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Research

Stabilizing mechanisms enable dioecious trees to maintain synchrony in spring budburst under climate warming

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Summary

- Climate change could reduce dioecious plant fitness if the phenology of males and females responds differently to temperature. However, the extent to which spring phenological responses to climate differ between sexes in wind-pollinated dioecious trees remains poorly understood.
- Here, we combined ground observations with climate-controlled experiments to investigate sexual differences in spring budburst in *Ginkgo biloba*, *Fraxinus chinensis*, and *Eucommia ulmoides*.
- In 96% of *in situ* cases, male trees initiated budburst earlier than females, on average by 3.0 ± 0.4 d. This disparity was more pronounced in warmer regions. The experiment indicated that background climate is a key predictor of sexual disparity in budburst, with the largest differences observed in twigs originating from regions with higher mean annual temperatures and precipitation. However, these disparities declined in areas where mean annual temperatures exceeded 17.1°C, indicating nonlinear trends. This pattern aligns with the warming treatments, where sexual disparities decreased under spring warming of 2–10°C.
- These results suggest that while sexual disparities can be larger in warmer climates, dioecious trees possess stabilizing mechanisms, including photoperiod and chilling requirements, to maintain synchrony under warming conditions. Our findings enhance understanding of sex-specific phenological responses to climate change, with important implications for future species conservation and ecosystem management.

Introduction

Climate change poses profound threats to plant fitness, population structure (Grimm *et al.*, 2013), and the functioning of ecosystems they sustain (Urban, 2015; Warren *et al.*, 2018). Dioecious plants are particularly vulnerable to changing environments due to the spatial segregation between male and female individuals (Hultine *et al.*, 2016). The temporal synchronization between male and female flowering is a critical adaptation that optimizes pollination efficiency and enhances reproductive success (Xie *et al.*, 2023; Buonaiuto, 2024). However, climate change has driven substantial shifts in the timing of spring leaf-out and flowering, potentially leading to asynchronous responses between males and females (Park *et al.*, 2022; Xie *et al.*, 2022; Wu *et al.*, 2025). Such mismatches can seriously impact plant fitness, reproduction, and species interactions (Urban, 2015; Koski *et al.*, 2018). Yet, sexual disparities in the responsiveness of spring phenology to climate change remain poorly understood (Buonaiuto, 2024).

The sexual reproduction of flowering plants highly depends on successful pollination (Albrecht *et al.*, 2012). In wind-pollinated dioecious species, anthers are exposed for only a short time before dehiscing and releasing pollen (Michalski & Durka, 2007). Previous studies indicated that male individuals typically flower earlier than females to ensure that pollen maturation coincides with female flowering (Yang *et al.*, 2022; Xie *et al.*, 2023). For instance, ground observations have shown that male *Ginkgo biloba* burst their buds 3–5 d earlier than females (Li *et al.*, 2009; Rasheid *et al.*, 2018). Even stronger sexual differences in flowering timing – exceeding 20 d – have been observed in *Populus*, based on digitized herbarium specimens (Xie *et al.*, 2023). Moreover, the extent of sexual separation has been reported to vary spatially, primarily driven by background climate factors such as mean annual temperature and total precipitation (Puixeu *et al.*, 2019; Xie *et al.*, 2023). Given the crucial control of background climate on spring phenology (Geng *et al.*, 2022; Wu *et al.*, 2023), the degree of sexual separation in spring phenology may vary along environmental gradients (Puixeu *et al.*, 2019). These dynamics highlight the need for a better understanding of sexual separation and its underlying mechanisms to assess the potential impacts of climate change on reproductive timing and success.

Global warming has led to earlier spring budburst in extratropical forests over the past decades (Piao *et al.*, 2019; Vitasse *et al.*, 2022). However, different species and phenological events exhibit varying sensitivities to temperature (Wu *et al.*, 2025), with events occurring earlier in the year generally responding more strongly (Zohner *et al.*, 2020; Xie *et al.*, 2022). In dioecious species, for instance, males, which flower earlier than females, often show greater sensitivity to temperature (Xie *et al.*, 2023). Under warming scenarios, this temporal separation between male and female flowering is projected to increase by an average of 8–28% (Xie *et al.*, 2023), potentially impacting sexual reproduction and gene flow (Petry *et al.*, 2016). However, these findings are mostly derived from field observations or digitized herbarium specimens, which may introduce uncertainties due to the complexities of natural environments (Yang *et al.*, 2022). Therefore, full-factorial manipulative experiments are urgently needed to better understand sexual dimorphism in phenological responses to climate change. Moreover, the considerable geographic variation in phenological responses (Shen *et al.*, 2015; Geng *et al.*, 2022) underscores the need to account for the influence of background climate factors, such as mean annual temperature and total precipitation (Gao *et al.*, 2020; Wu *et al.*, 2023).

Sexual dimorphism in other phenological phases, such as leaf-out, can also impact plant fitness (Guo *et al.*, 2023). In species that leaf out before flowering, leaf-out plays a critical role in supplying photosynthates essential for successful flowering (Tissue & Nobel, 1990; Davidson *et al.*, 2021; Guo *et al.*, 2023; Qiu *et al.*, 2024). Additionally, leaf-out timing can influence the pollination environment (Whitehead, 1969), especially in mixed-bud tree species like *G. biloba*, where leaves and sporophylls develop from the same buds and emerge in close spatial and temporal proximity. However, sexual differences in the timing of spring leaf and flower budburst remain poorly understood.

To address these knowledge gaps, we integrated multi-source ground observations of three dioecious tree species (*G. biloba*, *Fraxinus chinensis*, and *Eucommia ulmoides*) with a large-scale twig-cutting experiment on *G. biloba* across a broad environmental gradient to investigate the sequence of spring budburst and its spatial variation. We propose and test two main scenarios regarding the sequence of budburst: the Male first scenario (Fig. 1a–c), where males open their buds before females to optimize pollination efficiency (Wu *et al.*, 2022b; Xie *et al.*, 2023); and the Female first scenario (Fig. 1d–f), in which females burst their buds before males (Purrington & Schmitt, 1998). For each scenario, we examined three subscenarios of sex-specific responsiveness to temperature: If both sexes respond equally, the interval between their budburst dates remains constant (stable synchrony, Fig. 1a,d). If females are more sensitive, synchrony increases under the Male first scenario (Fig. 1b) and decreases under the Female first scenario (Fig. 1e). Conversely, if males are more sensitive, synchrony decreases under the Male first scenario (Fig. 1c) and increases under the Female first scenario (Fig. 1f).

Materials and Methods

Ground-sourced phenological observation

The following two ground-sourced phenology datasets were used in this study:

(**1**) The China Campus Phenology Observation Network (CCPON) was developed in 2020 and includes a total of 35 universities in China, covering temperate, subtropical, and tropical regions (102°51′00″E–125°16′19″E, 24°49′36″N–41°49′23″N, Supporting Information Fig. S1). *Ginkgo biloba* L. (hereafter referred to as *Ginkgo*) is a typical dioecious tree species widely distributed in China and commonly planted in other regions of the world (Zhao *et al.*, 2019) and has been extensively used in phenological research (Wu *et al.*, 2023). At each university, *c.* 10–40 adult male and female *Ginkgo* trees growing under similar environmental conditions were observed. Since *Ginkgo* leaves and sporophylls (reproductive organs) develop from the same bud, budburst timing was used to examine sexual differences and spatial variations. Budburst date was observed twice a week and defined as the day of the year (DOY) when at least 5% of the buds had visibly burst, based on visual inspection. A total of 2460 observations were recorded, including 1243 female and 1217 male observations. After quality checks, abnormal data and sites were excluded, and only those sites with both male and female individuals were included in the analysis.

(**2**) The Beijing Normal University (BNU) Phenology Observation Network was established in 2019 (116°24′00″E, 39°57′42″N, Fig. S1) and covers 27 species. For this study, we selected three dioecious tree species: *Ginkgo* (15 males and 17 females), *Fraxinus chinensis* Roxb. (5 males and 5 females), and *Eucommia ulmoides* Oliver. (6 males and 6 females). The timing of budburst was monitored two times a week for each individual tree. A total of 162 records were used, comprising 78 male and 84 female observations.

Twig-cutting experiment

To test the response of sexual differences in budburst under controlled conditions, we collaborated with 21 universities across temperate and subtropical regions (24°N–44°N, 102°E–126°E; Fig. S1) to conduct a twig-cutting experiment on *Ginkgo*. At each site, we selected *c.* 10 adult individual trees – 5 males and 5 females – with similar diameters at breast height. Eight healthy 40 cm twigs, each with a diameter of *c.* 0.5–0.8 cm, were collected from the southern canopy of each tree on two separate occasions: 19 December 2021 and 1 March 2022, respectively. In total, we collected 2144 twigs, including 1318 twigs from 21 sites in December and 826 from 11 sites in March. The collected twigs were disinfected in a hypochlorite solution and placed in 395 ml plastic bottles containing 265 ml of cool tap water inside

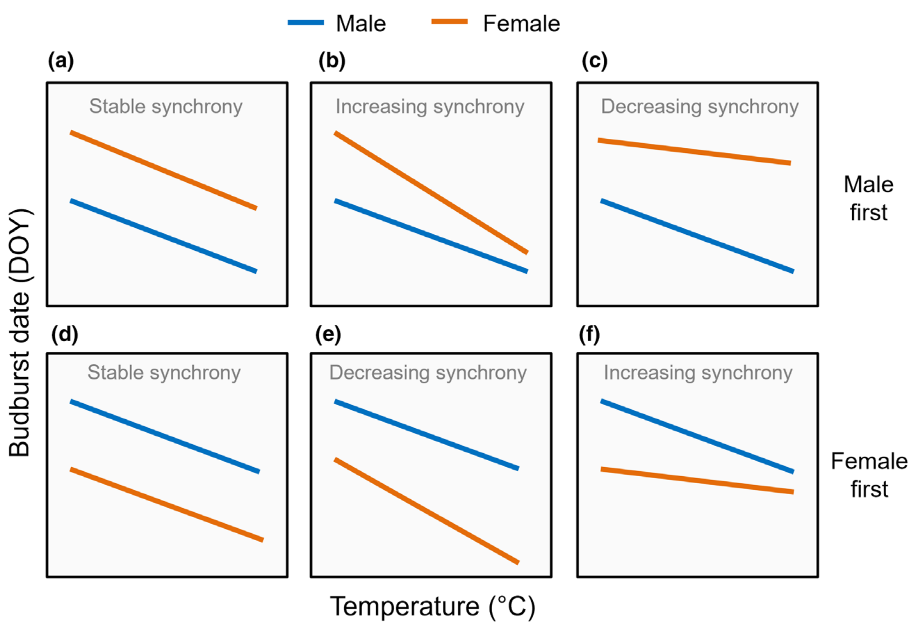

Fig. 1 Conceptual diagram of sexual dimorphism in spring budburst date of dioecious tree species. Two main scenarios are considered: the Male first scenario (a–c), in which males burst their buds earlier than females, and the Female first scenario (d–f), where females burst their buds earlier than males. Within each scenario, three subscenarios are explored based on the relative response of budburst to warming between males and females: (a, d) both sexes respond equally, maintaining stable synchrony, (b, e) females are more sensitive than males, and (c, f) males are more sensitive than females. Scenarios (c, e) decrease synchrony between sexes, whereas scenarios (b, f) promote more synchronized budburst under warming conditions. DOY, day of the year.

climate chambers (Wu *et al.*, 2022b). To prevent vessel blockage, the water was refreshed, and *c.* 2 cm was trimmed from the base of each twig every 1–2 wk.

Environmental treatment We conducted a full-factorial experiment in four climate chambers to assess the effects of temperature, photoperiod, and chilling on spring budburst. The climate chambers used for the experiment were located at BNU, each measuring 196 cm × 159 cm × 280 cm. The temperature treatments were based on ambient spring daily temperatures recorded at 3 h intervals from historical data in Beijing (1979–2018, Fig. S2) and used as the control chamber temperature (T_A). Three warming treatments were applied in three separate climate chambers. The first two simulated emission scenarios from the Intergovernmental Panel on Climate change (IPCC) Sixth Assessment Report (AR6), with temperatures set at +2°C and +5°C above ambient temperature (T_A), and the third chamber was set at +10°C above T_A to simulate extreme warming conditions that may occur in a particular year. Each chamber was subdivided into two sections using thick black cloth, which was opened and closed daily to create two contrasting photoperiod treatments for testing the effect of shorter day length: 14 h (P_{long}), representing late-spring conditions at our site, and 8 h (P_{short}), representing late-winter conditions (Wu *et al.*, 2023). Additionally, two chilling treatments were implemented based on the collection period of the twigs (Zohner *et al.*, 2016): low chilling, with twigs collected in early winter (C_{low}, 19 December 2021), and high chilling, with twigs collected in late winter (C_{high}, 1 March 2022). In total, 16 environmental treatment combinations (4 temperature × 2 photoperiod × 2 chilling) were applied in this experiment.

Each climate chamber was equipped with Philips Greenpower LED Toplighting, providing an illuminance of *c.* 161 µmol m^{-2} s^{-1}, which has been demonstrated to be sufficient for triggering photoperiodic responses in twig-cutting experiments (Du *et al.*, 2019; Wu *et al.*, 2023). Air temperature in each chamber was recorded at 30-min intervals using HOBO MX2202 loggers (Onset Computer Corporation, Bourne, MA, USA). Throughout the experiment, CO$_2$ concentration and relative humidity were maintained at *c.* 440 ppm and *c.* 40%, respectively, across all chambers.

Phenological observations We observed bud development every 3 d for each twig. The budburst date was defined as the day when the first bud scale opened and the leaf was partially visible (Vitasse, 2013; Wu *et al.*, 2023).

Climate data of provenances

Climate data for each provenance collection site were obtained from the China Meteorological Forcing Dataset (He *et al.*, 2020), provided by the Data Assimilation and Modeling Center for Tibetan Multi-spheres at the Institute of Tibetan Plateau Research, Chinese Academy of Sciences (http://data.tpdc.ac.cn/en/data/8028b944-daaa-4511-8769-965612652c49/). This dataset provides temperature and precipitation data at 3-h intervals, with a spatial resolution of 0.1°, covering the period from 1979 to 2018. We calculated the mean annual temperature, mean annual precipitation, temperature seasonality, and precipitation seasonality to characterize the background climate of each provenance site. Temperature seasonality was defined as the annual range between the highest and lowest daily temperatures (Wu *et al.*, 2023). Precipitation seasonality was calculated as the difference between the highest and lowest monthly precipitation values. Topographic elevation data were obtained from EarthEnv (https://www.earthenv.org/topography) (Amatulli *et al.*, 2018). The daily temperatures for 2021 and 2022 at the collection sites were obtained from the NOAA National Center for Environmental Information (https://ngdc.noaa.gov/).

Data analysis

The forcing requirement for each twig to achieve budburst was calculated using a sigmoidal function, as the rate of forcing accumulation varies under different temperature conditions (Hänninen, 1990; Wu *et al*., 2023):

$$\mathrm{GDD} = \sum_{\mathrm{Start}}^{\mathrm{Budburst}} \begin{cases} 0 & \text{if } T_{\mathrm{day}} < T_{\mathrm{base}} \\ \dfrac{28.4}{1+e^{-0.185\left(T_{\mathrm{day}}-18.4\right)}} & \text{if } T_{\mathrm{day}} \geq T_{\mathrm{base}} \end{cases}$$

where T_{day} represents the mean daily temperature from November 1 to the budburst date (including both field and indoor conditions), and the base temperature (T_{base}) is set at 0°C.

Paired *t*-tests were used to evaluate sexual differences in budburst date and their variations across temperature, photoperiod, and chilling treatments (Wu *et al*., 2023). The effects of mean annual temperature and total precipitation on spring budburst and their sexual differences were estimated using linear regression analysis. Photoperiod limitation on budburst was calculated as the day difference between the two photoperiod treatments (P_{short}–P_{long}). Chilling limitation on budburst was calculated as the day difference between the two chilling treatments (C_{low}–C_{high}). Synchrony in budburst, forcing requirement, and photoperiod and chilling limitations between males and females were analyzed using major axis regressions.

Variance inflation factor (VIF) analysis was conducted to assess multicollinearity among the selected climatic variables at the twigs' origin (Wu *et al*., 2022a), including mean annual temperature, total annual precipitation, temperature seasonality, precipitation seasonality, and elevation at the collection sites. All VIFs were smaller than 5, suggesting sufficient independence among the predictors. We then assessed the relative importance of each variable on sexual differences in budburst using random forest models. A nonlinear mixed effect model was used to test the robustness of the results (Rohner *et al*., 2018; Lai *et al*., 2023), with temperature and photoperiod treatments included as random effects. To further explore the interactive effects of the main drivers, we conducted recursive partitioning analyses using a decision tree algorithm (Hothorn & Zeileis, 2015; Ma *et al*., 2021). The minimum node size was set to 30% of the total sample size (50). All statistical analyses were conducted using R 4.2.2.

Results

Sexual separation in the timing of spring budburst across studied species *in situ*

Across all *in situ* site–species combinations, male individuals exhibited 3.0 ± 0.4 d (mean ± SE) earlier budburst than females ($P < 0.001$, Fig. 2a). Specifically, male *Ginkgo* individuals from CCPON burst their buds 2.5 ± 0.3 d earlier than females. In the BNU dataset, male individuals budburst 7.7 ± 0.3, 1.8 ± 1.3, and 4.7 ± 2.2 d earlier than females in *G. biloba*, *F. chinensis*, and *E. ulmoides*, respectively. Additionally, 96% of the site–species combinations showed earlier budburst in males, whereas only 4% showed earlier budburst in females (Fig. 2b).

We identified considerable spatial variation in budburst timing among CCPON sites, with budburst timing of both male and female individuals advancing linearly with mean annual temperature ($P < 0.05$, Fig. 2c). However, the rate of advancement was 9% higher in males compared to females ($P < 0.05$, Fig. 2c). Budburst timing advanced by 2.28 ± 0.47 d per degree warming for males and 2.10 ± 0.45 d per degree for females. At Jilin University – one of the coldest sites – the difference in budburst timing between males and females was only 1 d. This sexual separation significantly increased with rising mean annual temperature, with the time interval extending by 0.18 ± 0.08 d per degree warming ($P < 0.05$, Fig. 2c), consistent with the Male first, decreasing synchrony scenario (Fig. 1c). By contrast, no significant precipitation gradients were observed for the budburst timing of either sex or for the time interval between them ($P > 0.05$, Fig. 2d).

Sexual differences in budburst timing in the climate chamber experiment

Across all treatments in the first twig-cutting campaign, male twigs exhibited a significantly shorter time to budburst compared to females by an average of 5.6 ± 0.8 d ($P < 0.001$, Fig. 3a), aligning with findings from the *in situ* observations. Accordingly, the forcing requirement for budburst was also significantly lower in males than in females ($P < 0.001$), with accumulated growing degree days of 474 ± 9.4°C and 516 ± 9.2°C, respectively (Fig. 3c). When analyzed separately by environmental treatments, males consistently showed earlier budburst across all temperature, photoperiod, and chilling treatments (Fig. 3b). In addition, warmer temperatures, longer photoperiods, and higher chilling accumulation significantly shortened the time to budburst for both sexes (Fig. 3b). Specifically, under the +2°C, +5°C, and +10°C warming treatments, the time to budburst for males advanced by an average of 19.5, 40.2, and 60.7 d compared to the ambient temperature treatment, whereas females exhibited similar advancements of 19.4, 45.1, and 66.7 d, respectively. Long days shortened the time to budburst compared to short days, with a greater effect in females (12.8 d earlier) than in males (10.4 d earlier; $P < 0.05$). Longer chilling exposure shortened the time to budburst by 39.2 d for males and 40.8 d for females, respectively. Consistent findings were observed for forcing requirement (Fig. 3d).

Environmental treatments, that is, photoperiod and chilling treatments, also influenced the sexual differences in budburst timing (Fig. S3). A long 14 h photoperiod reduced the sexual difference in budburst by an average of 2.4 d compared to a short hour photoperiod ($P < 0.05$, Fig. S3a). However, the date of collection – a proxy for chilling accumulation – did not significantly affect sexual differences in budburst across all sites ($P > 0.05$, Fig. S3b).

Budburst timing and forcing requirements exhibited strong synchrony between male and female trees from the same location, such that a location with males that leaf out late and have high

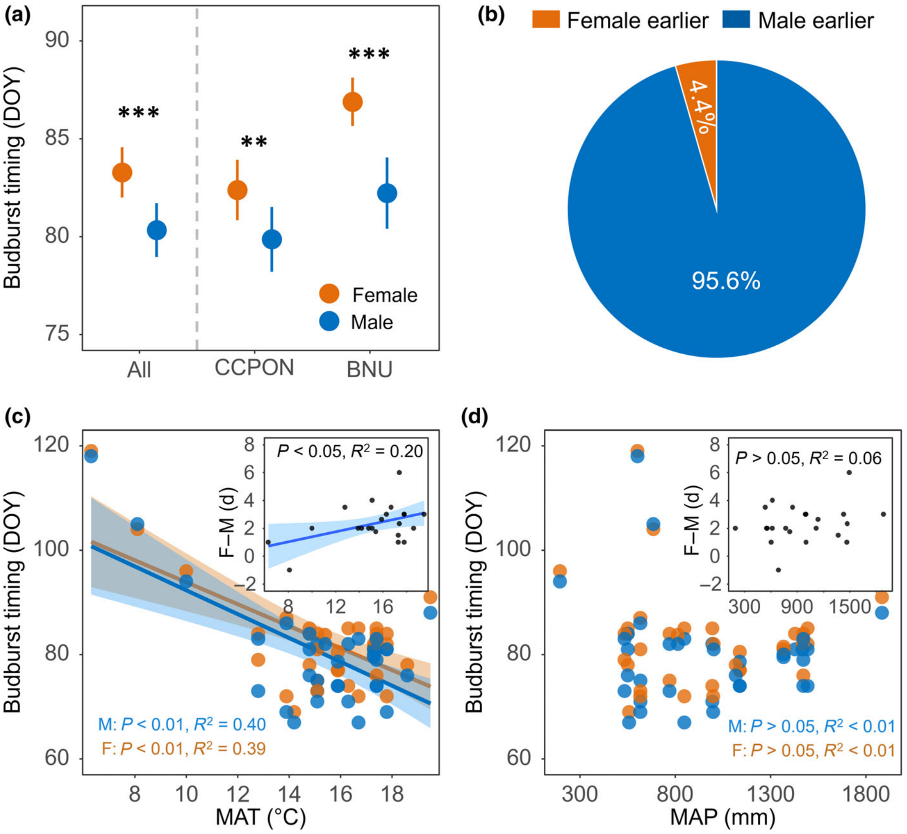

Fig. 2 Sexual separation in budburst timing based on field phenological observations. (a) Comparison of budburst timing between male and female individuals across all sites as well as within the China Campus Phenology Observation Network (CCPON), and the Beijing Normal University Phenology Observation Network (BNU). Data points represent mean values, with error bars indicating one SE. Asterisks denote significant differences between sexes based on a two-sided t-test (***, $P < 0.001$; **, $P < 0.01$). (b) Percentage of species–site combinations where males or females budburst earlier. (c, d) Relationships between mean annual temperature (MAT) and precipitation (MAP) with budburst timing for male and female individuals as well as the budburst interval between sexes (Female–Male (F–M)). Linear regression trend lines are shown with corresponding R^2 and P values, and shaded areas represent the 95% confidence intervals of the fitted models. DOY, day of the year.

forcing requirements is highly likely to also harbour late-leafing females, and vice versa (Fig. 4a,b). Similarly, photoperiod and chilling limitations on budburst were comparable between males and females from the same location (Fig. 4c,d). These findings suggest that despite sexual disparities in budburst timing, males and females exhibit similar environmental constraints, which may contribute to maintaining reproductive synchrony under varying climatic conditions.

Factors governing sexual differences in the timing of spring budburst

Mean annual temperature was identified as the best predictor of variation in sexual differences in budburst timing across locations, followed by mean annual precipitation and temperature seasonality (Fig. 5a). This was consistent with the mixed effect model (Fig. S4). Further examination through recursive partitioning analyses confirmed mean annual temperature as the primary driver of sexual disparities in budburst, with mean annual precipitation additionally modulating differences under colder conditions ($R^2 = 0.28$, Fig. 5b). Specifically, when mean annual temperature is below 17.1°C, higher temperatures and precipitation tend to amplify the difference (increasing from 0.2 to 13.7 d). However, when mean annual temperature exceeds 17.1°C, the sexual difference is minor (decreasing from 13.7 to 0.8 d), indicating nonlinear trends. Consistent nonlinear trends were found for the forcing requirement (Fig. S5).

Based on the climate groups determined by mean annual temperature and precipitation (Fig. 5b), we further investigated sexual differences in photoperiod and chilling limitations on budburst. We found no significant variation in photoperiod limitation between sexes across different climate groups (Fig. 6a). However, sexual differences in chilling limitation varied among climate groups (Fig. 6b). Specifically, when mean annual temperature was below 17.1°C, higher temperatures and precipitation tended to increase the sexual difference in chilling limitation. By contrast, when mean annual temperature exceeded 17.1°C, this disparity decreased, mirroring the observed patterns in budburst timing (Fig. 5b).

Discussion

Our field observations demonstrated that males consistently burst their buds earlier than females, with the temporal gap widening in regions with higher mean annual temperature. The full-factorial manipulative experiment confirmed this pattern, reinforcing the role of background climate – particularly mean annual temperature – as a key driver of sexual disparity in budburst timing. Notably, while individuals from warmer regions exhibited greater sexual disparity under controlled conditions, this difference diminished once the mean annual temperature at their sites of origin exceeded 17.1°C, suggesting a nonlinear response. The synchronous shifts in budburst timing and forcing requirements between male and female phenology in response to

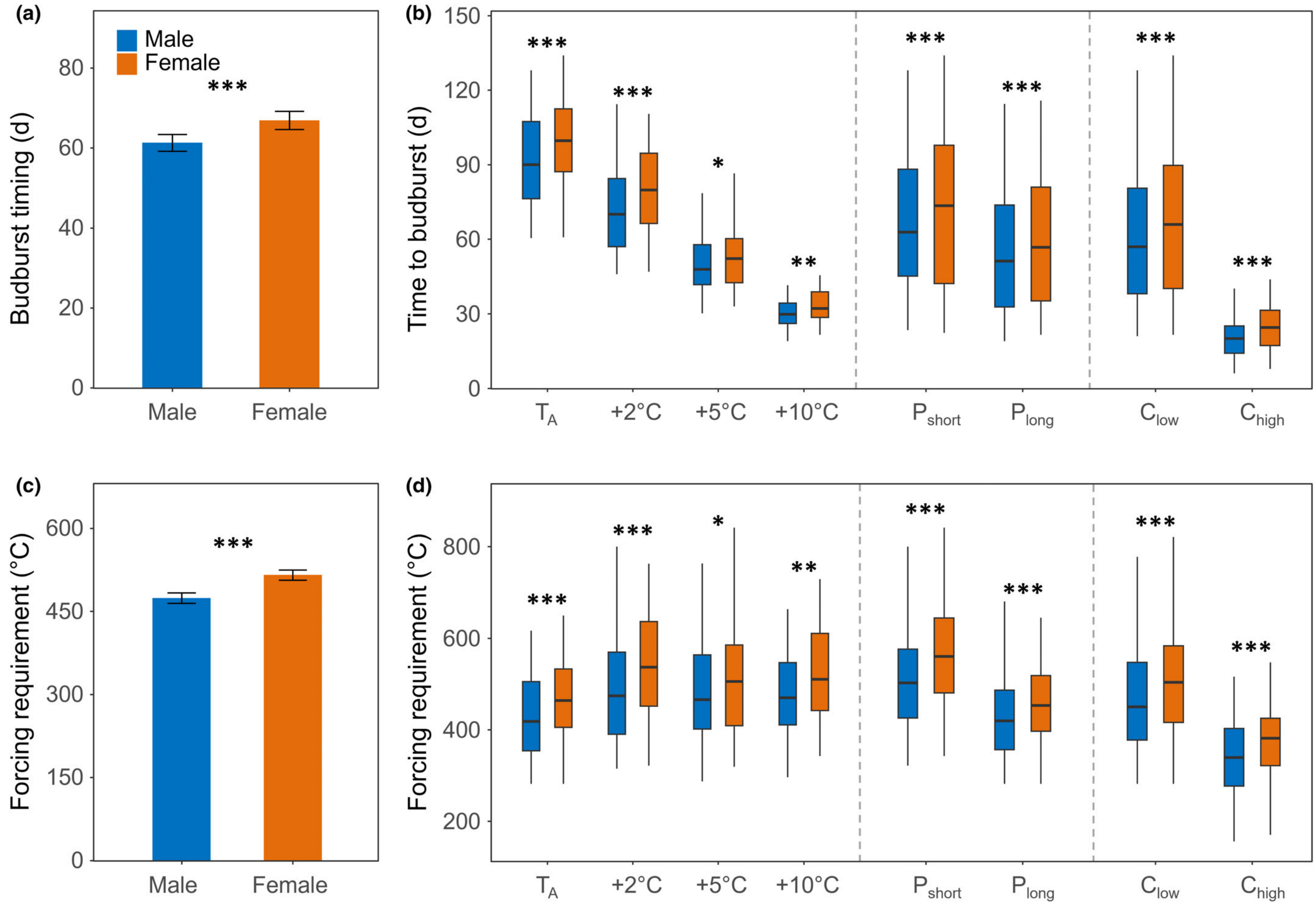

Fig. 3 Sexual differences in budburst timing and forcing requirement. (a, b) Budburst timing across all environmental treatments (a), and under different temperature, photoperiod, and chilling treatments (b). Budburst timing is represented as the time to budburst after being placed in chambers. (c, d) Forcing requirement across all environmental treatments (c), and under different temperature, photoperiod, and chilling treatments (d). The forcing requirement, calculated as the growing degree days needed to achieve budburst, was determined using a sigmoidal function. The bars and error bars represent the average and SE for each treatment. Boxplots show the first quartile, median, and third quartile, as well as the minimum and maximum values within 1.5 times the interquartile range. ***, **, and * indicate significant differences between sexes based on a two-sided *t*-test at $P < 0.001$; $P < 0.01$; $P < 0.05$, respectively.

environmental constraints, such as photoperiod and chilling, contribute to maintaining reproductive synchrony under varying climatic conditions. Under experimental warming, sexual disparities in budburst generally declined. These findings demonstrate stabilizing mechanisms that enable dioecious trees to maintain synchrony in spring budburst under extreme conditions, emphasizing their importance for plant fitness and ecological and evolutionary trajectories.

Temporal separation in spring budburst timing between male and female trees

Our observations that males consistently budburst earlier than females align with patterns observed in cottonwood species (*Populus*) from herbarium specimens and digitized photographs (Yang *et al.*, 2022; Xie *et al.*, 2023). One explanation for this sexual disparity relates to the pollination strategy of wind-pollinated dioecious species. Male reproductive success is often more constrained by mating opportunities than female success (Andersson & Iwasa, 1996; Munguia-Rosas *et al.*, 2011). By initiating budburst earlier, males can accumulate more photosynthates to support flowering, thereby increasing their chances of successful pollination through competition with other males for access to female gametes (Stanton, 1994; Skogsmyr & Lankinen, 2002). In addition, the anthers of wind-pollinated species are typically exposed for only a short period before dehiscing and releasing pollen (Michalski & Durka, 2007). To ensure that pollen maturation aligns with female flowering, earlier budburst in males helps maximize pollination effectiveness and reproductive success (Bogdziewicz *et al.*, 2020; Yang *et al.*, 2022).

Another possible explanation lies in the differing resource allocation strategies between sexes in dioecious species (Lloyd & Webb, 1977; Forrest, 2014). The timing of spring budburst is critical for enabling earlier photosynthetic carbon assimilation. Females typically allocate more resources to reproductive growth, including flowering, embryo development, fruit development,

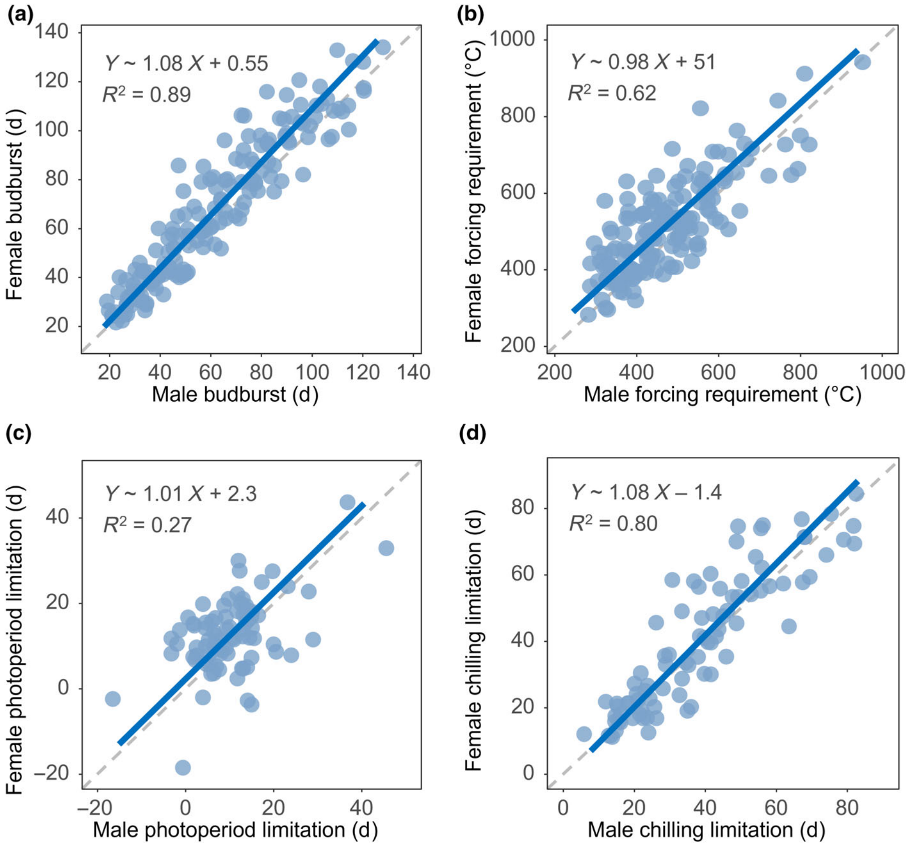

Fig. 4 Synchrony in budburst timing, forcing requirements, and environmental limitations between male and female trees from the same locations. The relationships between male and female trees are shown for budburst timing (days, a), forcing requirements (°C, b), photoperiod limitations (days, c), and chilling limitations (days, d). The dashed gray line represents the 1 : 1 relationship, whereas the blue line is fitted using major axis regression.

and seed dispersal (Xie *et al.*, 2023; Buonaiuto, 2024). By contrast, males' primary reproductive investment resides in flowering, so they allocate more resources to growth and budburst earlier. Additionally, the high reproductive investment of females increases the cost of mistimed flowering compared to males, leading them to adopt a more conservative phenological strategy that requires more forcing to achieve budburst (Forrest, 2014; Xie *et al.*, 2023), as demonstrated in our experiment (Fig. 3b). This effect may be more pronounced in species that produce metabolically costly fruiting bodies, which require greater reproductive investment compared to species with lower reproductive demands.

Spatial variation in sexual separation in spring budburst timing

We observed considerable spatial variation in the sexual disparity of budburst timing across observation sites, with this disparity increasing in warmer regions (Fig. 2c), which was also demonstrated in the experiment (Fig. 5b). This pattern arises from the divergent responses of budburst timing to local temperature between sexes, with males exhibiting greater variation along mean annual temperature gradients than females (Fig. 2c), likely due to differences in their forcing requirements (Fig. 3c). The larger sexual difference in chilling limitation on budburst in warmer regions may also contribute to the greater disparity observed in budburst timing (Fig. 6b). However, we did not observe evident spatial variation in the sexual difference in photoperiod limitation (Fig. 6a). Importantly, our findings show that when mean annual temperature exceeds *c.* 17°C, budburst differences between sexes diminish, along with the sexual difference in chilling limitation (Figs 5b, 6b). This suggests that while climate warming may initially amplify sexual dimorphism in phenology, trees may possess stabilizing mechanisms to maintain synchrony under extreme climatic conditions. The similar environmental constraints of photoperiod and chilling between males and females may also contribute to maintaining reproductive synchrony under varying climatic conditions (Fig. 4). Additionally, selective pressure may also contribute to synchrony, as females must largely advance flowering in very warm regions where conditions may become too hot for optimal fruit development later in the season (Higuchi *et al.*, 1998). These stabilizing mechanisms may have contributed to *Ginkgo*'s persistence for over 200 million years (Major, 1967; Zhao *et al.*, 2019).

Our study has some limitations that we want to acknowledge. The observational networks were operational for only a few years. Moreover, while our study focused on three widespread Asian species, the mechanistic responses assessed experimentally were limited to *G. biloba*. To build a more comprehensive understanding, future research should incorporate a broader range of dioecious species across diverse climatic regions and taxonomic groups to further examine potential phenological discrepancies between male and female plants under warming scenarios. Expanding these studies would also help identify populations

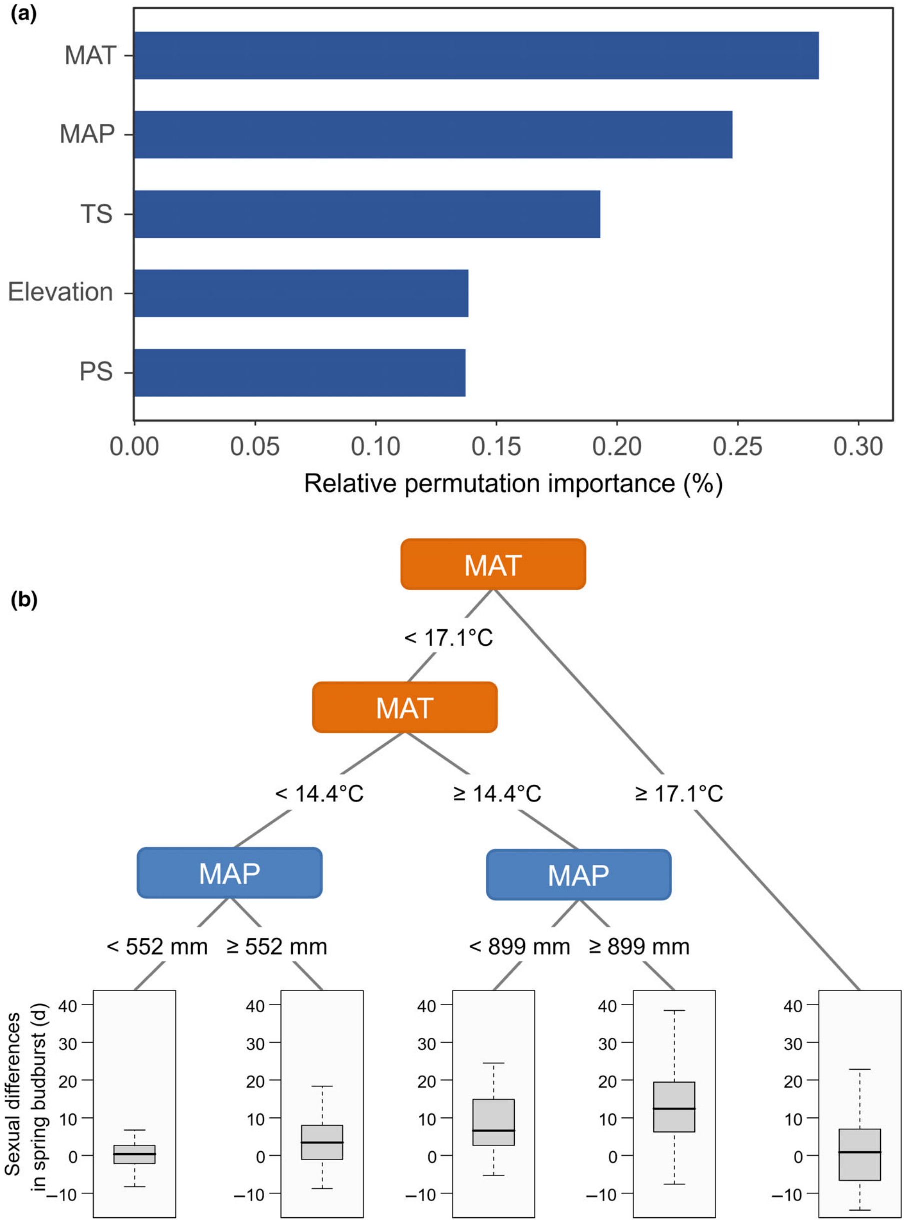

Fig. 5 Drivers of sexual differences in the timing of budburst in the experiment. (a) Relative permutation importance of the predictors included in random forest models ordered from greatest to least importance in determining the sexual differences in spring budburst. Six predictor variables, including mean annual temperature (MAT), mean annual precipitation (MAP), temperature seasonality (TS), precipitation seasonality (PS), and elevation were used in this analysis. (b) Recursive partitioning tree illustrating the effects of MAT and total precipitation on sexual differences in budburst timing. Boxplots show the first quartile, median, and third quartile, as well as the minimum and maximum values within 1.5 times the interquartile range.

most vulnerable to climate-driven phenological shifts and improve conservation strategies, such as assisted migration and genetic augmentation. Additionally, further research is needed to assess the ecological consequences of changing phenology patterns in dioecious species, particularly whether they can adapt to rapid environmental changes while maintaining reproductive synchronization. Integrating these studies with models of population structure and inbreeding depression (Cheptou & Mathias, 2001; Sargent *et al.*, 2006) could provide deeper insights into the long-term impacts of phenological shifts on population viability and genetic diversity.

Conclusions

Maintaining temporal synchrony in budburst is essential for effective pollination and reproductive success in dioecious, wind-pollinated plant species (Whitehead, 1969; Michalski & Durka, 2007). Disruptions in this synchrony could influence population growth, adaptive potential (Petry *et al.*, 2016), and species interactions (Forrest, 2014), leading to cascading effects that threaten biodiversity and ecosystem stability. Our large-scale observations and manipulative experiment revealed pronounced sexual dimorphism in the spring budburst timing of *G. biloba*, with males consistently initiating budburst earlier than females. This disparity was temperature-dependent, increasing in warmer climates but diminishing beyond a specific threshold, suggesting the presence of stabilizing mechanisms. Experimental evidence further supported this, as sexual disparities in budburst timing decreased under spring warming of 2–10°C. These findings suggest that, despite sexual disparities in budburst timing, males and females exhibit similar environmental constraints, which may help maintain reproductive synchrony under varying climatic

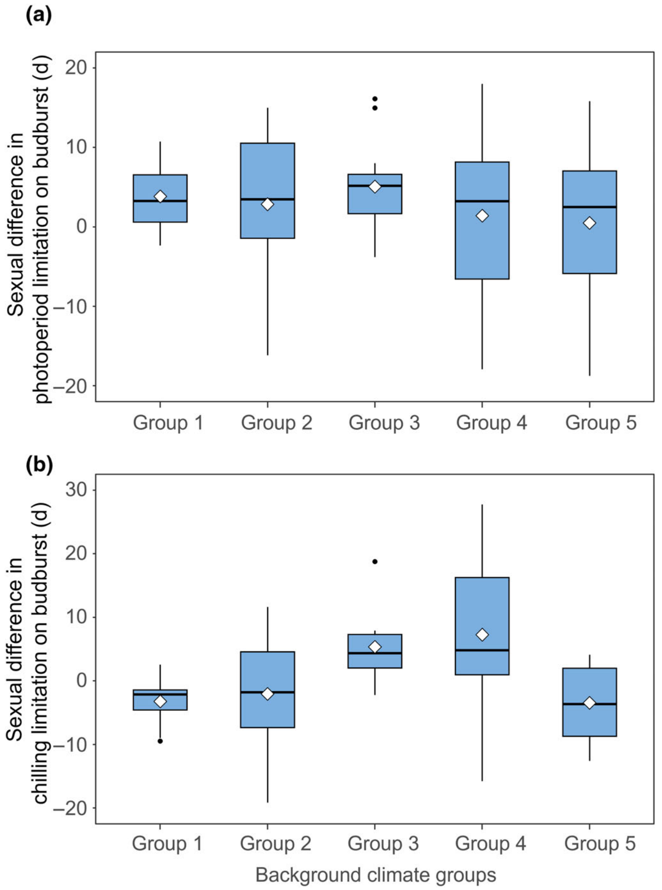

Fig. 6 Sexual differences in photoperiod (a) and chilling (b) limitations on budburst under varying background climates. Boxplots show the first quartile, median, and third quartile, as well as the minimum and maximum values within 1.5 times the interquartile range. The white square indicates the mean value. Background climate groups are based on the recursive partitioning tree in Fig. 5, determined by mean annual temperature (MAT) and mean annual precipitation (MAP): Group 1, MAT < 14.4°C and MAP < 552 mm; Group 2, MAT < 14.4°C and MAP ≥ 552 mm; Group 3, 14.4°C < MAT <17.1°C and MAP < 899 mm; Group 4, 14.4°C < MAT <17.1°C and MAP ≥ 899 mm; Group 5, MAT ≥ 17.1°C.

conditions. Accounting for sexual differences in budburst timing, their climatic dependencies, and the stabilizing mechanisms that mitigate extreme disparities is crucial for accurately predicting climate change impacts and assessing their ecological and evolutionary consequences. Overall, our findings provide key insights into how sexual differences mediate phenological responses to climate change, with important implications for reproductive success, species interactions, and population dynamics.

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Competing interests

None declared.

Author contributions

YHF and ZW conceived the study. ZW, YHF and CZ developed the methodology and analyses. ZW conducted the experiment in corporation with YG, SW and YN. ZW analyzed the data. ML, ZG, NW and HW helped with the experiment and data preparation. ZW, YHF, CZ and YV led the writing of the manuscript with input from DMB and LPCM. All authors discussed the results and reviewed the manuscript.

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Data availability

The datasets that support the findings of this study are available at https://github.com/Phenodoge/PhenoSexSynchrony.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Distribution of the observation and collection sites.

Fig. S2 Hourly temperature settings in the four climate chambers.

Fig. S3 Effect of photoperiod and chilling on the sexual differences in spring budburst timing.

Fig. S4 Relative effects of the predictors in determining sexual differences in spring budburst.

Fig. S5 Drivers of sexual differences in forcing requirements in the experiments.

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