling *et al.*, 1996; Richardson *et al.*, 2013; Wu *et al.*, 2019; Piao *et al.*, 2020). It also influences the regional and global climate by providing biophysical and biogeochemical feedback to the climate system (Peñuelas *et al.*, 2009; Piao *et al.*, 2019). Ground monitoring data and satellite-based vegetation indices have revealed significant tree phenological shifts in response to global warming worldwide (Peñuelas & Filella, 2001; Fu *et al.*, 2015b; Menzel *et al.*, 2020; Piao *et al.*, 2020; Wu *et al.*, 2021). For a given species, these phenological changes are generally greater at high latitudes and/or elevations (Vitasse *et al.*, 2018; Gao *et al.*, 2020). This phenomenon may be explained by the warming-induced environmental constraints affecting warm-edge species distributions. More specifically, the relatively short photoperiod and lack of chilling conditions prevent the complete release of winter dormancy. However, this possibility requires experimental verification.

Spring phenological sensitivity to warming (S_T; advancement of leaf unfolding in days per degree warming, days °C^{-1}) has declined significantly over the past few decades (Fu *et al.*, 2015b). This may be partially related to a shortening photoperiod, during which buds become sensitive to increasing temperatures and to the increasing thermal requirements for bud burst resulting from decreased chilling during winter (Fu *et al.*, 2019). Although chilling is essential for releasing tree buds from dormancy (Chuine *et al.*, 2010; Yu *et al.*, 2010; Baumgarten *et al.*, 2021), the effects of a shortened photoperiod on leaf unfolding and phenological sensitivity to warming remain unclear (but see Fu *et al.*, 2019). Indeed, for photoperiod-sensitive species, a shortened photoperiod may increase the amount of heat required for leaf unfolding, thereby delaying cell development. This so-called “photoperiod limitation” represents a protective mechanism enabling trees to avoid being damaged from frost events after warm spells in late winter and early spring (Korner & Basler, 2010; Fu *et al.*, 2019). Additionally, there are genetic differences underlying the diverse phenological responses among populations growing in contrasting climates (Vitasse *et al.*, 2013). There is very little available information regarding the possible genetic adaptations to specific photoperiods and whether populations growing in regions with the same photoperiod, but varying temperatures (i.e., along an elevational gradient), have evolved to adapt to differences in the interactive effects of temperature and photoperiod. How elevation alters spring phenological responses to climate change, especially under different photoperiods have received less attention and were urgently needed to be illustrated.

To predict future phenological shifts, manipulative experiments are particularly relevant as they allow us to investigate phenological responses beyond the range of historical temperature conditions and to change one environmental variable at a time in a controlled manner. Therefore, studies using twig cuttings under different chilling, forcing, and photoperiod conditions in climate chambers have recently been conducted (Flynn & Wolkovich, 2018; Baumgarten *et al.*, 2021). Twig cuttings have been proven to be a viable alternative to donor trees for phenological studies (Vitasse & Basler, 2014; Du *et al*., 2019).

Although most of the previous related studies focused on the phenological responses of European and North American angiosperms to climate change (Basler & Körner, 2012; Fu *et al*., 2019; Vitasse *et al*., 2009; Flynn & Wolkovich, 2018), there has been some research on deciduous gymnosperms and Asian species (Matsumoto *et al*., 2003; Kumar & Sati, 2016). Ginkgo (*Ginkgo biloba* L.), which represents a gymnosperm lineage, is a tall deciduous dioecious tree native to China, but it is currently distributed worldwide, including at various latitudes (Zhao *et al*., 2019). The morphology of this 'living fossil' species has essentially not changed for at least 200 million years (Major, 1967; Zhou & Zheng, 2003) and its ecological traits have been conserved since the late Cretaceous period (Royer *et al*., 2003). Its survival during the Quaternary glaciations depended on range shifts as well as environmental adaptations, some of which were related to robust responses to abiotic and biotic stresses (Zhao *et al*., 2019). Furthermore, a previous study suggested that the responsiveness of leaf unfolding to temperature may vary between male and female individuals (Sofi, 2018). Investigating the difference of ginkgo leaf unfolding responses to climate change between genders and the interplay with photoperiod is important for comprehensively characterizing the phenological responses of gymnosperms to ongoing climate change.

In this study, we conducted a manipulative experiment on the twigs of male and female ginkgo to evaluate the effects of two photoperiods (8 and 16 h) and three temperatures (10, 15, and 20 °C) on leaf unfolding, as well as the response differences along elevation gradients. We aimed to address the following scientific questions: 1) how the timing of leaf unfolding response to warming and photoperiod between male and female individuals, and 2) how the photoperiod affects the temperature sensitivity of leaf unfolding among different elevations. We tested the hypotheses that short photoperiod affects the phenological sensitivity to temperature (Flynn & Wolkovich, 2018; Fu *et al.*, 2019; Zohner *et al.*, 2016) and this effect may be larger in the provenances at lower elevations as a protective mechanism against warm events in late winter (Vitasse *et al.*, 2013); and 2) leaf unfolding dates and phenological sensitivity are similar between male and female individuals to safeguard reproductive success under different climatic conditions, but may differ among provenances (elevations).

2. Materials and Methods

2.1 Sample collection sites

Twigs were collected from adult ginkgo trees on 17–19 January 2021 on Tianmu Mountain (119° 23′ 47″–119° 28′ 27″ E, 30° 18′ 30″–30° 24′ 55″ N) in Zhejiang province in eastern China. More specifically, samples were collected at the following three elevations: E1: 344 ± 23 m, E2: 826 ± 39 m, and E3: 1,098 ± 41m. The aerial temperature 15 cm above ground and soil water content 8 cm below ground were recorded with an interval of 15 min using Temperature-Moisture-Sensor loggers (TMS-5; TOMST, Prague, Czech; Wild *et al.*, 2019). The mean values of both parameters for the three elevation classes were 15.75 ± 0.09 °C and 25 ± 1%, 13.43 ± 0.73 °C and 23 ±6%, 11.94 ± 0.21 °C and 29 ± 3%, respectively (Table S1). The dominant vegetation type on Tianmu Mountain is a subtropical evergreen and deciduous broad-leaved mixed forest at an elevation of 300–1,506 m. The occurrence of ginkgo in China, mostly cultivated, and the location of Tianmu Mountain is presented in Figure S1 (Gu *et al.*, 2021). According to our observation, cultivated ginkgo trees are bigger than 10 cm for trunk diameter.

2.2 Experimental design

At each elevation site, we selected 11 or 12 adult trees, ideally with an equal number of female and male individuals (Table S1). For each donor tree, we collected 12 healthy twigs (30–40 cm long) from the south-facing side of the crown. The twigs were pruned, cleaned, and disinfected with a commercial hypochlorite solution as previously described (Basler & Körner, 2012; Du *et al.*, 2019). The pruned twigs were placed in 395-ml plastic bottles, which were filled with tap water (up to 2/3 of the total volume), and then incubated in three climate chambers at Beijing Normal University. The water was changed every 2 weeks during the experiment. Additionally, the twigs were pruned 2 cm at the base to avoid the occlusion of vessels.

The climate chambers were set at 10 °C (T10), 15 °C (T15), and 20 °C (T20) for the duration of the experimental period. We used a constant temperature above 10 °C to avoid any confounding effects due to chilling (Baumgarten *et al.*, 2021). In each climate chamber (i.e., temperature treatment), two photoperiod treatments were set up by covering half of the samples with shade cloth daily for 16 h (P16; long photoperiod treatment) and 8 h (P8; short photoperiod treatment). Because we selected twigs from male and female trees at three elevations, analyses involved 36 combinations (three temperatures × two photoperiods × two genders × three elevations) with 11–12 twigs per treatment (one per tree). In each treatment, temperature sensors (HOBO MX2202) were used to record the air temperature every 30 min. The photoperiod treatment did not affect the temperature (Fig. S2). The photon flux in all chambers was approximately 161 μmol·m^{-2}·s^{-1}, which was consistent with that in an earlier investigation (Du *et al.*, 2019).

2.3 Phenology monitoring

We monitored the leaf unfolding of each twig every 3 days. The leaf unfolding stage was defined as the period in which at least one leaf was fully unfolded (Vitasse, 2013). During the phenological analysis, we also recorded the number of unopened buds and calculated the percentage of unopened buds per twig (i.e., number of unopened buds divided by the total number of buds on a twig).

2.4 Data analysis

The growing degree hours (GDH) were calculated as follows:

$$GDH = \sum_{t}^{LU}\left(\sum_{1}^{24}(T_{hour} - T_{base})\right) \; if \; T_{hour} > T_{base}$$

where T_{hour} is the hourly temperature and T_{base} is the base temperature, which was set at 0 °C on the basis of a previous study (Fu *et al*., 2016). The hourly accumulation of GDH was calculated from the start of the climate chamber experiment (23 January) to the date of leaf unfolding (LU). The treatment time to reach the leaf unfolding stage (i.e., the number of days from the start of the experiment until at least one leaf had unfolded) was recorded. Additionally, the requirement for the GDH of twigs during this period was calculated for each treatment. Moreover, the paired *t*-tests were used to evaluate the differences between the mean time of leaf unfolding, the GDH required for leaf unfolding, and the percentage of unopened buds under different treatments. Furthermore, as previous studies revealed that a certain amount of low temperatures during winter (higher than the freezing point, but lower than a temperature threshold), or called chilling, is required to break endodormancy (Cannell & Smith, 1983; Chuine *et al*., 2013; Fu *et al*., 2016), which occurred before January (Beil *et al*., 2021). After checking the temperature at the collection site, i.e. lower than 8 °C in January at Tianmu mountain, we therefore selected the 8 °C as the threshold following previous studies (Chuine, 2000; Fu *et al*., 2019), and estimated the chilling hours when the site hourly temperature was between 0 and 5 °C starting from 1 September to 31 December 2020. The temperature sensitivity of leaf unfolding (S_T) was calculated according to linear regression analyses of the leaf unfolding date of each twig and the temperature recorded by the HOBO sensors (Onset Computer Corporation, USA) for each treatment during the experimental period. Differences in the temperature sensitivity between the genders, elevations, and the photoperiod treatments were evaluated according to ANCOVAs, which can test the significance of both the slope and the intercept of the linear relationships (Fu *et al*., 2019). All statistical analyses were conducted using R (version 3.5.2).

3. Results

3.1 Differences in leaf unfolding between genders and provenance elevations

Leaf unfolding occurred slightly earlier on male twigs than on female twigs (1 day earlier) (Fig. 1a). The heat requirement (GDH) for leaf unfolding was also mildly greater for female twigs (14,334 ± 588 °C) than for male twigs (13,874 ± 551 °C) (Fig. 1b). In addition, there was no significant difference in S_T between female and male samples (i.e., 3.68 ± 0.22 days $°C^{-1}$ and 3.64 ± 0.17 days $°C^{-1}$, respectively; Fig. 2). We also found that the responses of leaf unfolding to photoperiod and elevation were same between male and female (Fig. S3). These results suggest that the leaf unfolding and its response to environment changes between male and female ginkgo trees are well synchronized. Therefore, in the following analysis of the effects of temperature, photoperiod, and elevation on leaf unfolding, we used the mixed dates across male and female samples.

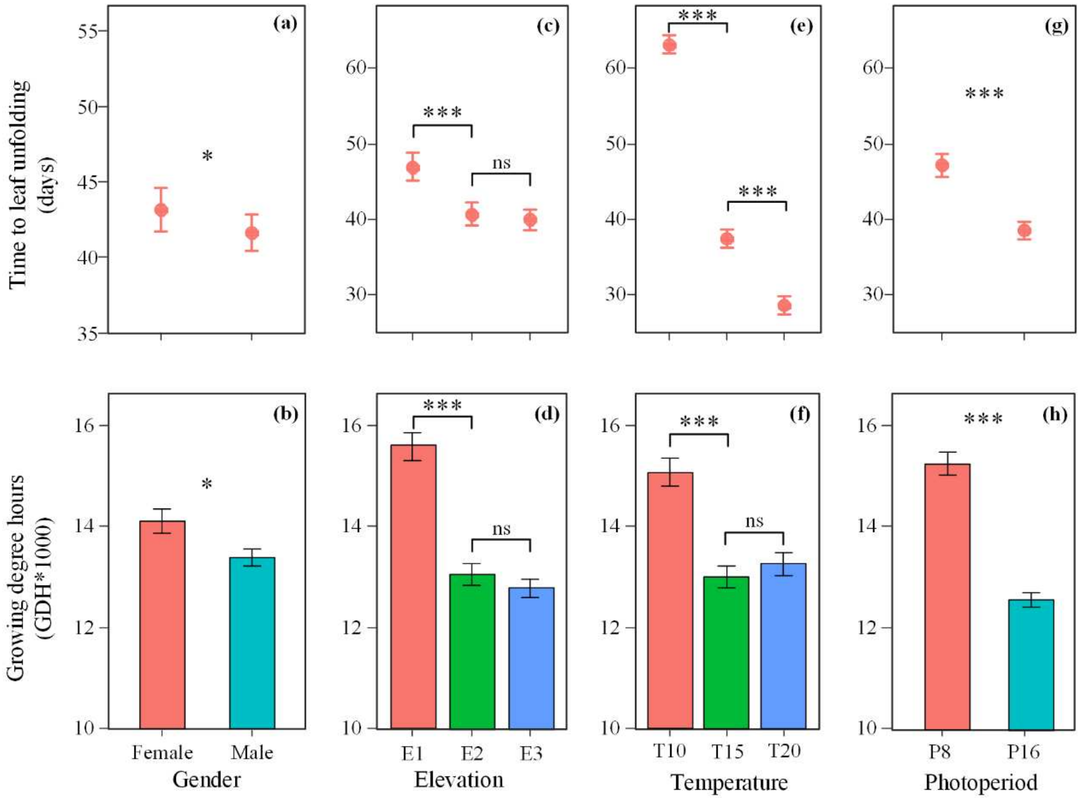

Fig. 1 Time to leaf unfolding for twigs (mean ± se, the upper panel), and heat requirement required for leaf unfolding (defined as the growing degree hours, the lower panel) for different gender (a, b), elevation (c, d), temperature (e, f) and photoperiod (g, h) treatments. "ns" indicates that no significant differences exist between two treatments, * and *** indicate $P < 0.05$ and $P < 0.001$, respectively.

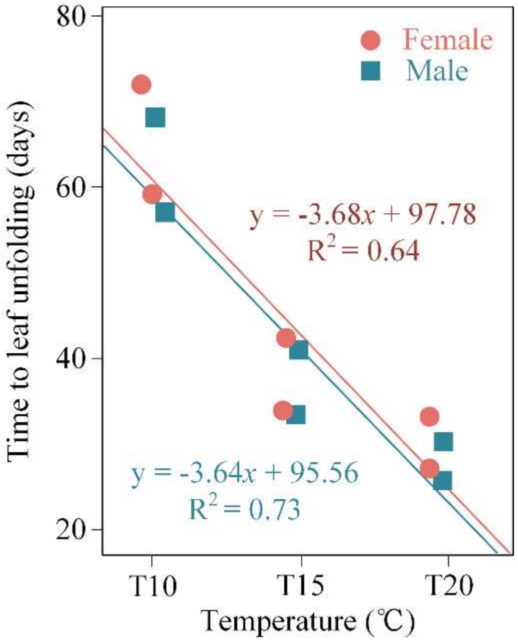

Fig. 2 Time to leaf unfolding for both male and female trees. The temperature sensitivity of leaf unfolding was determined as the slope of linear regression between temperature treatments and the time to leaf unfolding.

The time required to reach the leaf unfolding stage was significantly longer for the twigs collected at the lowest elevation (E1 = 50.5 days) than for the twigs collected at the two higher elevations (E2 = 41.3 days and E3 = 40.8 days; no significant difference) (Fig. 1c). The requirement of GDH at the leaf unfolding stage was greater for the E1 twigs (16,128 °C) than for the twigs from the two higher elevations (E2 = 13,161 °C and E3 = 13,024 °C; no significant difference) (Fig. 1d).

3.2 Temperature and photoperiod effects on leaf unfolding

Increases in the temperature and photoperiod significantly accelerated the leaf unfolding of all samples (Fig. 1e and g). Leaf unfolding occurred 36.1 and 26.7 days earlier for the twigs that underwent the T20 and T15 treatments, respectively, compared with the twigs that underwent the T10 treatment (Fig. 1e). Additionally, the heat requirement for leaf unfolding was significantly lower for the T15 and T20 treatments than for the T10 treatment (Fig. 1f). More specifically, the heat requirements were 13,345 °C and 13,445 °C at 15 °C and 20 °C, respectively, whereas it was 15,523 °C at 10 °C (Fig. 1f).

The time to leaf unfolding was shortened by 9.1 days following the long photoperiod treatment, which corresponds to a 2,837 °C decrease in the heat requirement (Fig. 1g and h). Interestingly, the long photoperiod significantly accelerated leaf unfolding for all temperature treatments, but this photoperiod effect decreased as the temperature increased. Compared with the effects of the P8 treatment, the P16 treatment shortened the time to leaf unfolding by 11.7, 8.0, and 5.1 days during the T10, T15, and T20 treatments, respectively (Fig. 3a). Consequently, the temperature sensitivity of leaf unfolding decreased from 3.98 days $°C^{-1}$ (P8 treatment) to 3.34 days $°C^{-1}$ (P16 treatment). Similar responses to the photoperiod were observed at each provenance (Fig. 3b). However, the photoperiod-related decrease in S_T was more pronounced for the E1 twigs (1.15 days $°C^{-1}$) than for the E2 (0.79 days $°C^{-1}$) and E3 (0.48 days $°C^{-1}$) twigs. This suggests the constraining effect of the short photoperiod increased as the elevation decreased.

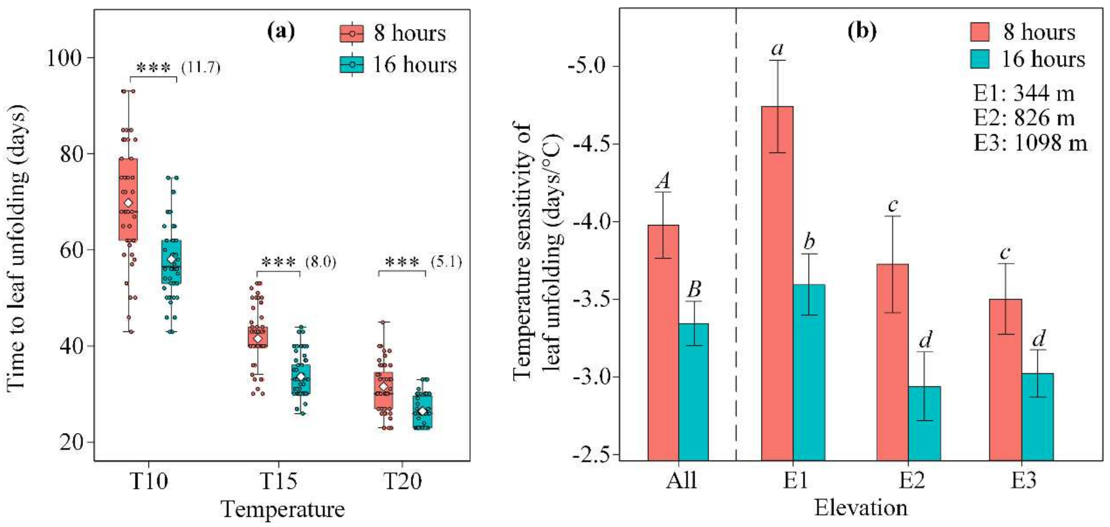

Fig. 3 Time (a) and temperature sensitivity (b) to leaf unfolding in the different temperature treatments under short (8 hours, P8) and long photoperiod (16 hours, P16) treatments. *** indicates significant difference between the two photoperiods ($P < 0.001$). The numbers in the brackets refer to the differences of time to leaf unfolding between the two photoperiod treatments. The different letters in (b) denote significant differences ($P < 0.05$) in the temperature sensitivity between the two photoperiods.

To clarify the potential mechanism mediating the leaf unfolding process, we examined the relationship between the forcing requirement (GDH) and chilling conditions (grouped according to the elevation). The chilling accumulation increased as the elevation increased, which further decreased the GDH required to induce leaf unfolding under both photoperiod treatments (Fig. S4).

3.3 Effect of the original elevation

The time to leaf unfolding was shortened by 2.0 days per 100 m increase in elevation for the T10 treatment, but by only 1.1 and 0.7 days for the T15 and T20 treatments, respectively (Fig. 4a). Similarly, the long photoperiod also reduced the advanced rate per 100 m increase in elevation from 1.3 days to 0.8 days for P8 and P16, respectively (Fig. 4b).

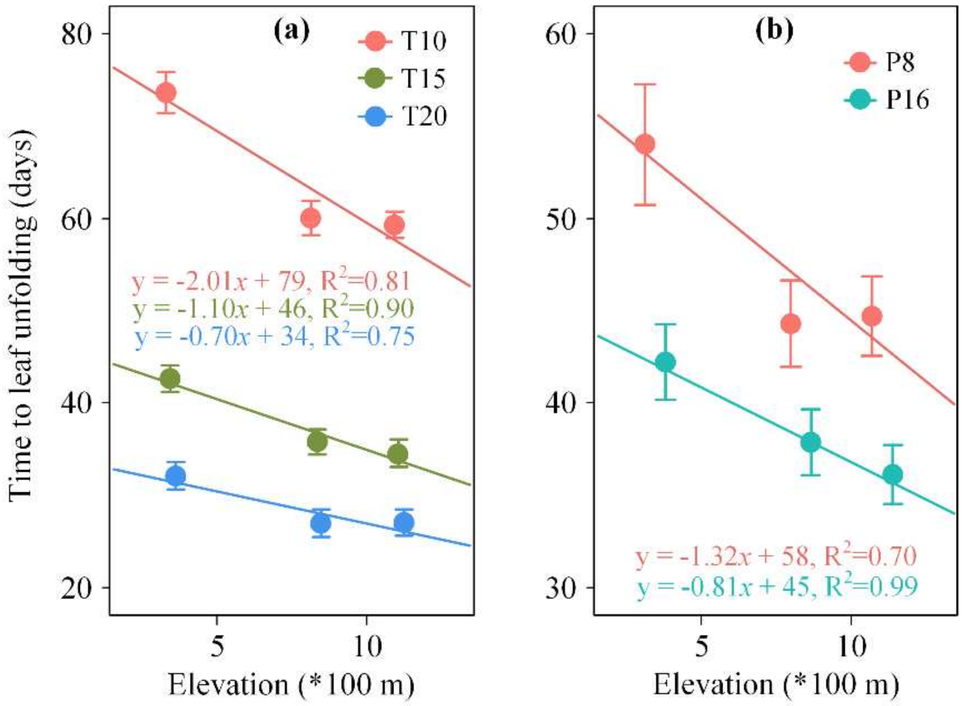

Fig. 4 Time to leaf unfolding under the different temperature and photoperiod treatments for the three elevation provenances. T10, T15 and T20 indicate the temperature treatments of 10 ℃, 15 ℃ and 20 ℃, respectively. P8, P16 represent the photoperiod treatments of 8 hours and 16 hours, respectively.

3.4 Effect of the photoperiod on twig vitality

The percentage of unopened buds per twig was significantly higher for the P8 treatment (29%) than for the P16 treatment (12%), irrespective of the temperature treatments and provenances (Fig. 5).

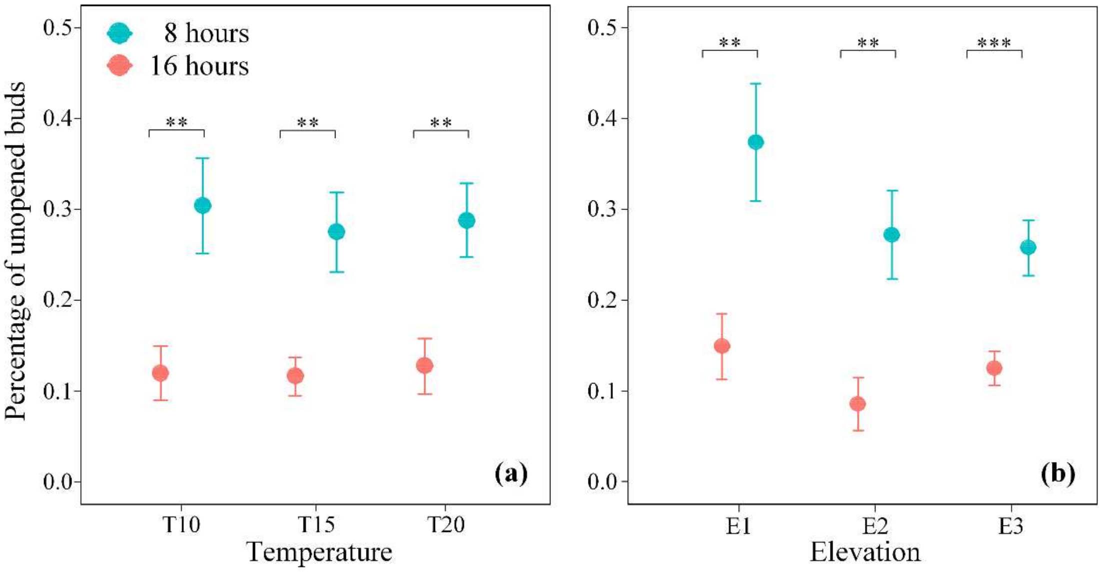

Fig. 5 Unopened bud percentage under different temperature (a) and elevation (b) treatments. ** and *** indicated significant difference existed between the two photoperiods at P <0.01 and P < 0.001 level, respectively.

4. Discussion

4.1 Effects of the temperature and photoperiod on leaf unfolding

Consistent with the findings of previous studies, we observed that temperature increases significantly shortened the time to leaf unfolding by accelerating the accumulation of the required heat (Flynn & Wolkovich, 2018; Fu *et al*., 2019). We also determined that the required GDH decreased with increasing temperatures, which is in accordance with the findings of an earlier large-scale investigation involving a remote sensing dataset (Fu *et al*., 2015a). However, our results challenged the linearity between the GDH requirement and leaf unfolding. Indeed, the heat accumulation rate may increase exponentially in the low-temperature range, and plateau above a certain threshold, as reflected by some phenological models (e.g., the sigmoid model; Chuine *et al*., 2010).

Our data imply that the likelihood of such a relationship increases for ginkgo trees with a stable heat requirement between 10 and 15 °C.

In addition to the temperature, the photoperiod reportedly regulates the spring phenology of trees in temperate and boreal regions (David & Christian, 2014; Fu *et al*., 2019; Way & Montgomery, 2015), with shorter photoperiods delaying bud development and longer photoperiods accelerating leaf unfolding, especially under low chilling conditions (Du *et al*., 2019; Flynn & Wolkovich, 2018; Heide, 1993; Laube *et al*., 2013; Caffarra *et al*., 2011). However, most of these studies were conducted using European or American species. Accordingly, relatively little is known about photoperiod effects on Asian species. In this study, consistent with the findings of the earlier related research, we revealed that increases in the photoperiod shortened the time to leaf unfolding of ginkgo. Interestingly, we also proved that the photoperiod effect differs substantially among populations along an elevational gradient, with the greatest photoperiod effect at the lowest elevation. This spatial variation in the photoperiod effects on spring phenology is consistent with that reported by Zohner *et al*. (2016). The differences in the responses of twigs to the photoperiod might be related to the winter conditions at the collection sites, with the most photoperiod-sensitive species generally originating from the regions with mild winters (Zohner *et al*., 2016). Additionally, the phenological sensitivity to temperature was highest for the twigs collected at the lowest elevation, regardless of the photoperiod treatment, which is consistent with the results of an earlier reciprocal transplant experiment involving seven tree species in the European Alps (Vitasse *et al*., 2013). Environment-induced local adaptations may explain this phenomenon. Trees at relatively high elevations may have adapted to be less responsive to temperature changes, enabling them to avoid the adverse effects of a late frost in the spring (Firmat *et al*., 2017; Vitasse *et al*., 2010).

Our study provides evidence that bud development is promoted by increases in the photoperiod, with an increase in the leaf unfolding rate, irrespective of the temperature, which is in line with the results of previous studies (Du *et al*., 2019; Zhang *et al*., 2021; Baumgarten *et al*., 2021). For example, Zhang *et al*. (2021) demonstrated that a long photoperiod can accelerate dormancy release and increase the bud burst rate, although the photoperiod effect varies considerably among species (Zhang *et al*., 2021; Fu *et al*., 2019). If the temperature continues to increase, the restrictive effects of daylength on leaf unfolding will likely increase, especially among plants distributed in the warm region, as revealed by a recent remote sensing-based investigation (Moon *et al*., 2021). Therefore, the interaction between the photoperiod and temperature should be analyzed more precisely and quantitatively to clarify ecosystem responses to climate change (David & Christian, 2014; Flynn & Wolkovich, 2018; Zhang *et al*., 2021; Fu *et al*., 2019).

4.2 Effects of gender and elevation on spring phenology

We detected leaf unfolding dates are slightly earlier on the male twigs than on the female twigs. Previous studies have found that compared with male gingko trees, the spring phenological events of female ginkgo trees are usually delayed by about 3–5 days (Sofi, 2018; Wang *et al.*, 2009). To increase the chances of a successful pollination, pollen maturation and dispersal should ideally occur after the ovules of female trees have formed (Wang *et al.*, 2009). Since the timing of flowering and leaf unfolding is synchronized, earlier leaf unfolding may lead to a more efficient pollination and reproduction. Additionally, the allocation of resources for reproductive activities in females (Lloyd & Webb, 1977) may result in delayed leaf development. However, we observed that almost all of the buds on the examined twigs lacked ‘flowers’ (strobiles), which may explain the small difference (only one day) in the leaf unfolding between the two genders. Interestingly, we found same response of leaf unfolding to warming, photoperiod and elevation between male and female, suggesting that leaf unfolding response to environment change are synchronized between male and female. These phenomena are likely related to the reproduction strategy of dioecious plants to ensure a higher overlap in the male and female reproductive periods. Because this study involved only one dioecious species, future investigations will need to include more species to analyze the gender-related phenological responses to ongoing climate changes.

Previous studies demonstrated that leaf unfolding is enhanced as the elevation decreases under natural conditions, primarily because of the temperate lapse rate (Dittmar & Elling, 2006; Vitasse *et al.*, 2009). In our climate chamber experiments, leaf unfolding occurred earlier on the twigs collected at higher elevations than on the twigs collected at the lowest elevation. This may be related to the insufficient chilling conditions at the lowest elevation when samples were collected. Indeed, sufficient chilling can effectively decrease the GDH requirement in temperate and subtropical regions (Du *et al.*, 2019; Flynn & Wolkovich, 2018; Laube *et al.*, 2013; Baumgarten *et al.*, 2021). It is likely that the relatively cold conditions at high elevations are sufficient for decreasing the amount of heat required for flushing. Moreover, to maximize the growing season, the environmentally induced adaptive plasticity of spring phenology at high elevations may further decrease the GDH requirement (Firmat *et al.*, 2017; Vitasse, Bresson, Kremer, Michalet, & Delzon, 2010). Therefore, plants growing at high elevations are likely to have a relatively long growing season in response to a warming climate (Vitasse *et al.*, 2010).

Interestingly, the temperature sensitivity decreased as the elevation increased, which is consistent with the findings of an earlier study (Vitasse *et al.*, 2013), but in contrast to the results of other studies (Chapman, 2013; Gao *et al.*, 2020). We assumed that the chilling requirement can be fulfilled more easily at high elevations, leading to an increase in the sensitivity of leaf unfolding to the temperature (Du *et al.*, 2019; Flynn & Wolkovich, 2018; Laube *et al.*, 2013). However, we observed that although gingko leaf unfolding responded quickly to the temperature, the temperature sensitivity decreased along the elevation gradient, similar to the results of earlier research in eastern Europe (Vitasse *et al.*, 2013; Gao *et al.*, 2020). This negative relationship may be attributed to the decrease in spring radiation as the elevation increases (Gao *et al.*, 2020), but this obviously cannot explain our experimental results. Alternatively, increasing temperature variances at high elevations may help to explain our results (Lechowicz, 1984; Wang *et al*., 2014). Trees growing in area with larger magnitude of temperature variance such as high-elevation may have adapted to the unstable temperature conditions and therefore have lower temperature sensitivity to warming (Lechowicz, 1984; Wang *et al*., 2014). In addition, the temperature sensitivity is dependent on both the changes in heat accumulation and the time, although the heat requirement for budburst is larger at the low elevation, the warm temperature may fulfill the requirements with a short time period, and thus result in a higher temperature sensitivity at the low elevation. Furthermore, an active selection for reducing temperature sensitivity or a more stringent interaction with photoperiod in high elevation might further minimize the damage risk induced by late spring frosts (Vitasse *et al*., 2013). Because populations from the margins of warm and cold regions may respond differently to environmental cues and warming, predicting phenological events for these populations might be challenging. A limitation of the present study was the use of twigs for the controlled analysis of different temperature and photoperiod gradients. The constant temperature and photoperiod treatments during our experiments do not accurately reflect natural conditions. This high level of experimental control did enable better attribution of plant responses to specific environmental cues, however, but should be supplemented in the future with experiments under more natural environmental conditions to also reveal natural feedbacks (De Boeck *et al*., 2015).

5. Conclusions

Our study revealed that the sensitivity of gingko leaf unfolding to the temperature and photoperiod decreases as the elevation increases, possibly because of local adaptations that protect against a late frost in the spring. Our experiment indicated that bud development is mainly affected by the temperature, but other climatic conditions (e.g., chilling) and the photoperiod can significantly change the heat requirement for leaf unfolding. Therefore, phenological models should account for population-specific responses to the temperature by considering local adaptations, especially along bioclimatic gradients (e.g., across elevations). Additionally, although the differences between genders were small in this study, our results demonstrated the gender-synchronous response of leaf unfolding under changing environments and also provide new insights into the phenological responses of dioecious plants to climatic variations. Future research should focus on the interactive effects of the photoperiod and temperature, the differential responses of genders, as well as gymnosperms and angiosperms, and the environmental plasticity of plants to elucidate leaf phenological responses to climate change.

Author Contribution Statement

Y.H.F. and Y.P.Z conceived the ideas and designed methodology; Y.P.Z and C.F.L collected the twig at Tianmu Mountain; Z.W., S.W and Y.G collected the data of leaf phenology; Z.W. and Y.H.F. analyzed the data and led the writing of the manuscript, in corporation with Y.P.Z and C.F.L; J.T., H.D.B. and Y.V. advised on experimental method, research design and assisted in writing the manuscript; Z.W. and C.F.L contributed equally to this work. All authors contributed critically to the drafts and gave final approval for publication.

Data availability statement

The datasets that support the findings of the current study are available from the corresponding author on reasonable request.

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