Intertwining of fecundity, sexual and growth selection on spring phenology along an altitudinal gradient of European beech (*Fagus sylvatica* L.).

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ABSTRACT

Background: fecundity and viability selections on phenological traits are difficult to distinguish in plants, where vegetative and reproductive phenologies are closely synchronized. Moreover, interference with sexual selection may arise from assortative mating (the positive correlation between mates for flowering time). This study aims at disentangling these components of selection on spring phenology in European beech.

Methods: the timing of budburst (TBB) was surveyed in 147 and 192 adult trees in two natural populations at low and high elevation respectively along an altitudinal gradient. Male and female individual fecundities were estimated using paternity and parentage analyses of 1414 seeds and 473 seedlings, which also allowed mating networks to be inferred. Morever, TBB and growth was surveyed in 1552 and 1709 seedlings originating from the same populations and planted in a common garden.

Results: assortative mating occurred only at low elevation, where spring phenology was also more spread out. Phenological mismatch reduced male but not female fecundities at both plots, indicating sexual selection to maximize mating opportunities. At high elevation, directional fecundity selection for earlier TBB occurred both through male and female fecundities. At low elevation, directional fecundity selection for earlier spring phenology was mitigated by a positive association between TBB and fecundity in the smaller trees. In the common garden, early budburst was associated to higher seedling growth.

Discussion: This study showed that sexual selection arising from assortative mating could drive stabilizing selection on TBB through the male function, while selection through female fecundity predominantly selects for earlier TBB. This intertwining of sexual and fecundity selection calls for an integrative approach to predict the evolution of spring phenology under changing climate.

Keywords: budburst phenology, selection gradient, assortative mating, Bateman's gradient, parentage/paternity analyses, Mixed-Effect Mating Model (MEMM), Fagus sylvatica

Introduction

Many changes in phenology (i.e., the timing of biological events) were observed during the last decades and attributed to climate change (Parmesan & Yohe, 2003). In particular, leafing, flowering and fruiting records advanced significantly in temperate zones (Menzel et al., 2006), consistent with the rise of spring/summer temperatures. Besides the plastic component of these phenological shifts, rapid evolution of phenological traits in response to selection has also been reported (Franks, Sim, & Weis, 2007; Hamann, Weis, & Franks, 2018). However, it is still largely unknown to what extent microevolution may contribute to the response of plants' populations to climatic variation (Merilä & Hendry, 2014). Moreover, in many plants, vegetative phenology (the timing of germination, stem and leaf development) and reproductive phenology (the timing of flowering and fruiting) are tightly synchronized throughout the yearly cycle. Hence, selection on phenological traits is likely to be the complex outcome of viability selection (selection for phenotypes that increase survival), fecundity selection (selection for phenotypes that increase fecundity) and sexual selection (selection arising from competition for mating partners or their gametes) (Figure 1). This study aims to account for these different components while investigating selection on phenology in a temperate tree species along an altitudinal gradient.

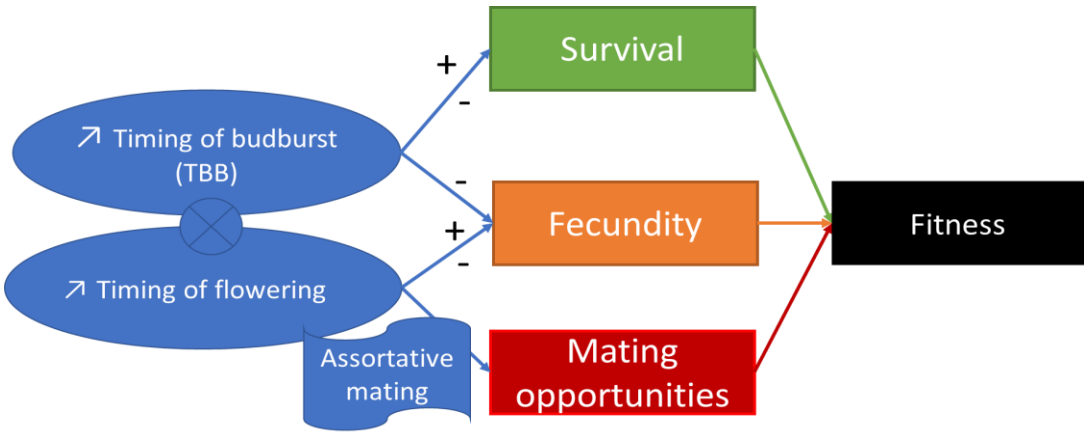

Figure 1 – A schematic representation of the expected relationships between vegetative/flowering phenology and fitness at individual level. Delayed timing of budburst (TBB) is expected to increase survival through frost avoidance, but also to decrease fecundity and survival through reduced length of the growing season, and hence reduced reserves. Delayed timing of flowering is expected to increase fecundity by allowing more resources to be accumulated and invested in reproduction, however at the cost of reduced time for seed maturation. Finally, synchronized flowering with the other individuals in the population (i.e., assortative mating) is expected to maximize the number of mates. The combination of these different selection components determines the optimal values of TBB and flowering times, i.e. those maximizing fitness. Moreover, the timing of budburst and flowering are usually closely physiologically synchronized throughout the plant yearly cycle.

Most selection studies on the timing of flowering have been conducted in short-lived herbaceous plants (Geber & Griffen, 2003; Munguía-Rosas et al., 2011), while the adaptive value of vegetative phenology has been mainly investigated in long-lived forest trees (Alberto et al., 2013). In both cases, stabilizing selection is the most straightforward expectation within-population, considering life history in temperate ecosystems. In the former case, this is because the fecundity benefits of flowering early (sufficient time for seed maturation) are expected to balance with those of flowering later, as early reproduction usually entails reproducing at a small size with limited resources available for offspring production. Note that this expectation could be different for long-living plants for which resources have been accumulated previous year (Hacket-Pain et al., 2018). Yet, early flowering plants are generally found to be favored (Geber & Griffen, 2003; Munguía-Rosas et al., 2011), an apparent paradox for which Austen et al. (2017) proposed four explanations: (1) selection through other fitness components may counter observed fecundity selection for early flowering; (2) asymmetry in the flowering-time–fitness function may make selection for later flowering hard to detect; (3) flowering time and fitness maybe condition-dependent; and (4) selection on flowering duration is largely unaccounted for.

Regarding vegetative phenology in long lived plants, stabilizing selection is expected to be driven by the balance between the benefits of: (1) avoiding frost damages on vegetative and reproductive organs, especially in early spring (viability selection); and (2) maximizing the duration of the growing season, which determines the resource level acquired by photosynthesis (viability and fecundity selection). More complex situations may occur when other abiotic or biotic stresses are considered (e.g. early flushing may amplify drought effects, Meier et al. 2021). Common-garden experiments generally demonstrate significant genetic differentiation of phenological traits between tree provenances along environmental gradients, suggesting that the differences in climatic conditions led to the evolution of different phenological schedules contributing to populations' local adaptation (Alberto et al., 2013). However, experimental selection studies on vegetative phenology lag behind (but see Bontemps et al. 2017; Alexandre et al. 2020). A recent simulation study with a process-based phenological model accounting both for fecundity and viability selection predicted selection towards earlier TBB across a climatic gradient, and realized TBBs always later than the value conferring highest fitness in different tree species (Gauzere et al., 2020). Moreover, these simulations showed that the strength of this selection was stronger at high than low elevation, i.e., in the conditions where the growing season is more limiting for the maturation of fruits.

Compared to fecundity or viability selection, the role of sexual selection on the evolution of phenology remains understudied, even though the existence of sexual selection in plants is now widely acknowledged (Moore & Pannell, 2011). Yet, assortative mating for flowering phenology, that is the positive correlation between male and female flowering time across mated pairs, is obligate in plants (Weis, Nardone, & Fox, 2014), and may lead to sexual selection, depending on the overlap in individual flowering phenologies within the population. Moreover, phenological assortative mating is by nature density-dependent, as any individual synchronized with the rest of the population will gain opportunities for mating (Weis et al., 2005). Hence, assortative mating is expected to lead to stabilizing selection towards an optimal timing of flowering maximizing mating opportunities, depending on the frequency distribution and late and early phenotypes. Finally, due to anisogany (the higher cost of producing female versus male gametes), male reproductive success is generally expected to be more limited by mating opportunities than than by investment in each gamete, whereas female reproductive success should depend on their ability to produce viable ovules and seeds rather than on the probability of having ovules fertilized (one of Bateman's principles; Bateman 1948; Tonnabel, David, & Pannell, 2019). These contrasting challenges could lead to different patterns of selection on phenology through male and female reproductive functions.

Distinguishing fecundity from sexual selection on phenological traits may be particularly challenging, as both jointly act within a single reproduction episode. However, while fecundity selection can occur even under unlimited access to mates, sexual selection involves limited mating opportunities. Hence, the relationship between phenology and fitness (e.g., phenotypic selection analyses, Lande and Arnold 1983) is considered to inform about the joined effects of fecundity and sexual selection (i.e., natural selection), while the relationship between phenology-related mating opportunities and fitness (e.g., Bateman's gradient analyses, Bateman 1948) informs about sexual selection on phenology. Selection gradients (the regression coefficients of relative fitness on a trait) are among the widely used metrics to measure selection in natural populations (Kingsolver et al., 2001). They determine the direction and the pattern of the selection and, when regressing multiple traits, have, at least in theory, the ability to distinguish direct selection on the trait from the indirect selection coming from correlation with other traits (Lande & Arnold, 1983). Using quadratic regressions instead of simple linear regressions also permits to detect and test stabilizing selection (Lande & Arnold, 1983). Finally, standardized selection gradients provide a dimensionless measure of selection strength that can be used for comparisons across traits, populations or species (Kingsolver et al., 2001).

This study takes advantage of the extensive physiological knowledge on a major monoecious tree, the European beech, and of a well-studied altitudinal gradient in South-Eastern France, to estimate selection gradients on phenological traits. European beech is an early flushing deciduous species (Davi et al., 2011), sensitive to frost damages (Lenz et al., 2013).We expect intense selection for early phenology at high elevation, due to high constrains on the length of the growing season. Along the studied gradient, vegetative phenology was monitored both *in situ* and in a common garden of maternal progenies (Oddou-Muratorio et al., 2021). Previous studies showed that, *in situ*, budburst occurs ~9.8 days earlier at the lower elevation plot compared to the upper elevation plot (Davi et al., 2011), but that, in the common garden, the lower plot is ~2.1 days late compared to the upper plot (Gauzere et al., 2020). Phenotypic selection

analyses conducted at the lower plot found that growth and reproductive (seed set) performances could be maximized either by a water-uptake strategy, including early budburst, or by a water-saving strategy, including late budburst (Bontemps et al., 2017). Finally, male and female fecundities were estimated for all the adults in the lower and upper plots through paternity or parentage analysis of germinated seeds and established saplings (Oddou-Muratorio et al., 2018). These fecundity estimates are effective, i.e. they relate to the effective amount of pollen achieving successful pollination, and to the effective number of seeds achieving successful germination and establishment in the population.

The specific objective of this study was to investigate simultaneously fecundity, sexual and viability selection on spring phenology in both the upper and lower plots along the altitudinal gradient. We used paternity analyses to estimate the strength of assortative mating, and we investigated whether male and female fecundities were affected by mating opportunities (sexual selection) by regressing fecundity on the phenological mismatch within mating neighborhood. We estimated fecundity selection on phenology by regressing male and female effective fecundity on TBB. Finally, viability selection was estimated in the common garden by analyzing the relationship between TBB and seedlings growth, under the hypothesis that vigor (i.e. growth capacity) is positively associated with viability (Collet & Le Moguedec, 2007).

Methods

Studied species and sampling design

The European beech is a monoecious, wind-dispersed, predominantly outcrossed tree species (Gauzere, Klein, & Oddou-Muratorio, 2013). Male and female flowers are borne on the same branches and open as the leaves unfold (Nielsen & Schaffalitzky de Muckadell, 1954; Packham et al., 2012), between April and May. Beech is protogynous, i.e. male flowers produce pollen after the peak of receptivity of the stigmas of the same plant (Nielsen & Schaffalitzky de Muckadell, 1954).

Mont Ventoux is located at the warm and dry southern margin of the European beech distribution, and the climate is typical of low altitude mountains with Mediterranean influences (weather station of Mont Serein, 1 445 m a.s.l., 1993–2006; mean annual temperature of 6.8◦C and mean annual rainfall of 1300 mm). On the northern face of Mont-Ventoux, the beech forest ranges almost continuously from 750 to 1700 m above sea level. This steep elevation gradient provides almost linear variation in mean temperature and humidity with elevation (Cailleret & Davi, 2011). We studied two plots at opposite positions along an altitudinal gradient, named N1 (1.3 ha; 1,020 m a.s.l.), and N4 (0.8 ha; 1,340 m a.s.l.).

In 2009, one large masting event occurred, which provided a unique opportunity to collect seeds and monitor regeneration. All potentially reproductive trees were mapped, measured and sampled for genetic analyses (164 at plot N1 and 365 at plot N4). Mother-trees were chosen among the trees with medium to high seed production, ensuring a minimal distance of 10 m between two mother-trees, and covering the whole plot area. Open-pollinated seeds were collected from 20 mother-trees at each plot (40 families in total for this study) and sown in the greenhouse. The common-garden experiment was arranged in 50 complete blocks, each block including two seedlings per family (Oddou-Muratorio et al. 2021). Among these open-pollinated seedlings, we genotyped 694 seedlings from plot N1 and 720 seedlings from plot N4 (~35.3 seedlings per mother tree). Additionally, in September 2010, we sampled and genotyped seedlings which originated from the same reproduction event in 2009 and germinated in situ in spring 2010 (223 seedlings at plot N1 and 250 seedlings at plot N4). Spring at year 2009 was not colder as an average year considering the mean and minimal temperatures from March to June (Fig. S1). Not late frosts (ie temperatures <-4°C after budburst) were observed in 2009 at any site.

Phenology measurement

In beech, the flowering phenology is hard to follow because (1) it occurs when leaves are spread out, (2) the succession of the flowering stages is rapid, and (3) the reproductive organs are small. However, as in oaks (Franjic et al., 2011), reproductive buds open very shortly after leafing (Nielsen & Schaffalitzky de Muckadell, 1954). Therefore, we employed budburst phenology as a proxy of reproductive phenology.

The budburst was surveyed in situ in spring 2009 on 147 adult trees in population N1, and 192 adult trees in N4. The budburst phenology was characterized using the five stages described by Davi *et al*. (2011): 1) dormant buds; 2) swelling buds; 3) broken bud scales; 4) emerging leaves; 5) spread out leaves (Fig. S2). The phenological stages of each adult tree were noted on 15 different dates in population N1 (between

the 23th of March and the 4th of May 2009), and on 13 different dates in population N4 (between the 24th of March and the 5th of May 2009). First, a phenological score sum (PSS) was computed for each tree as the sum of the phenological stages observed over all of the dates: the higher the PSS at a given date of measurement, the earlier and quicker was leaf unfolding (Bontemps et al., 2017). We also used a linear interpolation to estimate the timing of budburst (TBB) as the date of passage (number of days since 1st January) from stage 2 to 3, stage 3 being one the most sensitive stage to frost damages. Finally, we computed the spread of budburst for each adult tree from the temporal sequence of phenological scores, as the number of days where the phenological stage was >2 and ≤4 (i.e., the duration of stage 3).

The budburst was also surveyed ex situ for seedlings in the common garden using five stages (Gauzere et al., 2016). The phenological stages were noted on 4 different dates (between the 5th and 26th of April 2011). We also used linear interpolation to estimate TBB as the date of passage (number of days since 1st January) from stage 2 to 3.

Seedlings growth measurements

We measured seedlings diameter ($D2011_{start}$, $D2011_{end}$) and height ($H2011_{start}$, $H2011_{end}$) in the common garden on two dates (respectively April 2011, and September 2011). This allowed us to estimate diameter growth in 2011 as GrowthD = $D2011_{end}$ - $D2011_{start}$ and height growth in 2011 as $H2011_{end}$ - $H2011_{start}$. In total, growth was measured in 2011 for 1552 seedlings originating from 20 families at plot N1, and for 1709 seedlings originating from 20 families at plot N4. Note that the trial was divided in two contrasted experimental conditions: "watered" (from block 1 to 25, 1652 seedlings) versus "water-stressed" (from block 26 to 50, 1609 seedlings).

Mating opportunities and assortative mating estimation

Direct observation of mating or pollination events being impossible in this anemophilous species, we used phenological mismatch as a proxy of mating opportunities. We computed the sum of phenological mismatches between each adult tree *i* and its neighbors in a radius R as: $|PMis_i|_s = \sum_{j\ in\ R}|TBB_i - TBB_j|$, with R-values of 20, 50, 75 or 100 m. Note that similar |PMis|s values can be obtained either with a low density and large asynchrony or with a high density and low asynchrony. We also computed a mean phenological mismatch $|PMis|_m$, weighted by density. We hypothesize that the greater the phenological mismatch, the lower the opportunities of mating. Note also that using absolute mismatches implicitly assumes a symmetric effect asynchrony (earlier and later trees plays the same role for mating opportunities).

We estimated the strength of assortative mating as the correlation in vegetative phenologies between mates. We used the genetic data of maternal progenies (seedlings of the common garden) and adults trees to run paternity analyses and identify mating pairs, i.e. the most likely father siring a known mother. The genotypes of seeldings and adults were scored at a combination of 13 microsatellite loci (Oddou-Muratorio et al., 2018). The number of alleles observed in each cohort was greater than 106. Paternity assignments were conducted using the maximum-likelihood procedure implemented in the software CERVUS v.3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007). Likelihood scores, based on allele frequencies in the experimental population, were calculated for each seed /potential father couple. To determine whether the paternity of each offspring could be assigned to the father with the highest likelihood, we used the difference in likelihood scores (ΔLOD) between the two most likely pollen donors. The critical value (ΔC) of ΔLOD below which paternity/parentage could not be assigned at 80% was determined using a distribution of Δ obtained from 5 000 simulated mating events. This distribution was generated using the following simulation parameters: 1% of genotyping error and no unsampled parents (Oddou-Muratorio et al, 2003).

Fecundity estimation

We used the male and female fecundities estimated using the spatially explicit mating model described in Oddou-Muratorio et al. (2018). Briefly, this model considers mating and dispersal events in a hermaphroditic plant population, and allows individual fecundities to be estimated together with mating system parameters, using genotypes and positions of potential parents and their offspring. It is implemented in a Bayesian framework in the MEMM software. First, the individual male fecundities were estimated with MEMM, jointly with the pollen dispersal kernel, the selfing rate and the pollen migration rate, from the open pollinated seeds with known mother tree. Second, female effective fecundities were

estimated jointly with male fecundities, the pollen and seed dispersal kernels, the selfing rate and the pollen and seed migration rates, from one-year established seedlings without any known parent.

Remarkably, MEMM estimates of fecundity account for the effect of the relative positions of putative parents and offspring, while at the same basic fecundity, putative parents closer to an offspring would have a higher parentage probability in uncorrected models. Hence, by using MEMM, estimates of fecundity are not sensitive to spatial biases due to sampling design, or edge effects. Moreover, MEMM estimates of fecundities are *effective*: male fecundity is a proxy of the effective amount of pollen achieving successful pollination, and female fecundity is a proxy of the effective number of seeds achieving successful germination and establishment in the population. Therefore, these estimates account for the individual effects (maternal or genetic) that modify the success of mating, including differences in pollen tube growth, seed abortion (for male fecundity) and in seed maturation, germination or early survival during the post-dispersal processes (for female fecundity). Effective fecundity provides more realistic estimates of individual plant contribution to the next generation than simpler estimates, such as fruit or seed set. Finally, MEMM estimates of fecundity are relative, and consider uncertainty in parentage reconstruction. Indeed, MEMM does not categorically assign parents to offspring, but rather consider the likelihood of all adults to be the parent of each offspring, accounting for the genotypes of adults and offspring and allowing genotyping errors.

Estimations were performed separately on each plot. The MCMC procedure to estimate individual fecundities and mating system parameters is described in details in Oddou-Muratorio et al. (2018). For this study, we analyzed only the adult individuals for which vegetative phenology was monitored, that is: 147 among the 164 adults at plot N1, and 192 among the 365 adults at plot N4.

Fecundity and sexual selection analyses on adult trees, *in situ*

To investigate fecundity selection, we used selection gradient analysis (Lande & Arnold, 1983), with MEMM estimates of fecundity as the response variable, and TBB as the predictor. To investigate sexual selection, we used Bateman gradient analysis (Bateman, 1948; Tonnabel et al., 2019), with a proxy of mating opportunities as predictor (here, phenological mismatch), and MEMM estimates of fecundity as the response variable. Moreover, because fecundity variations are shaped by many other factors besides phenology, we included size and competition effects and their interactions using a hierarchical procedure, and selected the most parsimonious model to estimate the effect of phenology.

Models for directional fecundity selection

For each sex (male and female) and each plot (N1 and N4), seven hierarchical models were compared. We first fitted a baseline model M1 including only the predictor of interest (TBB):

$$\text{(1)} \qquad \text{M1.fec: } \ln(\text{F}) = \alpha + \beta TBB + \varepsilon$$

where F is the fecundity, α is the origin of the regression, β is the directional selection gradient on TBB, and ε is the residual.

In a previous study not including phenological traits (Oddou-Muratorio et al., 2018), we showed that both female and male fecundities increased with tree size and decreased with density and competition in the neighborhood. As selection probably simultaneously acts on these different correlated characters (phenology, size, competition), we fitted three models including size or/and competition variables, in addition to TBB:

$$\text{(2)} \qquad \text{M2.fec: } \ln(\text{F}) = \alpha + \beta TBB + \gamma Size + \varepsilon$$

$$\text{(3)} \qquad \text{M3.fec: } \ln(\text{F}) = \alpha + \beta TBB + \delta Compet + \varepsilon$$

$$\text{(4)} \qquad \text{M4.fec: } \ln(\text{F}) = \alpha + \beta TBB + \gamma Size + \delta Compet + \varepsilon$$

Note that several variables were used to measure size and competition (Table 1 and Oddou-Muratorio et al. 2018). Tree size was assessed by measuring the Diameter at Breast Height (Dbh), but as beech sometimes produces stump shoots resulting in multiple stems, we measured both the maximum Dbh (MaxDbh) and the sum of Dbh (SumDbh) of all the stems produced by a given genotype. Competition on each adult tree was assessed using (1) beech density in a radius of 20 m (ConDens20), (2) a competition

index integrating the density and diameter of beech competitors in a radius of 20 m (ConMartin20) and (3) tree stature (a class variable with 3 levels: dominant, codominant, and suppressed). Based on the previous results of Oddou-Muratorio et al. (2018), we chose the most pertinent variable for each sex and plot (Table 1).

Table 1: Variables included in the phenotypic selection analyses. The size and competition variables were identified as best predictors (BestPred) of female fecundity (*F*♀) and male fecundity (*F*♂) in the previous study of Oddou-Muratorio et al. (2018).

Category	Variable name	Variable definition	BestPred	Range at N1	Range at N4
Phenological mismatch (PMis)	$\lvert PMis \rvert_s$*	Sum of phenological mismatch in a 20 m radius	Not included	4-222.37	6.7 -445.8
	$\lvert PMis \rvert_m$	Mean phenological mismatch in a 20 m radius	Not included	1.57-10.48	0.37-7.83
Size	MaxDbh (cm)	Maximum diameter of the clonal copies	*F*♀	10.1-45.4	9.2-28.3
	SumDbh (cm)	Sum of diameters of the clonal copies	*F*♂	11.6-231.6	10.2-127.1
Competition	ConDens20	Conspecific local density in a radius of 20 m	*F*♀	2-50	6-115
	TotMartin20	Total competition index in a radius of 20 m	*F*♀ and *F*♂	8.7-100	43.0-127.4
	Stature	dominant, codominant, or suppressed	*F*♂	44, 51, 52, resp.	35, 66, 91, resp.

* this variable was retained as the estimator of phenological mismatch in the fecundity and sexual selection analyses of this study.

Finally, we also fitted three models including two-way interaction terms between TBB and size/competition covariates, in order to account for possible changes in the relationship between TBB and fecundity depending on size or competition:

$$\text{(5)} \quad \text{M5.fec: } \ln(F) = \alpha + \beta TBB + \gamma Size + \delta Compet + \kappa TBB \times Size + \varepsilon$$

$$\text{(6)} \quad \text{M6.fec: } \ln(F) = \alpha + \beta TBB + \gamma Size + \delta Compet + \lambda TBB \times Compet + \varepsilon$$

$$\text{(7)} \quad \text{M7.fec: } \ln(F) = \alpha + \beta TBB + \gamma Size + \delta Compet + \kappa TBB \times Size + \lambda TBB \times Compet + \varepsilon$$

We compared these seven hierarchical models based on the Akaike information criterion (Akaike, 1987) corrected for small sample size (AICc, Burnham and Anderson 2002), and we selected the most parsimonious model, denoted BestFec-Model in the following.

Models for stabilizing fecundity selection

A quadratic effect of TBB can also be included to estimate stabilizing selection through a bell-shaped response function (Lande & Arnold, 1983). This was done for the BestFec-Model selected above.

Models for sexual selection

We followed the same strategy as above and for each sex (male and female) and plot (N1 and N4), we fitted seven similar models but replacing TBB by PMis, the phenological mismatch between each tree and its neighbors in a 20 m radius. For instance, for the first model:

$$\text{(8)} \quad \text{M1.sex: } \ln(F) = \alpha + \beta PMis + \varepsilon$$

We compared the seven models based on the AICc, and selected the most parsimonious model, denoted BestSexSel-Model in the following

Fecundity versus sexual selection and compound models

We finally fitted a compound model, derived from the BestFec-Model but adding PMis as predictor, and we compared the BestFec-Model, the BestSex-Model, and the compound model.

Variable transformation and model fitting

Note that MEMM estimates of fecundity are relative, as required for selection gradient estimation (Lande and Arnold 1983). Moreover, the fecundities were log-transformed to approach Gaussian distribution and to account for the higher variance associated to higher fecundities. Besides, all the predictor variables (including TBB and PMis) were scaled to mean zero and unit variance. Such transformation of the predictor variables allows improving the interpretability and comparability of the estimated regression coefficients, especially when interactions are present (Schielzeth 2010). Once the best model selected, we estimated the standardized selection gradients on fecundity by fitting this selected best model without log-transformation of fecundity.

All models were fitted using the *lm* function implemented in R-base. Model comparisons was performed using the *aictab* function of the R package 'AICcmodavg' (Mazerolle, 2020). For the best models, the residuals were visually inspected through a plot of residuals vs predicted. All the analyses are available as online supplementary material (file SelectionAnalyses_adult.html).

Growth selection analyses on seedlings, in the common garden

As the common garden was designed to minimize seedlings mortality, we focused on growth as a performance trait related to viability, which is particularly expected when competition is homogeneous among seedlings (Collet & Le Moguedec, 2007). We used the following mixed model to investigate the effect of phenology and plot on annual growth in diameter and height (respectively *GrowthD* and *GrowthH*) during year 2011:

$$\text{(9)} \qquad \mathrm{M1}_{\mathrm{viabSel}}\text{: GrowthD or GrowthH} = (TBB\ in\ Plot) + D2011 or H2011 + \ Family + Block$$

Where D2011 (respectively H2011) is the initial diameter (respectively height) in spring 2011, introduced to account for difference of vigor among seedlings. We tested for the effect of TBB nested within plot (N1 or N4) to account for the fact that the effect of TBB on growth may differ among plots (knowing moreover that TBB is on average higher for seedlings at plot N1 as compared to plot N4, Gauzere et al. 2020a). Family (the maternal family of the seedlings) and Block (the trial unit to which seedlings belongs to) were introduced as random factors to remove undesirable variation in growth related respectively to genetic variation for phenology and microenvironmental effects (e.g., half the bocks received a water-stress treatment).

We also tested another mixed model:

$$\text{(10)} \qquad \mathrm{M2}_{\mathrm{viabSel}}\text{: GrowthD/H} = (TBB\ in\ Plot \times\ Treatment) + D/H2011 + \ Family + Block$$

where the treatment (watered vs water-stress) was specified as a fixed effect, in order to investigate whether the effect of TBB on growth may differ among plots and among treatments.

These models were fitted with the function lmer in lme4 package. All the analyses are available All the analyses are available as online supplementary material (file GrowthSelectionAnalysis_seedlings.html).

Results

Interindividual variations in phenology and fecundity

TBB ranged from 103 to 121 (over 17 days) at plot N1 (with a mean TBB on the 20th of April) and from 117 to 130 (over 13 days) at plot N4 (with a mean TBB on the 4th of May; Fig. S3). A smaller inter-trees variance of TBB was observed in Plot N4, with an important proportion of trees with the same TBB of 124. At plot N1, larger trees had an earlier budburst ($corr_{TBB\text{-}circ}$ = -0.15, p-value=0.007), while there was no significant relationship between size and TBB at plot N4 ($corr_{TBB\text{-}circ}$ = -0.02, p-value=0.12). The within-tree spread of budburst was higher at plot N1 (mean spread = 4.8 days) than at plot N4 (mean spread=2.9 days, Fig. S4A). Trees with a later budburst also showed a higher spread of budburst at plot N1 ($corr_{TBB\text{-}spread}$ = 0.57, p-value< 10^{-3}) but not at plot N4 ($corr_{TBB\text{-}spread}$ = 0.09, p-value=0.20; Fig. S4B).

The distribution of male fecundities estimated by MEMM were strongly L-shaped (Fig. S5A). At plot N1, male fecundities (MF) varied from 0.013 to 10.5 (median = 0.33) and 97 trees (66%) had a non-negligible

male fecundity. At plot N4, male fecundities varied from 2.10^{-3} to 16.6 with MEMM (median = 0.016) and 69 trees (36%) had a non-negligible male fecundity.

The distribution of female fecundities estimated by MEMMseedlings was also strongly L-shaped (Fig. S5B). Female fecundities varied from 3.10^{-3} to 13.3 at plot N1 (median = 0.014), and from 3.10^{-4} to 25.8 at plot N4 (median = 0.005). The number of trees with a non-negligible female fecundity was 55 (37%) at plot N1, and 30 (16%) at plot N4.

Assortative mating and phenological mismatch

A total of 713 seedlings could be assigned to a most-likely father: 397 among the 694 genotyped seedlings at plot N1 (57%) and 316 among the 720 genotyped seedlings at plot N4 (44%). The correlation in TBB between mating pairs was significantly positive at plot N1 (ρ=0.196, p<0.001) but not significant at plot N4 (ρ=-0.11, p=0.09). Accordingly, at plot N1, the joint distribution of parent pairs' phenological score (PSS) differed from the expectation under random mating (Fig. 2).

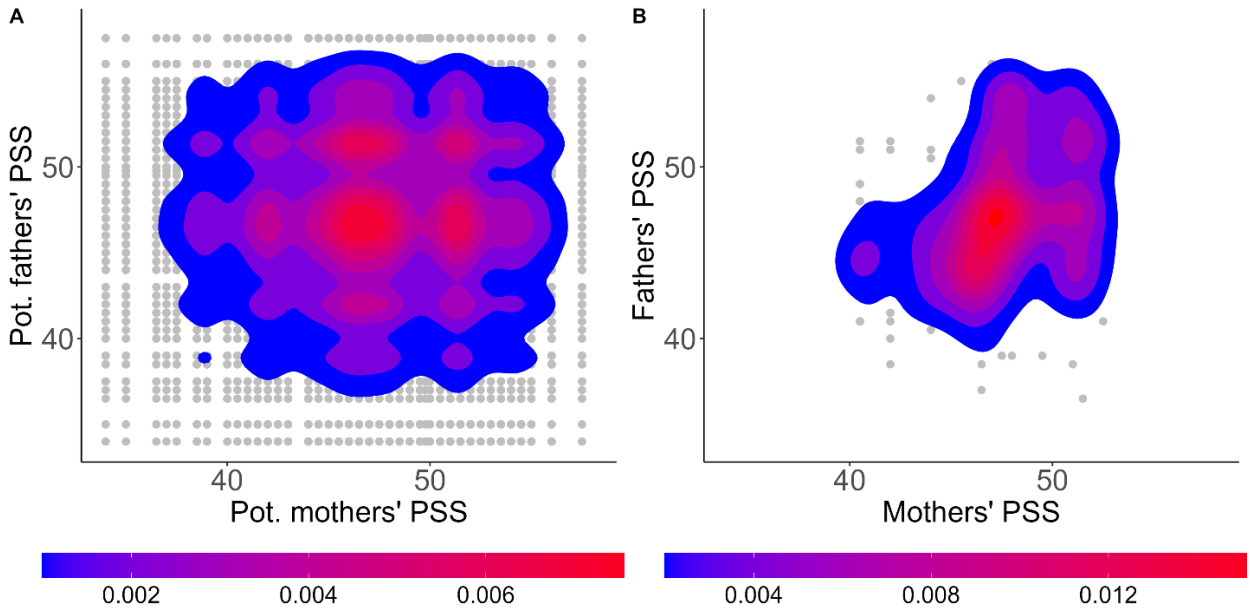

Figure 2 – Join distribution of parent pairs' phenological score (PSS) under random mating (A), and in realized mating events (B) at plot N1. A. The density of the data cloud was computed under the hypothesis that each tree mates once as male and once as female with all possible trees. B. Paternity analyses of seeds sampled on mother-tree allowed to identify mates' pairs. See also Fig. S6.

The phenological mismatch with neighbors, measured by $|PMis|_s$ and $|PMis|_m$, was slightly higher at plot N1 as compared to N4 (Fig. S7, S8): for instance, in a 20 m radius, $\mu_{|PMis|m}$ = 4.52 days at plot N1 while $\mu_{|PMis|m}$ = 2.12 days at plot N4. The phenological mismatch was more variable at plot N4 than at plot N1: for instance, in a 20 m radius, $cv_{|PMis|s}$ = 1.05 at plot N4 versus $cv_{|PMis|s}$ = 0.54 at plot N1. For the following analyses, we selected $|PMis|_s$ in a radius of 20 m as the best estimator of phenological mismatch (i.e., the estimator with the highest variation, Table S1) and we denote it PMis in the following for the sake of simplicity. At plot N1, PMis decreased with increasing phenological spread ($corr_{PMis\text{-}spread}$ = -0.23, p-value=0.004) while a reverse trend occurred at plot N4 ($corr_{PMis\text{-}spread}$ = 0.12, p-value=0.09, Fig. S9A). Finally, the relationship between TBB and PMis was quadratic, with a TBB value minimizing PMis (Fig. S9B), which is expected if the TBB variable is not strongly spatially structured (Fig S10).

Fecundity selection on TBB

We selected the most parsimonious model among the seven that were fitted (Table S2), and visually checked inspected the residuals (Fig. S11). Then, considering this best model, we evaluated the strength and direction of directional fecundity selection on TBB through the effect of TBB on fecundity (Table 2). For female fecundity at plot N1, the best model included TBB, size, competition and interactions among TBB

on the one hand and size and competition variables on the other hand. Delayed TBB significantly decreased female fecundity for the larger trees, but tended to increase it for smaller trees (Fig. 3A). Delayed TBB significantly decreased female fecundity for the more competed trees (Fig. 3B). Overall, we estimated a marginally significant directional selection gradient for earlier TBB (β_{TBB} = -0.37, p = 0.07; Table 2), i.e. for a tree with average DBH and average competition.

Table 2. Fecundity selection on female (*F*♀) and male (*F*♂) fecundities at plots N1 and N4. Fecundity selection on phenology was assessed through the effect of TBB on fecundity, accounting for joint effect of size and competition (see variable names in Table 1). We selected the most parsimonious regression model for each sex and plot (Table S2) to estimate the effect's coefficient and Sum Of Squares (SOSq) associated with each term. Significance of the effect (AIC and *p*-values) was assessed with the R function *"drop1"*. *F*♀ and *F*♂ were log-transformed.

Term	Coefficient	SofSq	AIC	p-value
F♀, plot N1	Adjusted R^2=0.1414; p-value<0.001 ; AIC_{ref}=262.85			
TBB	-0.37	18.91	264.24	0.072
MaxDbh	0.78	61.06	271.53	0.001
ConMartin20	0.06	0.36	260.91	0.802
TBB:MaxDbh	-0.88	54.02	270.34	0.003
TBB:ConMartin20	-0.55	32.28	266.59	0.019
F♀, plot N4	Adjusted R^2=0.145; p-value<0.001 ; AIC_{ref}=370.43			
TBB	-0.34	21.97	371.09	0.071
MaxDbh	0.78	87.06	380.67	0.000
ConDens20	-0.37	19.26	370.68	0.091
F♂, plot N1	Adjusted R^2=0.119; p-value<0.001; AIC_{ref}=112.01			
TBB	-0.08	0.88	110.44	0.519
SumDbh	0.56	45.31	130.54	<0.001
F♂, plot N4	Adjusted R^2=0.176; p-value<0.001; AIC_{ref}=336.80			
TBB	-0.41	31.78	340.50	0.019
SumDbh	0.46	33.64	340.83	0.015
Stature	Dom : 0.31	96.33	349.60	<0.001
	Cod : 0.74			
	Suppr : -1.05			

For female fecundity at plot N4, the best model included TBB, size, competition and interaction among TBB and size, but this interaction term was not significant. As the second-best model, without the competition term, performed nearly as best (ΔAICc = 0.56), we favored parsimony and kept it. Delayed TBB and increased competition tend to decrease female fecundity (β_{TBB}=-0.34, p=0.052; $\beta_{ConMartin20}$=-0.43, p=0.05), while female fecundity increased with tree size (β_{MaxDbh}=0.78, p<0.001).

In the best model for male fecundity at plot N1, the effect of TBB was not significant, while male fecundity markedly increased with tree size (β_{SumDbh}=0.56, p<0.001). By contrast, the effect of TBB was significant in the best model for male fecundity at plot N4 (Fig. 4B), and delayed TBB decreased male fecundity (β_{TBB}=-0.41, p=0.019). Male fecundity also increased with tree size (β_{SumDbh}=0.46, p=0.015), and depended on tree stature, with higher fecundity for codominant and dominant trees.

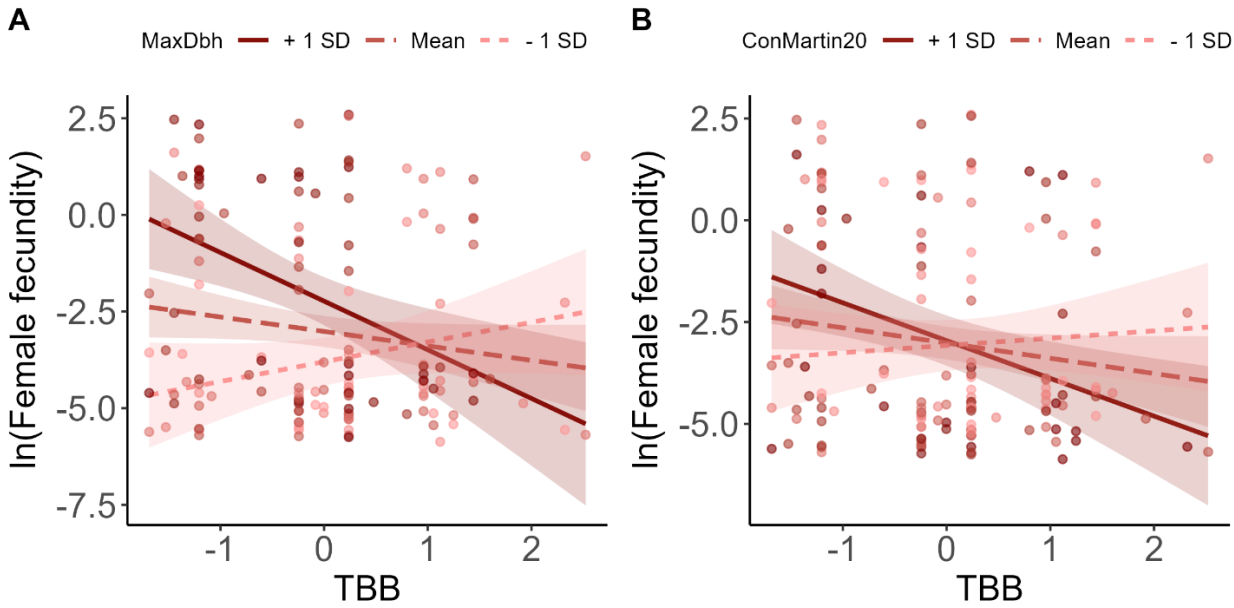

Figure 3 – Interaction effects between TBB and size (A) or competition (B) on female fecundity at low elevation (plot N1). Predicted regression lines are plotted for three values of each moderator variable, corresponding to +/- 1 standard deviation from the mean. Confidence interval at 95% are shown around each regression line. Dots are the observed values

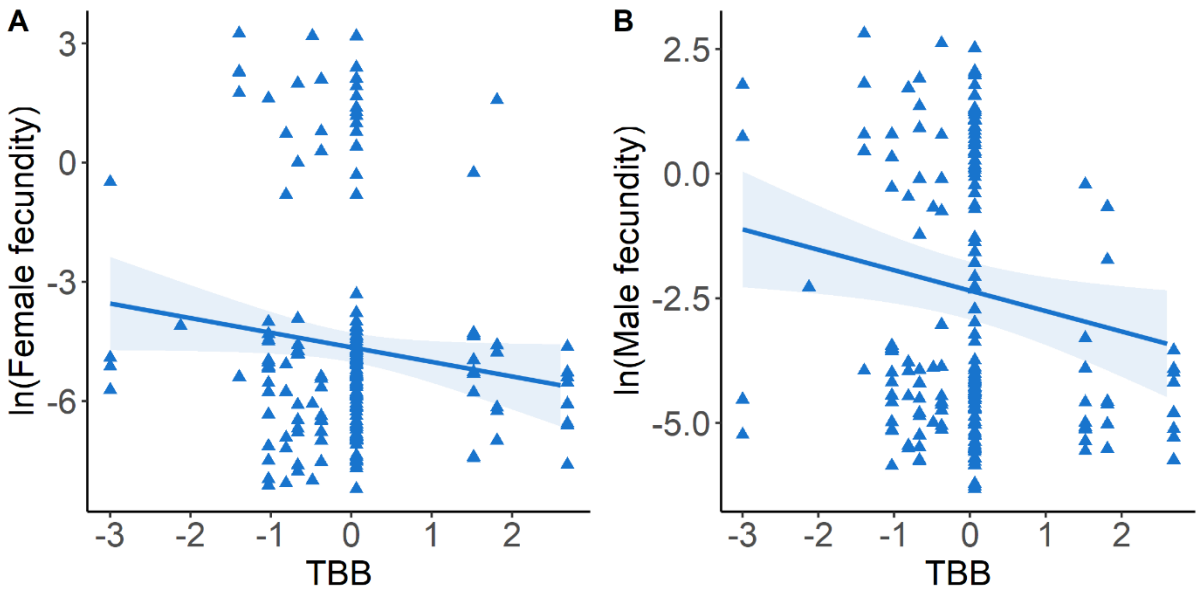

Figure 4 – Relationship between TBB and female fecundity (A) or male fecundity (B) at high elevation (plot N4). The lines are the predictions with their 95% confidence intervals, and triangles are the observed values.

Sexual selection

Following the same strategy as for fecundity selection, we selected the most parsimonious model among the seven that were fitted (Table S3), checked the residuals (Fig. S11), and evaluated the strength and direction of sexual selection through the effect of PMis on fecundity (Table 3). Only male and not female fecundities were significantly affected by PMis (Fig. 5). In the best models, male fecundity decreased with increasing PMis both at plots N1 (β_{PMis}=-0.44, p<0.001) and N4 (β_{PMis}=-0.45, p<0.001). Besides, male fecundity increased with SumDbh at both plots, and for tree with codominant stature at plot N4.

Table 3: Sexual selection on female (*F*♀) and male (*F*♂) fecundities at plots N1 and N4. Sexual selection on phenology was assessed through the effect of phenological mismatch (PMis) on fecundity accounting for joint effect of size and competition. We selected the most parsimonious regression model for each sex and plot (Table S3) to estimate the effect's coefficient and Sum Of Squares (SOSq) associated with each term. Significance of the effect (AIC and *p*-values) was assessed with the R function *"drop1"*. *F*♀ and *F*♂ were log-transformed.

Term	Effect	SofSq	AIC	p-val
F♀, plot N1	Adjusted R^2= 0.088; p-value<0.001; AIC_{ref}=268.86			
PMis	0.13	2.34	267.25	0.536
MaxDbh	0.85	95.81	282.07	<0.001
F♀, plot N4	Adjusted R^2= 0.117; p-value<0.001; AIC_{ref}=373.91			
PMis	-0.2908	16.119	374.27	0.128
MaxDbh	0.9382	167.782	395.14	<0.001
F♂, plot N1	Adjusted R^2=0.199; p-value<0.001; AIC_{ref}=98.04			
PMis	-0.4436	28.305	110.44	<0.001
SumDbh	0.6072	53.031	121.963	<0.001
F♂, plot N4	Adjusted R^2=0.181; p-value<0.001 AIC_{ref}=335.73			
PMis	-0.45	37.64	340.50	0.010
SumDbh	0.42	28.28	338.84	0.026
Stature	Dom : 0.31	117.71	352.18	<0.001
	Cod : 0.86			
	Suppr : -1.17			

Finally, we tested compound best models, where both TBB and PMis were included as factors in the best model for fecundity selection. Only for male fecundity at plot N4 the compound model outcompeted the BestFec and BestSex models (Table S4). The effect of TBB in the compound model (β_{TBB}=-0.34, p=0.045) was very similar to that of TBB in the BestFec model, showing that sexual selection does not affect the estimate of fecundity selection. For female fecundity, the effect of PMis was not significant in the compound model and the effect of TBB did not differ from that of the BestFec models, showing that sexual selection does not affect the estimate of fecundity selection. For male fecundity at plot N1, the effect of TBB was not significant in the compound model and the effect of PMis did not differ from that of the BestSex model.

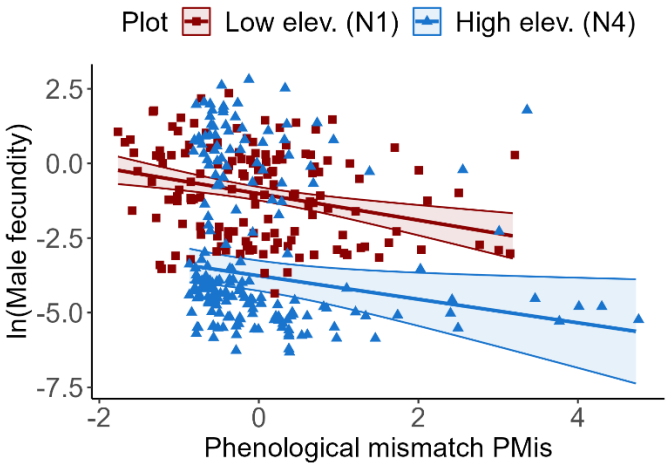

Figure 5 – Relationship between phenological mismatch and male fecundity (Bateman's gradient). The higher the phenological mismatch, the lower the opportunities for mating. The phenological mismatch, PMis, was estimated as the sum of absolute difference in TBB between a tree and each of its neighbors in a 20 m radius. Symbols represent observed values (square: plot N1; triangle: plot N4) and lines are the prediction of the best sexual selection model.

Stabilizing selection and selection gradients on phenology

We found no evidence of stabilizing selection on TBB through a significant effect of TBB² neither on female nor on male fecundity (Online Appendix 1). However, the significant effect of PMis on male fecundity at plot N1 and N4 results de facto in a stabilizing selection on TBB since lower PMis are obtained for average TBB (e.g. quadratic relationship between PMis and TBB, Figure S8B).

Standardized selection gradients (Table S5) show that selection for earlier TBB through female fecundity was higher at plot N4 (β_{TBB}'=-0.43) than at plot N1 (β_{TBB}'=-0.24). Selection for earlier TBB through male fecundity at plot N4 was within the same order of magnitude (β_{TBB}'=-0.30) than through female fecundity. Finally, the directional selection for reduced phenological mismatch with neighbors was higher at plot N1 ($\beta_{|PM|}$'=-0.40) than at plot N4 ($\beta_{|PM|}$'=-0.16).

Impact of phenology on seedlings growth

Growth selection analyses revealed a significant effect of TBB on seedlings growth (Table 4): both diameter and height growth significantly decreased with delayed budburst (increasing TBB). Moreover, growth increased with increasing initial size, and growth was reduced for seedlings originating from plot N4 compared to those from plot N1. As expected, the variance in growth was significantly structured by block and family (Table S6). A more detailed analysis showed an expected strong negative effect of water stress on growth. Moreover, the negative effect of delayed budburst on growth (albeit much lower than that of treatment) was higher in the water-stress treatment (Table S7).

Table 4: Selection on seedling growth in diameter (Dgrowth) and height (Hgrowth). Selection on phenology was assessed through the effect of TBB on seedling growth, accounting for the effects of plot (N1 or N4), initial size (initD or initH), and common garden design (with Block and Family included as random effects). The global significance of each fixed term was assessed based on the Sum Of Squares (SoSq) and the F-value (F-test), while the effect of TBB within each plot was assessed based on the t-value (Student test). See Table S7 for a more complex model including treatment.

A- Diameter growth

Term	npar	SoSq	F-value	p-value	Effect	St. error	t-value	p-value
Plot	1	2.86	4.12	0.042	-0.345	0.387	-0.89	
initD	1	228.76	330.18	<0.001	0.291	0.016	18.66	
Plot:TBB11	2	15.80	11.40	<0.001	N1: -0.015	0.006	-2.80	0.005
					N4: -0.021	0.005	-3.94	<0.001

B- Height growth

Term	npar	SoSq	F-value	p-value	Effect	St. error	t-value	p-value
Plot	1	67549	11.83	0.001	-76.940	35.256	-2.18	
initH	1	17753	3.11	0.078	0.046	0.020	2.33	
Plot :TBB11	2	53894	4.72	0.009	N1: -0.188	0.511	-0.37	0.712
					N4: -1.545	0.504	-3.07	0.002

Discussion

Mating opportunities limit male fecundity, and drive stabilizing selection on TBB

This study is among the first ones to show and estimate assortative mating on spring phenology in a tree species. Moreover, and consistently with Bateman's principle, we found that increasing phenological mismatch with neighbors, as a proxy of decreasing mate opportunities, affected male but not female fecundity. Hence, variation in phenology among trees within stand create opportunities for sexual selection, and could drive stabilizing selection on TBB through the male function.

Assortative mating has important evolutionary and ecological consequences (Jiang et al. 2013), and assortative mating for phenological traits in particular can markedly affect the evolutionary response to climate change (Godineau, Ronce, & Devaux, 2021; Soularue & Kremer, 2014; Whittet et al., 2017). However, the standard measure of assortative mating based on the observation of individual synchronicity of flowering schedules (Weis et al., 2014, 2005) is hardly applicable in forest trees. Hence, the potential assortative mating for phenological traits had been mostly investigated between tree populations, by measuring the difference in the timing of pollen shedding among oak populations along temperature clines (Whittet et al., 2017) or by inferring the latitudinal origin of pollen in pine open-pollinated progenies grown in common gardens (Nilsson, 1995). Another common approach is to estimate mating system parameters using genetic markers; such studies proposed assortative (respectively disassortative) mating as a general mechanism driving higher (respectively lower) relatedness between mated individuals than expected by chance (Hardy et al., 2019; Ismail & Kokko, 2020; Monthe et al., 2017). Here, we applied the approach widely used in animal species to quantify assortative mating (e.g., Jiang et al. 2013): we first used paternity analyses to infer mated pairs *a posteriori*, and secondly, we computed the correlation of spring phenology across members of mated pairs. This is one of the rare studies to our knowledge to evaluate effective assortative mating for spring phenology in a tree species, by combining budburst phenology data and marker-based paternity analyses (see also Gérard et al. 2006; Lagache et al. 2014; Larue et al. 2022). Our approach showed significant assortative mating for spring phenology at the lower plot, where budburst spread over 17 days. The correlation in vegetative phenology between mating pairs was moderate (ρ=0.19) as compared to the range reported in the literature (e.g. 0.05–0.63 within the same old-field community, Weis et al. 2014). At the upper plot, it is likely because leaf unfolding was quicker (with a 13 days range) that assortative mating was absent, or too weak to be detected.

As we expected, because of assortative mating, we found that a timing of flowering synchronized with close neighbors maximizes mating success through the male function, but does not significantly affect the female fecundity. Indirectly, this favors intermediate timing of bud burst since in absence of a strong spatial structure, males with intermediate TBB are those most synchronized with their neighbors. To our knowledge, this study is the first to test and validate the Bateman's principle in a tree, likely due to the difficulty of estimating the number of mates in these species producing a large number of offspring. We used the phenological mismatch as a proxy of (potential) mating opportunities rather than the mating success that could have been estimated based on paternity analyses (Tonnabel et al., 2019), because our sampling design, with only 35.3 seeds per mother tree, may underestimate the contribution of rare fathers. The effect of phenological mismatch could be related to stabilizing selection on TBB, since the phenological mismatch is a quadratic function of TBB. However, and surprisingly, we did not retrieve the expected consequence of a significant quadratic relation between effective fecundity and TBB. This could be due to different abilities to detect significant linear coefficients (from the slope of the regression line) as compared to quadratic coefficients (from the curvature of the fitness surface).

The observed effect of mating opportunities on MEMM-estimates of fecundity is counter-intuitive, as these estimates are claimed to be effective estimates of basic fecundity (Oddou-Muratorio et al., 2018). This is likely because the effect of the phenological mismatch is not included in the MEMM model we used; thus, any effect of phenological mismatch on individual reproductive success will be retrieved into the estimate of individual fecundity. In the same way that MEMM models the effect of the relative positions of putative parents and offspring on fecundity through the pollen dispersal kernel (spatial assortative mating), we could also model in MEMM the effect of phenological mismatch on fecundity (temporal assortative mating, as done in Gérard et al. 2006; Gleiser et al. 2018; Larue et al. 2022). Thereby, the estimated fecundity would no longer depend on the mating opportunities. This option would be interesting to include in future developments of MEMM.

Earlier budburst increases female fecundity and seedling growth, with contrasted effects of drought stress

We showed that earlier budburst increased the female fecundity at both elevations (of all trees at high elevation, and of the larger trees at low elevation) and the male fecundity only at high elevation. This finding is consistent with the pervasive phenotypic selection for early reproductive phenology documented in plants (Austen & Weis, 2015; Geber & Griffen, 2003; Munguía-Rosas et al., 2011). Moreover, the selection strength on female fecundity was higher at high than at low elevation, and the additional selection on male fecundity in plot N4 suggested even stronger 'global' fecundity selection at high than low elevation. These results are consistent with the physiological expectation that the length of the growing season strongly constraint the resource level acquired by photosynthesis, particularly at high elevation. They also support the simulation study of Gauzere et al. (2020a) showing that selection for earlier budburst dates is stronger in conditions more limiting for reproductive development, i.e., here in cold environments.

At low elevation, directional fecundity selection for later budburst was found only for the larger trees, while the smaller ones tend to show the reverse pattern, or at least, no gain in fecundity with early budburst. These contrasted selection gradients on TBB among neighboring trees suggest that different ecological strategies exist within the same drought-prone population, likely due to some micro-environmental heterogeneity. These two strategies can be related to the "growth-stress survival" trade-off (Grime, 1977; Grubb, 1998), whereby slower development (small trees) and delayed budburst can be viewed as a drought tolerance strategy. Indeed, a previous study at the same low elevation plot found that trees displaying late budburst were also associated with low size, low leaf water content and other traits symptomatic of a water-saving strategy (e.g., high leaf mass per area; Bontemps et al. 2017).

Finally, we found selection for earlier spring phenology using growth measurements from a common garden experiment, with seedling showings early budburst also having higher growth. This association was slightly stronger in the drought-stress treatment of the experiment, which is at odd with the above-described "growth-stress survival" trade-off. These results for seedlings in the common garden can be interpreted as the fact that an early budburst allows seedlings to start photosynthesizing when the conditions are the most optimal for growth (i.e., before drought) and can be view as a "drought-escape" strategy. Our findings hence suggest that the patterns of selection on phenology may change across ontogenic development, eventually due to different challenges in terms of survival (and fecundity) for juvenile versus mature trees (Vitasse 2013).

Implications of the observed selection on spring phenology for beech adaptation to climate variations

A main result of this study is that early phenology is associated to high reproductive outputs and high seedling growth in the studied European beech population. As hypothetised by Austen et al. (2017), we nonetheless found two evidence of selection countering this observed fecundity and growth selection for early phenology: first, stabilizing selection through assortative mating for male mating success, and second, selection for later TBB through female fecundity as a drought-tolerance strategy. Moreover, as we estimated selection during a single reproductive episode, we can not exclude that other selection patterns may be observed in different years. In particular, we may not have been in favorable conditions to observe selection for later budburst driven by late frosts, as they did not occur in the year when we sampled seeds and seedlings for this study. Selection for later budburst through late frosts damages can be expected as a strong selection force (by reducing the photosynthetic capacities of adult trees and hence their seeds' development and maturation and/or reducing seedling survival) but occurring only occasionally in balance

of other selections that apply every year with a more moderate intensity. Alternatively, it is also possible that other selective forces, non-acconted for in this study or in general, may contribute to the observed general pattern of selection for earlier TBB. For instance, in this multispecies ecosystem at ecological margin between Mediterranean and mountainous climates, interspecific competition could participate to shape patterns of selection, and reinforce the trend for beech to flush earlier than competing species (Palacio-Lopez et al., 2020).

On a quantitative point of view, the standardized directional selection gradients on phenology estimated in this study (β') ranged between -0.43 and -0.24. This indicates a rather strong magnitude, taking the meta-analysis of Kingsolver et al. (2001) as a reference (where a mean $|\beta'|$-value of 0.22 was found across all traits, with a median $|\beta'|$-value of 0.08 for life-history/phenological traits). This metanalysis also reported higher value of $|\beta'|$ for selection via fecundity or mating success (median $|\beta'|$ = 0.18) than for selection via survival (median $|\beta'|$ = 0.09), supporting the strong directional selection estimated here. Such selection gradient may contribute to the fast adaptation of beech populations to the multiple effects of ongoing climate change, and in particular to 1) the likely emergence of late frosts pressures paralleling the advanced spring season at the high elevation site and 2) the increasing risk of hydraulic failure with increased summer drought at the low elevation site, which may reshuffle the respective benefits of the drought-resistance and drought-escape strategies. However, integrating the different significant selection gradients on TBB estimated through male fecundity, female fecundity and seedling growth at each plot and predicting the population response to selection would require a dedicated, forward modelling approach that was out of the scope of this study.

Spring phenology has been defined as a "magic trait", which affects fitness through its influence on growing season and simultaneously contributes to non-random mating (Servedio et al., 2011; Soularue & Kremer, 2014). Previous simulations studies have demonstrated how environmental variation can cause populations to diverge for a selectively neutral trait that causes assortative mating (Kirkpatrick 2000 ; Soularue and Kremer 2012). Consequently, some patterns of clinal genetic variation of phenological traits observed in forest trees can be generated solely by the effects of assortative mating and gene flow, without divergent selection. When both divergent selection and assortative mating for TBB occur, Soularue and Kremer (2014) predicted that genetic clines can either be inflated or constrained by assortative mating, depending on species life history. However, these simulation studies considered simple, single-trait models of divergent selection, where a single optimal value maximizes fitness within each population. The intertwining of sexual, fecundity and viability selection on phenology and the variation of fitness landscapes for budburst along temperature and drought gradients demonstrated in this study call for integrative, mechanistic and multidisciplinary studies of phenological shifts. Only such approaches can account for the complexity of fitness landscapes, identify the factors limiting response to selection, and ultimately anticipate the increased extreme selective pressures associated with the ongoing climate change.

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Data, scripts, code, and supplementary information availability

Data (two files: dataAdultField.txt and dataGrowthCommonGarden.tab), Scripts of statistical analyses (two files: SelectionAnalyses_adult.html and GrowthSelectionAnalysis_seedlings.html) and Supplementary figures and tables (one file: SuplMaterial_V10.pdf) are available online: https://doi.org/10.57745/ZVPNXX.

Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article. S.O.M and E.K. are recommenders of PCIEvolBiol.

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Authors contributions

Research conceptualization: S.O.M, A.B., E.K., J.G. Design or development of research methods and tools: S.O.M, E.K., A.B., J.G. Data collection, analysis, management, interpretation: S.O.M, A.B., E.K., J.G. Validation: S.O.M, E.K. Manuscript writing: S.O.M. (first draft), E.K., J.G., A.B.

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