New Phytologist

Research

Climate lags and genetics determine phenology in quaking aspen (*Populus tremuloides*)

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Received: *2 September 2022*
Accepted: *19 February 2023*

New Phytologist (2023)
doi: 10.1111/nph.18850

Key words: carbon allocation, drought, genome-wide association, landscape genetics, phenology, ploidy level, remote sensing, sex.

Summary

- Spatiotemporal patterns of phenology may be affected by mosaics of environmental and genetic variation. Environmental drivers may have temporally lagged impacts, but patterns and mechanisms remain poorly known.
- We combine multiple genomic, remotely sensed, and physically modeled datasets to determine the spatiotemporal patterns and drivers of canopy phenology in quaking aspen, a widespread clonal dioecious tree species with diploid and triploid cytotypes.
- We show that over 391 km^2 of southwestern Colorado: greenup date, greendown date, and growing season length vary by weeks and differ across sexes, cytotypes, and genotypes; phenology has high phenotypic plasticity and heritabilities of 31–61% (interquartile range); and snowmelt date, soil moisture, and air temperature predict phenology, at temporal lags of up to 3 yr.
- Our study shows that lagged environmental effects are needed to explain phenological variation and that the effect of cytotype on phenology is obscured by its correlation with topography. Phenological patterns are consistent with responses to multiyear accumulation of carbon deficit or hydraulic damage.

Introduction

Canopy phenology describes the timing of leafing for plants (Rathcke & Lacey, 1985; Fenner, 1998). Variation in canopy phenology occurs across space and time (Tang *et al.*, 2016; Park *et al.*, 2021) and may be linked to environmental factors, for example, photoperiod, temperature, and precipitation (Piao *et al.*, 2019). However, the effect of the environment on phenology may be temporally lagged, depending on the underlying ecophysiological processes. Additionally, genotypes within a species may respond differently to the same environmental cues. Priorities now include understanding how plant ecophysiology could influence phenology (Kikuzawa, 1995; Cleland *et al.*, 2007; Zani *et al.*, 2020), the timescales over which environmental factors act (Park *et al.*, 2021), and the role of intraspecific genetic variation (Vitasse *et al.*, 2013; Fitzpatrick & Keller, 2015).

Environmental predictors of phenology

Environmental stressors may affect phenology (Piao *et al.*, 2019) because leaves may make a greater contribution to plant performance at certain times of year, or represent a risk at others (Kikuzawa & Lechowicz, 2011). For drought, drier conditions could lead to earlier greendown dates or shorter growing season lengths, as leaves are dropped as 'hydraulic fuses' (Wolfe *et al.*, 2016; Hochberg *et al.*, 2017). This drought deciduousness could contribute to performance by reducing the risk of hydraulic damage or high respiration costs when carbon gain would have a high water cost (Marchin *et al.*, 2010; Pivovaroff *et al.*, 2016; but see Borchert *et al.*, 2002). Drought deciduousness could also be an adaptive response to reduce herbivory (Aide, 1992), or a non-adaptive response to hydraulic failure (Kaufmann, 1982; Bigler & Vitasse, 2021). Alternatively, drier conditions could also lead to later greendown dates or longer growing season lengths if leaves are instead grown for longer periods of time when photosynthesis rates are lower (due to closed stomata) (Zani *et al.*, 2020).

For heat, higher extremes could lead to later or earlier greenup dates. More extreme heat could drive earlier greendown dates and lower maximum greenness if heat damages leaf tissues, leads to reduced photosynthetic efficiency, or increases respiration costs

relative to carbon gain (Jolly *et al.*, 2005). Or, higher extremes could also drive later greendown dates, lower maximum greenness, and longer growing season lengths if leaves are instead retained for longer periods of time to compensate for the effects of reduced photosynthetic efficiency and higher respiration costs (Zani *et al.*, 2020). Nonextreme heat could also drive later greendown dates and higher maximum greenness if heat brings environmental conditions closer to temperatures optimal for photosynthesis (Keenan *et al.*, 2014). If warmer spring weather is also associated with more extreme weather excursions, then earlier greenup dates could also lead to canopy frost damage and compensatory phenology.

For snow, late snowmelt dates necessarily provide a lower bound on greenup dates (Canaday & Fonda, 1974), while early snowmelt dates could drive early greenup dates if melting is also associated with warming that enhances photosynthetic efficiency, or if carbon gain under high soil moisture is most efficient (Livensperger *et al.*, 2016). Later, snowmelt might also drive higher maximum greenness if plants invest more resources in leaves and perform better during wet years that are likely to be favorable to growth (Walker *et al.*, 1995; Campbell, 2019). Early greenup dates relative to snowmelt may also increase the risk of frost damage to stems or complete loss of canopies (Sperry & Sullivan, 1992).

Phenology responses to the environment may include multiyear lags (Fu *et al.*, 2014; Keenan & Richardson, 2015). Lagged environmental effects occur in long-lived woody plants where carbon deficits may persist over multiple years (Sherry *et al.*, 2011; Prather *et al.*, 2023). Lagged environmental effects could lead to shifts in phenology depending on the availability of stored carbon and future environmental conditions (Trugman *et al.*, 2018; Kannenberg *et al.*, 2020). For example, high stem hydraulic damage in one year (perhaps caused by high leaf production or leaf water use) or heat stress (Peltier *et al.*, 2021) can cause reduced hydraulic capacity in future years unless carbon is reinvested in repairing or regrowing xylem. This in turn could cause more conservative water use in future years (associated with later greenup date and earlier greendown date, and lower maximum greenness or leaf area index; Anderegg *et al.*, 2019), or less conservative water use and extended phenology to make up for prior losses (Jump *et al.*, 2017). Nonstructural carbohydrate (NSC) remobilization may also mediate these lagged responses (Peltier *et al.*, 2016, 2021) because NSCs are used during periods when carbon demand exceeds supply, such as during drought and early in the growing season.

Lagged phenological responses may yield variable performance depending on the suitability of future environmental conditions (Duputié *et al.*, 2015). For example, in a wet year, a lagged phenological response comprising a long growing season could enhance performance through increased carbon gain, or, in a dry year, that same lagged response could decrease performance through further hydraulic damage. Lagged effects of environmental stress commonly lead to multiyear lags in tree mortality (Anderegg *et al.*, 2013; Trugman *et al.*, 2018), suggesting that phenological lags are usually insufficient to compensate for the effects of prior damage.

Genetic and size-based predictors of phenology

Heritability is important for phenology as it describes the degree of genetic determination of phenology across individuals in a population and enables evolutionary response to natural selection. High heritability of phenology has been found in several species, likely as a result of within-species evolution across populations or habitats (Atwell *et al.*, 2010; Vitasse *et al.*, 2013; Duputié *et al.*, 2015; Hultine *et al.*, 2020).

Key genetic features may also play important roles in phenology. Individuals with differing cytotypes (the 'ploidy level', or the number of chromosome copies) may have different life history trade-offs, leading to variation in carbon gain and water use, and thus in phenology. Polyploid individuals (with greater gene expression) typically have 'faster' life histories and more risky phenology than diploid individuals in stressful environments across species (Comai, 2005; Van de Peer *et al.*, 2021). In dioecious species, sex may also influence carbon gain and water use (Shine, 1989; Dawson & Ehleringer, 1993; Petry *et al.*, 2016), impacting phenology. For example, if females have greater reproduction costs (Cipollini & Whigham, 1994), they may have more conservative canopy phenology than males in stressful environments, as in *Silene latifolia* (Purrington & Schmitt, 1998), or they may have longer growing seasons and greater total carbon gain to support seed production, as in *Rubus chamaemorus* (Ågren, 1987). However, in other species, no sex differentiation of phenology has been observed (Milla *et al.*, 2006; McKown *et al.*, 2017).

Plant size could also interact with the environment to influence phenology, because shifts in carbon gain or water loss can differentially impact performance in larger individuals (Seiwa, 1999; Osada & Hiura, 2019). Larger individuals may have proportionately more stored carbon to draw upon, as well as deeper roots for greater water access, and can buffer risks; alternatively, larger individuals may have higher hydraulic risks due to needing to support proportionately greater metabolic costs than smaller individuals. These size-dependent factors may then influence carbon deficit accumulation and recovery.

Study system

This study assesses the spatial and temporal responses of canopy phenology (greenup date, greendown date, growing season length, and maximum greenness) to environmental stressors (drought, heat, and snow) and genetic factors in quaking aspen (*Populus tremuloides* Michx. (Salicaceae)). This broadleaf tree species has a range spanning northern Canada to central Mexico (DeByle & Winokur, 1985) and occurs in numerous habitat types (Mitton & Grant, 1996). Leaves are primarily flushed in spring, though additional flushing occurs continuously, and in response to stressors like defoliation (St Clair *et al.*, 2009).

Aspen genetics are complex. First, aspen can grow in large spatially extensive clones or as solitary individuals. Second, individuals vary in cytotype. Individuals have either diploid (n = 2) or triploid (n = 3) ploidy level (Einspahr *et al.*,

1963). Triploids have more resource-acquisitive life histories (Benson & Einspahr, 1967; Greer *et al.*, 2017) than diploids, and are at higher risk of mortality in hotter and drier environments (Dixon & DeWald, 2015; Blonder *et al.*, 2021a,b). Third, the species is dioecious (Einspahr, 1960). Differentiation of morphology by sex in aspen is poorly described and has been considered absent by some (Einspahr, 1960), while others have found greater clone size, ramet numbers, basal area, and growth rates for females (Grant & Mitton, 1979; Sakai & Burris, 1985; Mitton & Grant, 1996). Variation in clonality, cytotype, and sex can occur within populations (Mock *et al.*, 2008; Blonder *et al.*, 2021b), with females (Grant & Mitton, 1979) and triploids (Blonder *et al.*, 2021b) more common at low elevation.

Aspen leaf phenology is highly variable. Greenup and greendown date variation occurs within and across landscapes (Kanaga *et al.*, 2008; Meier *et al.*, 2015; Fig. 1a,b,g,h). Drought deciduousness can also occur, primarily in stressed individuals, where leaves change become brown but persist on stems (Fig. 1c,d). The environmental cues that influence phenological responses remain under investigation in aspen (Meier *et al.*, 2015) and its close relative, *Populus tremula* (Luquez *et al.*, 2008; Strømme *et al.*, 2017; Michelson *et al.*, 2018). In provenance trials, greenup date is responsive to frost risk (Li *et al.*, 2010; Ding & Brouard, 2022), while greendown date is responsive to day length and nighttime warmth (Schreiber *et al.*, 2013). Growth is reduced by heat (Hogg *et al.*, 2008), drought (Anderegg *et al.*, 2013), spring frosts (Sutton & Tardif, 2005; Birch *et al.*, 2022), and herbivory (Marchetti *et al.*, 2011; Seager *et al.*, 2013). Phenology may also be linked to mortality. Mortality is sometimes preceded by several years of reduced leaf area index (sparse canopies; Fig. 1e,f) and early deciduousness, in which leaves are lost before normally senescing (Kaufmann, 1982). Defoliation can lead to very low growth in one year (Hogg *et al.*, 2002) followed by higher canopy investment in the next year (Eisenring *et al.*, 2022), potentially yielding structural overshoot (Trugman *et al.*, 2018).

From a genetic perspective, phenology in aspen often differs among clones within populations (Egeberg, 1963; Barnes, 1969; Jelínková *et al.*, 2014). Genetic trade-offs exist between phenology and growth (Ding & Brouard, 2022). The heritability of phenology was 20–30% in one common garden study (Ding *et al.*, 2020), 30–50% in a different common garden study (Kanaga *et al.*, 2008) and a field study (Drobyshev *et al.*, 2019), or 50–90% in clonal trials (Gylander *et al.*, 2012; Ding & Brouard, 2022).

To quantify variation in quaking aspen, we asked: (1) How much variation in aspen phenology exists across space, time, and key genetic features (cytotype and sex)? Then, to determine whether these patterns are associated with genetic differences, we asked (2) what fraction of phenological variation is explained by allelic genetic variation? Last, to explain how different predictors might independently or interactively influence phenology, we asked (3) how spatiotemporal mosaics of environmental and genetic variation combined to influence phenology; and are environmental effects temporally lagged?

Materials and Methods

Study region

The study was carried out in a 391 km^2 region near Gothic, CO (Supporting Information Fig. S1a). Elevations range from 2678 to 4104 m and include desert, montane, and alpine habitats. Mean daytime high temperatures in the Gothic range from −5°C in January to 20°C in July. High aspen canopy damage was observed in the early 2000s in this region (Worrall *et al.*, 2008; Marchetti *et al.*, 2011).

Genetic data

In the summer 2018, we permanently tagged and georeferenced a set of 503 sites located within aspen forests of the study region, most of which were randomly located (Blonder *et al.*, 2021b; Fig. S1b). At each plot, we obtained canopy leaves from a focal tree and extracted DNA, which was then passed through a RAD-Seq sequencing pipeline (Blonder *et al.*, 2020). Data were then used to assign each tree a cytotype using inferred allelic proportions and allelic ratios in a Bayesian framework (Gompert & Mock, 2017); data in Blonder *et al.* (2020). Sex was assigned using the *TOZ19* male-specific gene (Pakull *et al.*, 2015), which is known to be reliable in *Populus* (Pakull *et al.*, 2011), and also in diploid aspen (Bidner, 2021). It is assumed to be valid in triploid aspen. However, results should be interpreted with caution as genetic control of sex can fail, with intersex individuals also occurring (Mennel, 1957; Pauley & Mennel, 1957).

Topographic data

In June 2018, the study area was overflown by the NEON Airborne Observation Platform, yielding hyperspectral (380–2500 nm) reflectance imagery and LiDAR data covering the region at 1 m spatial resolution (Chadwick *et al.*, 2020a). Topographic data were derived from the above LiDAR data and used to calculate elevation, slope, and cosine aspect (−1 = south-facing; +1 = north-facing) at 1 m resolution over the study area (Fig. S2a–c).

Cover and canopy height data

From the hyperspectral dataset above, we applied random forest machine learning algorithms to produce a map of aspen cover at 1 m resolution (Chadwick *et al.*, 2020b; Brodrick *et al.*, 2021). From the LiDAR dataset, we obtained a map of canopy height that was then clipped to aspen cover (Goulden *et al.*, 2020; Fig. S2d).

Cytotype maps

We used 1 m resolution maps of aspen cytotype covering the entire study region clipped to aspen cover, based on the spectral separability of canopy reflectance (Fig. S2e). Ensembles of artificial neural network machine learning models were trained on

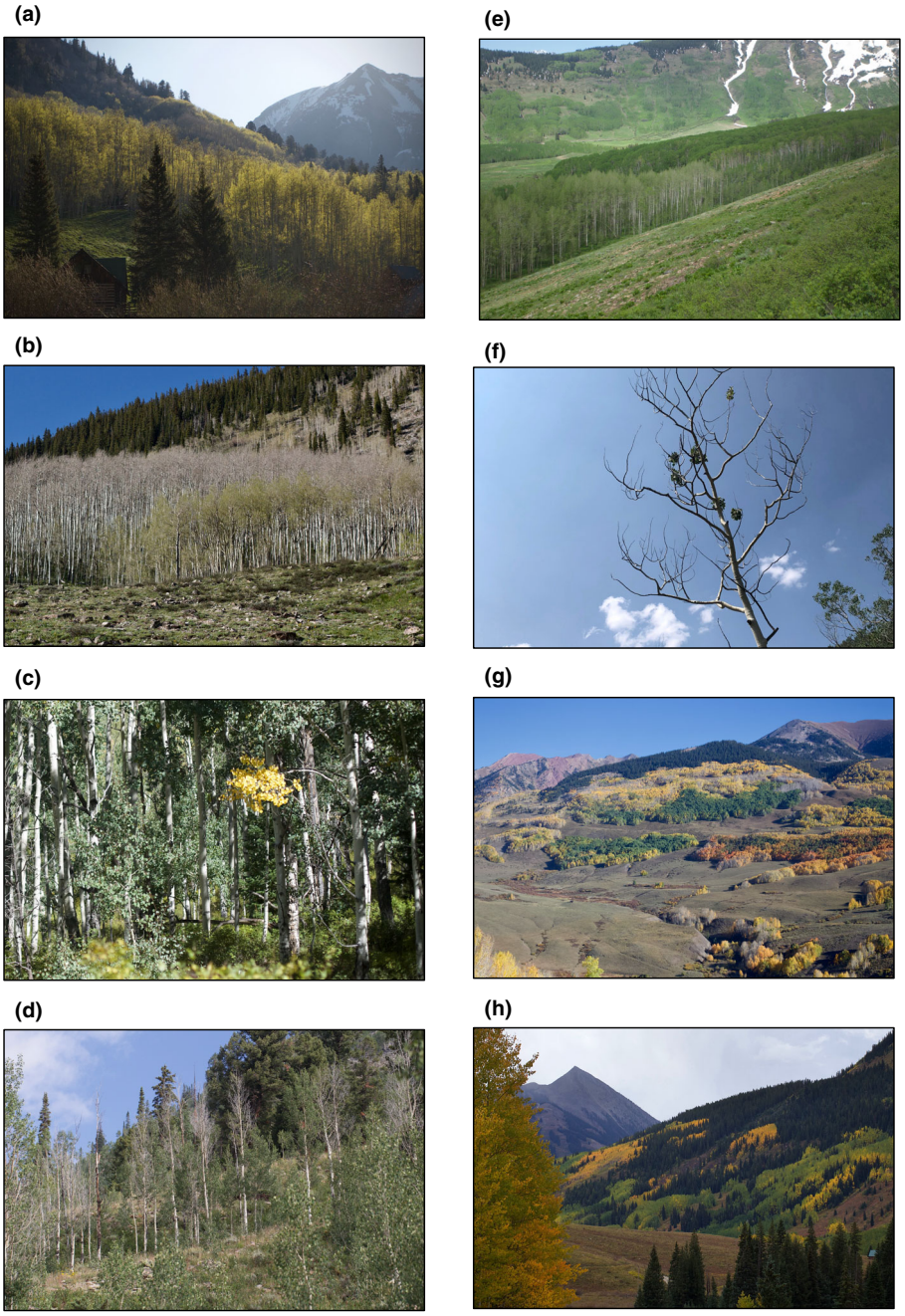

Fig. 1 Example photographs of phenological variation in quaking aspen forests in southwestern Colorado, including (a, b) greenup date, (c, d) growing season length, (e, f) maximum greenness, and (g, h) greendown date. Dates of images are (a) 4 June 2015; (b) 4 June 2015; (c) 5 September 2016; (d) 21 August 2021; (e) 11 June 2017; (f) 11 August 2021; (g) 22 September 2018; and (h) 24 September 2016. All images are from within the study extent except (c), which is from *c*. 10 km to the southwest. Image (c) likely depicts a stressed branch that will die back the next year, and image (d) may actually depict full stem mortality; (e, f) may also represent stems that will soon die.

hyperspectral data as predictors and genetic data (as above) as responses and then predicted out over the whole landscape. Aspen in the study region is 57.5% triploid and 42.5% diploid. Detailed results were reported in Blonder *et al.* (2021a) and Brodrick *et al.* (2021).

Temperature data

Daily maximum temperature data were obtained from the *gridMET* data product at 4 km resolution at daily intervals (Abatzoglou, 2013; Fig. S3a). This dataset blends spatial attributes of gridded climate data from PRISM (Daly *et al.*, 1997) with temporal attributes from regional reanalysis (NLDAS-2; Mitchell *et al.*, 2004). For each year, 99% upper quantile values were extracted for 2013–2019.

Soil moisture data

We obtained simulations of subsurface water transport at 1 km spatial resolution and hourly temporal resolution from 2013 to 2019 over the Upper Gunnison River Basin using the PARFLOW-Community Land Model (CLM) model (Tran *et al.*, 2022). PARFLOW-CLM solves three-dimensional variably saturated flow in the subsurface, integrated with physical-based overland flow (Kuffour *et al.*, 2020). Land surface processes are coupled with PARFLOW through the CLM (Lawrence *et al.*, 2019). The combined model

solves the coupled water–energy balance at the land surface including snow accumulation and melt, infiltration, root water uptake, plant transpiration, interception, and bare soil evaporation. This approach explicitly calculates lateral groundwater flow and dynamic interactions between groundwater and surface water. Landcover is assumed to be constant over the simulation runs, so the approach does not account for vegetation dynamics. Results have been validated against streamflow and soil moisture sensors. We used these simulations to obtain values of volumetric soil moisture at 0.1 m depth. For each of the 2012–2019 water years (e.g. for the 2012 water year from 1 October 2012 to 30 September 2013), we calculated the 1% quantile soil moisture value within each year as a metric of drought extremity (Fig. S3b) and the maximum consecutive number days with soil moisture below 20% as a metric of drought duration (Fig. S3c). Pixels experiencing no droughts within a year were set to a value of 0 for drought duration.

Snowmelt data

Snowmelt timing maps at 500 m spatial resolution were obtained on an annual basis from 2013 to 2019 covering the study area (Fig. S3d). Maps are based on the Moderate Resolution Imaging Spectroradiometer (MODIS) 8-d composite snow-cover product MOD10A2 collection 6. The maps were created by conducting a time-series analysis of these snow maps to identify the day of year of snowmelt on a per-pixel basis. Snowmelt was defined as a snow-free reading following two consecutive snow-present readings for each pixel. Data from 2013 to 2018 were publicly available (O'Leary *et al.*, 2020), while 2019 is reported here.

Phenology data

Phenology estimates were obtained using a continental-scale land surface phenology algorithm and data product based on harmonized Landsat 8 and Sentinel-2 imagery (Bolton *et al.*, 2020), available in Friedl (2020). The algorithm creates a time series of vegetation indices from satellite imagery, which are then used to estimate the timing of vegetation phenophase transitions at 30 m spatial resolution. In deciduous forests, the algorithm achieves $R^2 > 0.9$ relative to ground observations. We assigned greenup date as the day of year on which 15% maximum greenness was reached ('OGI'), greendown date as the day of year on which 85% of maximum greenness decrease was reached ('OGMn'), growing season length ('GSL') as the difference between the 50% greenup and greendown dates, maximum greenness as the maximum value of EVI2 ('EVImax'), a two-band enhanced vegetation index (dimensionless from 0 to 1). Greenness changes were calculated as EVI2 values within each growing season. Values were only used when quality flags were 'high' or 'moderate' with no interannual gap-filling. Estimates were obtained for 2016–2019 (Fig. 2).

Data aggregation

For all analyses in this study except as described below, we reprojected all spatial data products to a UTM Zone 13N projection and mean-aggregated to 30 m resolution, matching the original properties of the phenological datasets. In the case of 1-m resolution binary predictors (aspen cover, cytotype), we calculated fractional values (fraction aspen cover and fraction diploid cytotype). We restricted analysis only to those pixels that had ≥ 50% fractional aspen cover, yielding a total of $n = 3188$ pixels. Pixels with < 100% fraction aspen cover are mixed with meadow, willow, conifer forest, or other vegetation types (Brodrick *et al.*, 2021). For analyses in Question 1 and Question 2 integrating cytotype and sex data, we restricted analyses to only ground-based sites whose genetic data included successful cytotype and sex classifications, because sex could not be remotely sensed (pilot analyses not shown). We then restricted analyses only to those locations whose overlaying phenology datasets met the quality criteria described above, as well as ≥ 25% fractional aspen cover. A lower fractional cover threshold was selected as a compromise between lower spatial replication and lower spectral purity. To assess sensitivity of results to threshold values, we replicated all analyses at 30% and 70% cover thresholds.

Statistical analyses – ranges of variation (Question 1)

For the remotely sensed dataset including cytotype but not sex, we reported descriptive statistics for each phenology metric across all years and across cytotype fractions. For the ground-based dataset including cytotype and sex, we repeated the above descriptions also disaggregating by sex. Patterns are reported descriptively, as inferential statistics are more appropriate when applied to a more covariate-rich model (Question 3).

Statistical analyses – heritability of phenology (Question 2)

We extracted phenology metrics over each plot for four consecutive years (2016–2019). For all the above data, values were retained in the analysis when remotely sensed fractional aspen cover within a 30 m pixel above the plot was ≥ 25% and RAD-Seq data were available. After quality control, 78% ($n = 392$) of plot-year-variable samples were retained. The analysis also was repeated using more conservative 30%, 50%, and 70% aspen cover thresholds, but we focus on the 25% results because the larger sample size yielded lower error in estimates.

Genomic variation and environment are both spatially structured in natural populations, and both contribute to phenotypic effects. We therefore used several spatially differentiated but temporally constant variables describing the physical environment at each plot to help correct for environment-driven effects on phenology. Some variables were available from Blonder *et al.* (2021b), comprising elevation, slope, cosine aspect, summer insolation, mean diameter of breast height of trees in the plot, canopy openness, regolith type, and rock unit type. Using datasets described above, we also included values of maximum temperature, snowmelt date, drought duration, and drought extremity at 0–3 yr lags. We fit a linear regression model using each of the four phenology metrics as outcomes, and the environmental variables, along with sex and year as fixed-effect predictors. Samples missing sex ($n = 29$) were assigned a mean value of 0.5. Samples were subset by cytotype: diploid ($n = 38$), triploid ($n = 338$), or combined (including unknown cytotypes).

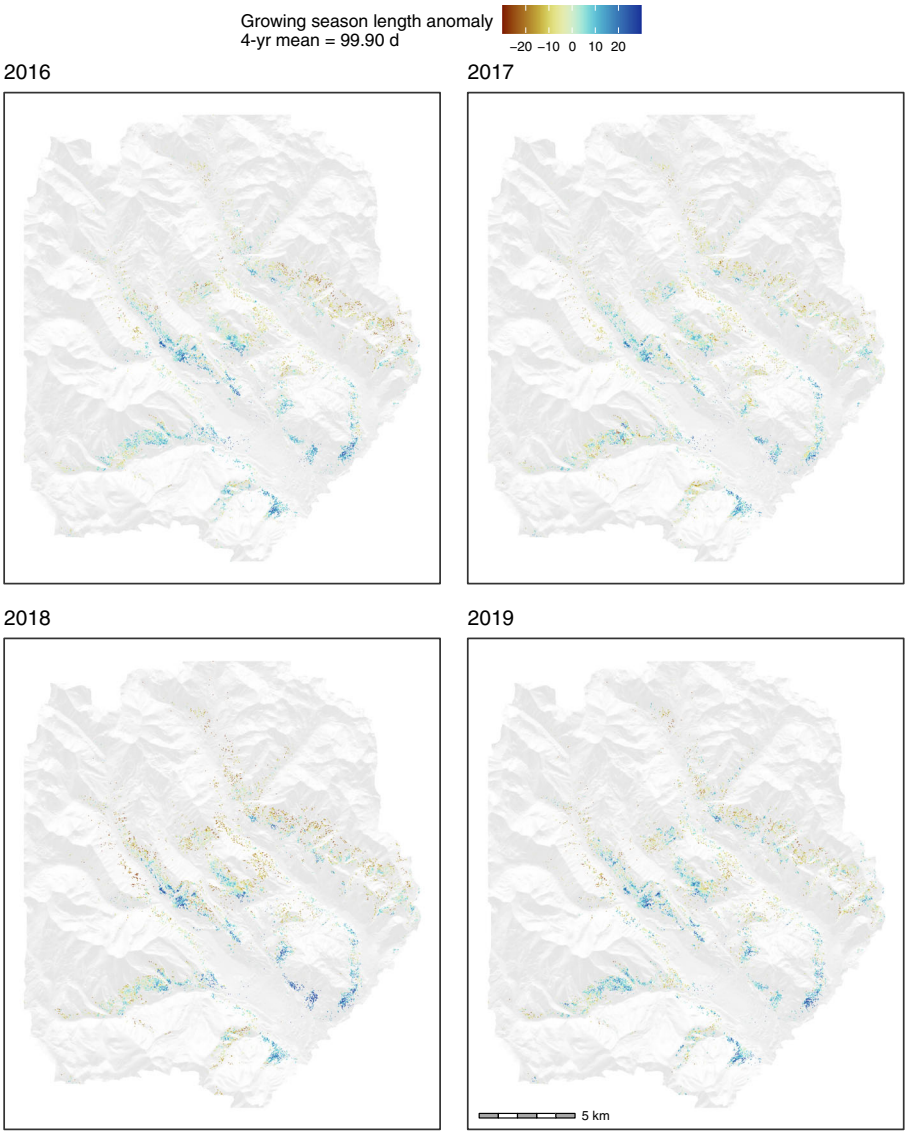

Fig. 2 Variation in phenology across 2016–2019 within quaking aspen forests in the study extent, as illustrated for growing season length. Each panel is shown as an anomaly relative to the 4-yr spatiotemporal mean. Values are overlaid on a topographic base map. Similar plots for other phenology metrics are shown in Supporting Information Figs S4–S6.

The residuals of the linear regression were then interpreted as the variable to be explained in terms of heritable genetic components. We applied a genome-based linear mixed model (Yang *et al.*, 2011) to the above residuals. This method utilizes a genetic relatedness matrix and a restricted maximum likelihood approach to estimate the proportion of phenotypic variance explained by all genotyped single-nucleotide polymorphisms (SNP) in a set of samples (SNP-based heritability). We constructed the genetic relatedness matrix using 6173 high-quality SNPs with allele frequency ≥ 0.5% from the RAD-Seq data aligned to assembled RAD-Seq contigs. The proportion of variance in the phenology measurements attributable to the measured genetic variance across each of the sample sets was then the operational definition of heritability.

Statistical analyses – environmental and genetic drivers of phenology (Question 3)

Using the remotely sensed dataset including cytotype but not sex, we built ensembles of random forest models to determine which predictors or interactions among predictors influenced each phenological metric. This analysis was not repeated for the ground-based cytotype and sex dataset due to insufficient sample size. Analyses were carried out using the RANGER R package (v.0.13.1; Wright & Ziegler, 2015) using a maximum tree depth of 10, a maximum of 1000 trees, and model structure:

$$\begin{aligned}\text{Phenology metric}(t) \approx\ & \text{Cytotype} + \text{Canopy height}\\ & + \text{Elevation} + \text{Slope} + \text{Cosine aspect}\\ & + \text{Maximum temperature}(t, t-1, t-2, t-3)\\ & + \text{Snowmelt date}(t, t-1, t-2, t-3)\\ & + \text{Drought extremity}(t, t-1, t-2, t-3)\\ & + \text{Drought duration}(t, t-1, t-2, t-3) + t\end{aligned}$$

The notation (t) indicates the year for which the response variable is extracted, and the notation (t, $t-1$, $t-2$, and $t-3$) indicates that values are extracted for year t as well as the three prior years. Three years of lags were used to maximize data

availability. The predictor t (as factor) was included in the model to account for additional temporal effects. Predictors with no (t) notation are assumed to be constant over the study duration. Data for all 4 yr in which phenology data were available were included (2016–2019), resulting in predictor data from 2013 to 2019 being used.

For each phenology metric, an ensemble of 10 models was then fitted to the data using a spatial cross-validation approach to minimize the effect of spatial autocorrelation and avoid overfitting. The spatial data were gridded into 100 × 100 m subregions. In each model, 80% of the subregions were randomly selected, and all pixels within these selected regions were then used for model training, while remaining pixels (in the other 20% of subregions) were then used for model testing. Performance was calculated as the R^2 between observed and predicted values in cross-validation, and reported as the mean value across the ensemble of models. Variable importance was calculated using a node impurity statistic.

We then visualized model predictions as partial dependence plots using the PDP R package (v.0.7.0; Greenwell, 2017), which show the marginal effect of a set of predictors (each continuous predictor and year) on each response variable. Estimates for each response variable at each predictor combination were then combined across ensemble models and reported as mean ± SD.

Results

Ranges of variation (Question 1)

Across time and space, greenup dates varied (2.5–97.5% quantile range) from 125 (5 May) to 156 (5 June) (median 138, 18 May), while greendown dates varied from 265 (22 September) to 322 (18 November) (median 284, 11 October). Growing season length varied from 88 to 128 d (median 106). Maximum greenness varied from 0.47 to 0.68 (median 0.58).

The four phenology metrics showed spatial (Figs 2, S4–S6) and temporal heterogeneity (Fig. 3). Greenup date and greendown date were latest in 2019, earliest in 2018, and more average in 2016–2017. Growing season length was highest in 2018 but showed less variation among years. Maximum greenness was highest in 2019 and also showed less variation among years.

Cytotype influenced phenology (Fig. 3a). Greenup dates were generally earlier for triploid cytotypes by *c.* 2–3 d, though the effect was strongest in 2018 and 2019. Greendown dates were 4–5 d later for triploids in 2018, while there was less variation in other years. Growing season was longer by 7–10 d in triploids across all years. Maximum greenness was also higher in triploids in most years by *c.* 0.03–0.05.

Sex also influenced phenology (Fig. 3b). Females had greater growing seasons lengths by 10–15 d and earlier greenup dates by 7–10 d, and also earlier greendown dates by 3–5 d. There was no clear impact on maximum greenness. Results were largely consistent across years and were similar for diploid and triploid cytotypes.

The above results were similar across aspen cover thresholds (Figs S7, S8).

Across the 2013–2019 interval, there was substantial climate variation (Fig. S3). 2014 and 2018 were warm years (2018 especially so), while 2015 was relatively cold, and others were closer to average. The warmest conditions were generally present in the southeastern area of the study extent across time. Soil moisture variation was more spatially heterogeneous with the highest 1% quantile values in some of the headwater valleys in the northwestern part of the study extent, though also on south-facing slopes in the southwestern part of the study extent. 2015–2017 were relatively wet, while 2018–2019 was dry. Snowmelt date was often earliest on south-facing and lower-elevation slopes, but showed additional patchiness that varied from year to year. 2019 had the latest snowmelt date, while 2014 and 2017 were also relatively late. 2018 had early snowmelt everywhere and 2015 in many locations.

Heritability of phenology (Question 2)

Heritability ranged from 0.31 to 0.61 (interquartile range across all cytotypes and phenology metrics; Fig. 4). The highest value was found for maximum greenness for all cytotypes (0.72), and the lowest value was found for greendown date for diploids (< 0.001). Aside from the outlying low values estimated in the small (n = 38) diploid set, all other estimates were > 0.36. The growing season length for diploids was actually the highest (0.77) but confidence intervals were very wide. Heritability estimates decreased at higher cover thresholds due to the decrease in sample size (Figs S9–S11).

Cytotype influenced phenology heritability, with diploids showing lower values than triploids (except for growing season length). This could be biologically relevant, or could be an artifact of the smaller sample size in diploid compared with triploid analyses, which is also reflected in the larger confidence intervals for diploid estimates.

Environmental and genetic drivers of phenology (Question 3)

The model of maximum greenness (Fig. 5, first row) had a R^2 of 0.64 ± 0.009 (ensemble mean ± SD; Fig. S12). The most important predictor was cytotype (fraction diploid), which had a negative effect (−0.03; predicted change in response variable over predictor range). Temperature also had a negative effect (−0.02) at 0-yr lag and a positive effect of +0.01 at 1-yr lag (though only above 30°C). Snowmelt date had a negative effect (−0.02) at 1-yr lag. Minimum soil moisture had a positive effect primarily at 3-yr lag (+0.01). Other climate predictors had more limited importance. Canopy height had a positive effect (+0.02), while topographic predictors had more limited effects. There was a moderate effect of year (0.01).

The model of growing season length (Fig. 5, second row) had a R^2 of 0.79 ± 0.008 (Fig. S12). The most important predictor was snowmelt date at 2-yr lag (−5 d). The next most important predictors were snowmelt date at 0-yr lag (−5 d), elevation (−4 d), snowmelt date at 3-yr lag (−3 d), and maximum temperature at 0-yr lag (+2 d). Cytotype had a negative effect (−1 d). There was no effect of year.

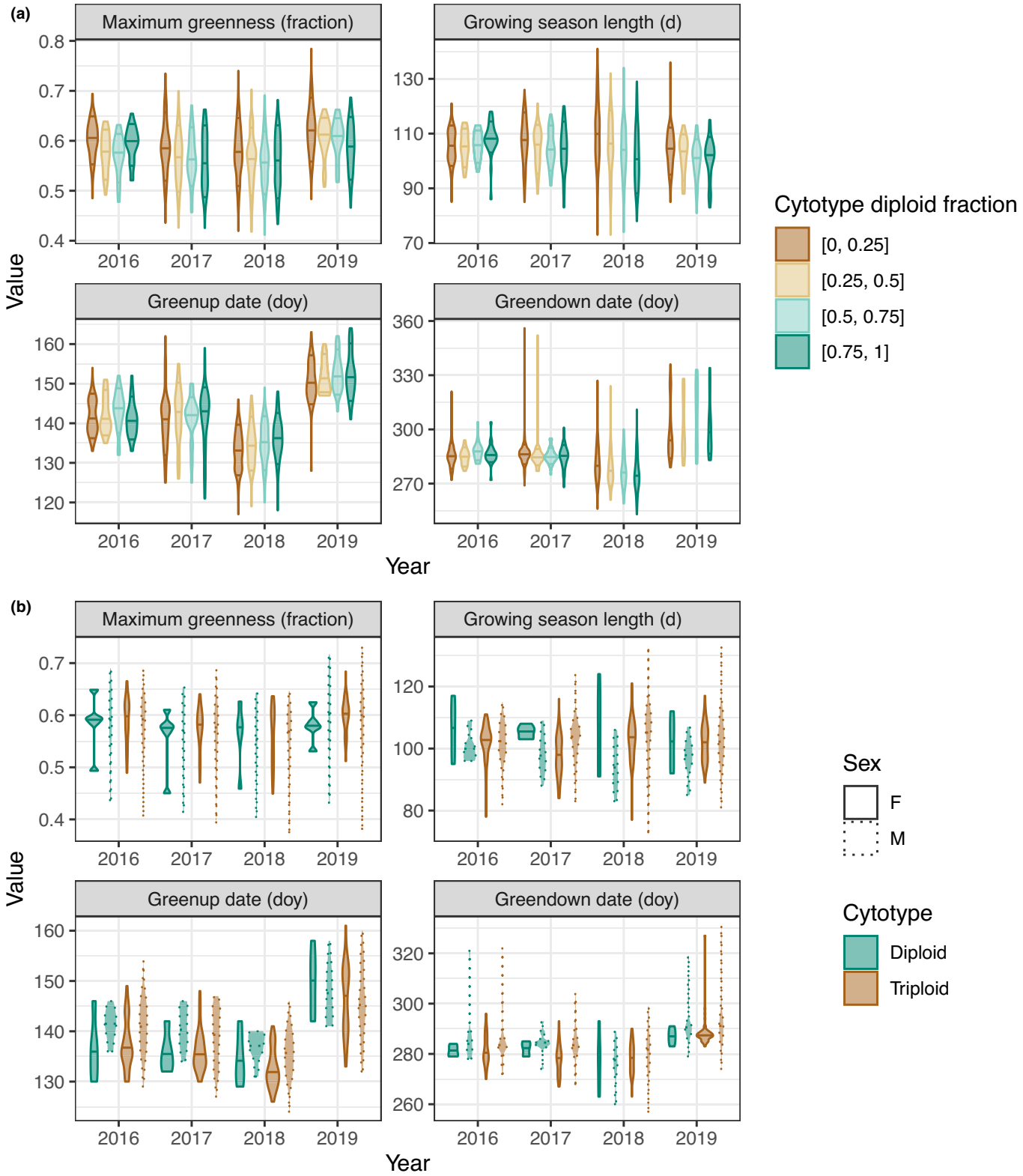

Fig. 3 (a) Ranges of variation in phenological metrics across cytotype and year, when using the full remotely sensed dataset. (b) Ranges of variation in phenological metrics across cytotype, sex, and year, when using ground-based sex dataset paired to remotely sensed pixels. Data comprise pixels containing > 50% fractional aspen cover. In both panels, violin plots show 10%, 50%, and 90% quantiles as horizontal lines. The acronym 'doy' indicates day of year since 1 January.

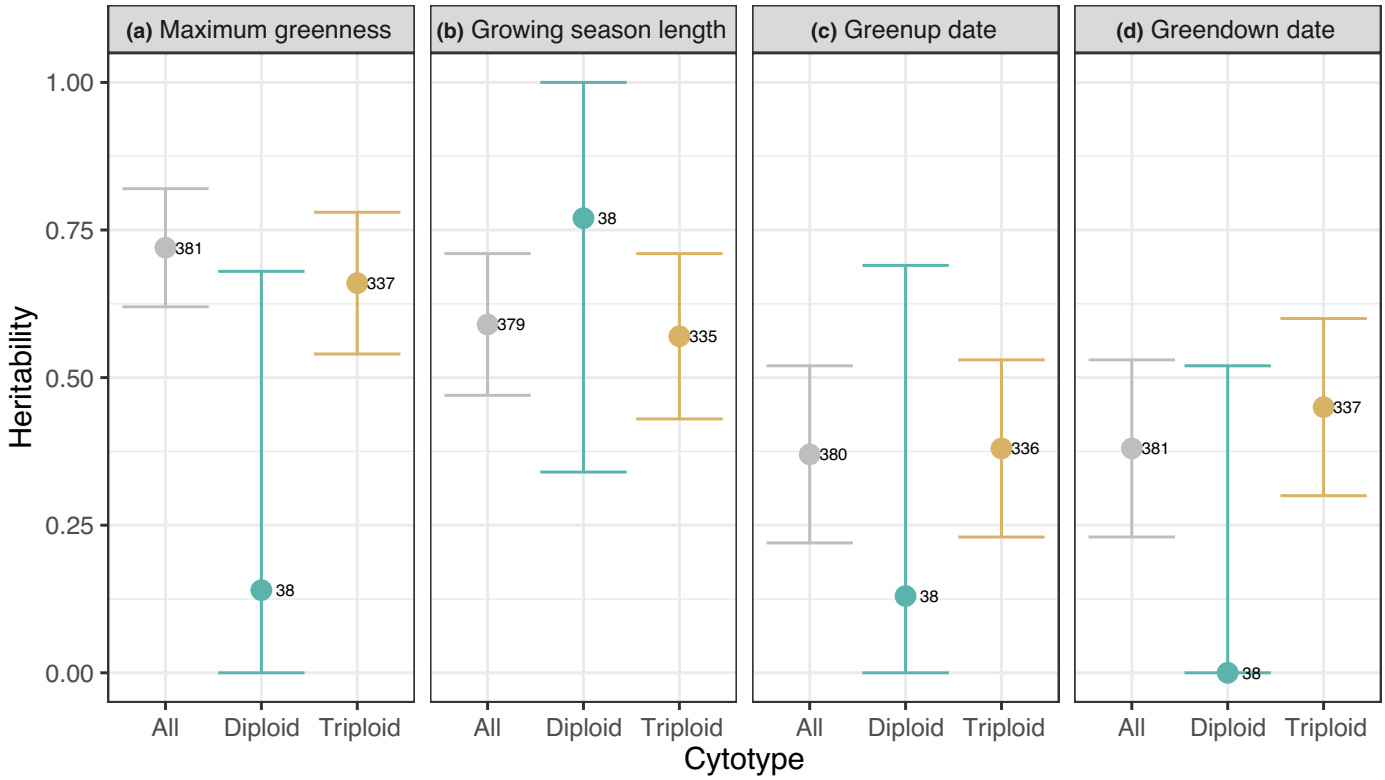

Fig. 4 Heritability of (a) maximum greenness, (b) growing season length, (c) greenup date, and (d) greendown date. Estimates are for genotyped samples within sites containing ≥ 25% fractional aspen cover, subset by ploidy (all samples including samples of undetermined cytotype, diploid only, and triploid only). Bars show 95% confidence intervals; inset numbers indicate sample sizes.

The model of greenup date (Fig. 5, third row) had a R^2 of 0.91 ± 0.002 (Fig. S12). The most important predictor was snowmelt date at 0-yr lag (+7 d). The next most important predictors were maximum temperature at 0-yr lag (−4 d), snowmelt date at 3-yr lag (+2 d), snowmelt date at 1-yr lag (−1 d), and maximum temperature at 1-yr lag (+1 d). Cytotype had a negligible effect. There was also substantial variation among years (6 d).

The model of greendown date (Fig. 5, fourth row) had a R^2 of 0.79 ± 0.013 (Fig. S12). The most important predictor was snowmelt date at 1-yr lag (−6 d). The next most important predictors were snowmelt date at 0-yr lag (+4 d but only above 130 d), maximum temperature at 1-yr lag (+2 d), cosine aspect (−4 d), and snowmelt date at 2-yr lag (−2 d). Cytotype had a negligible effect. There was also substantial variation among years (6 d).

The large year effects observed for greenup date and greendown date also indicate that other temporally variable factors not captured in this analysis are important. Additionally, some of the less important predictors still have substantial importances when summed together or considered interactively, for example, indicating a role for topography on most aspects of phenology, as well as other lagged environmental effects. Partial dependence plots and importance estimates for all predictors are provided in Figs S13 and S14.

The above results were similar when using other cover thresholds (Figs S15–S18 for partial dependence plots, Figs S19, S20 for R^2 estimates, and Figs S21, S22 for variable importances).

Discussion

Ranges of variation (Question 1)

Across 4 yr of time and 391 km^2 of space, we found multiweek variation in greenup and greendown dates and growing season lengths and also substantial heterogeneity in spatial patterning. Spatial and temporal heterogeneity of 2–3 wk in aspen spring phenology has been previously reported (Baker, 1921; Cottam, 1954; Egeberg, 1963; Barnes, 1969; Morgan, 1969; Meier *et al.*, 2015; Ding *et al.*, 2020), somewhat lower than observed here.

Triploid cytotypes had greater maximum greennesses, earlier greenup dates, later greendown dates, and longer growing season lengths. The more extended growing season and higher greeness for triploids provide a mechanism (more carbon gain for longer time periods) for the greater annual growth rates observed in triploids (Benson & Einspahr, 1967), as well as their higher water use efficiency and gas exchange rates (Greer *et al.*, 2017). Later greendown date also potentially provides a mechanism (maladaptive deployment of leaves in stressful conditions) for the higher mortality for triploids in hotter and drier environments (Blonder *et al.*, 2021b).

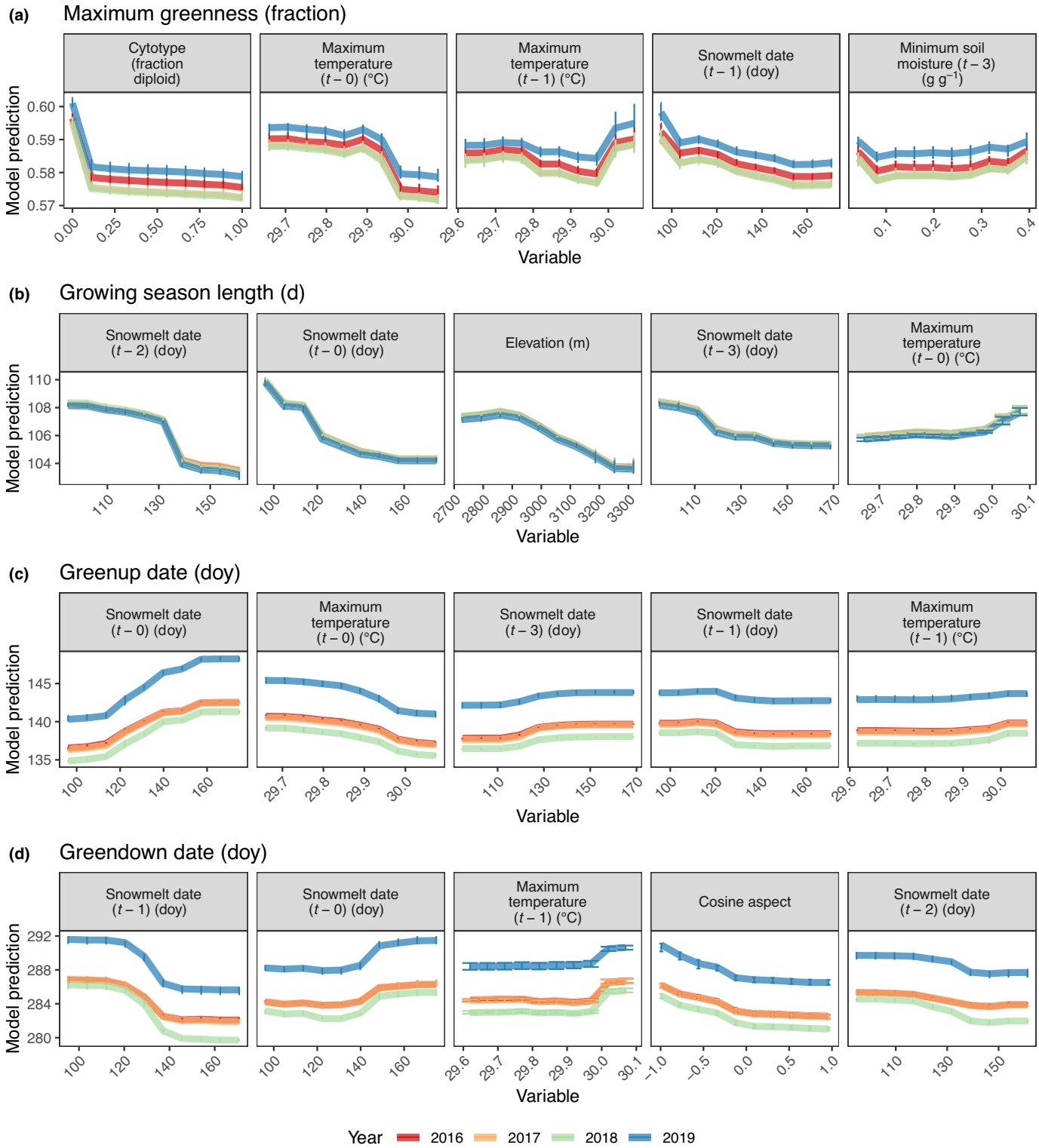

Fig. 5 Effect of genetic and environmental predictors on phenology metrics: (a) maximum greenness, (b) growing season length, (c) greenup date, and (d) greendown date. Partial dependence plots show the independent effect of each predictor and arise from ensembles of spatially cross-validated random forest models (shown as mean ± SD). Parenthetical *t* notation indicates the number of years by which the predictor is lagged relative to the response. The five most important predictors for each metric are shown, ranked from left (most important). Supporting Information Fig. S14 shows responses for all predictors. The acronym 'doy' indicates day of year since 1 January.

Sex influenced greenup date, greendown date, and growing season length more than maximum greenness. Females generally had longer growing seasons but also earlier greendown dates than males, regardless of year or cytotype. Our results are suggestive of occupation of less stressful environments, and shifts in ecophysiology, in the sex that has greater reproductive demands (Sakai & Burris, 1985). The limited number of plots for which we were able to pair sex data with phenology data prevented further analyses.

Heritability of phenology (Question 2)

The heritabilities reported here have similar ranges to previously reported values, but differ in interpretation. Ideally, heritability is assessed within a common garden or laboratory setting, where environmental conditions can be controlled and samples can be randomized to avoid spatial autocorrelation of the genetic variation. Our estimates adjust for these parameters statistically rather than experimentally. Values correct for the influence of the physical environment, but are not narrow-sense heritabilities *sensu* (Ding & Brouard, 2022). They reflect the proportion of the variance explained by genetic variance given the measured environmental variation, which likely does not reflect the overall contribution of genetics to phenology.

There was evidence for substantial phenotypic plasticity of phenology. The large ranges of interannual variation in phenology we observed within individual plots should be interpreted as plasticity, under the reasonable assumption that there is limited genetic turnover in 30 m canopy pixels over 4 yr. These results challenge the use of phenology for delineating boundaries between aspen genotypes (Barnes, 1969). Heritable and plastic components to these responses could potentially be conflated in field observations. Nevertheless, sharp phenology transitions between forest patches may still be useful markers of clone boundaries, as it is difficult to postulate environmental factors that could drive such responses. Indeed, we observed substantial sub-100 m scale spatial patterning in phenological metrics, consistent with such clone boundaries.

Phenology may evolutionarily respond to natural selection because of its high heritability. Responses may occur over relatively short timescales. Although clonal growth is common in aspen and clones may persist for hundreds to thousands of years (Mitton & Grant, 1996), sexual reproduction, especially postfire, is now acknowledged to also be frequent (Landhausser *et al.*, 2019). Responses may occur for both cytotypes. While triploids are expected to be sterile due to chromosome pairing problems in odd-numbered cytotypes (Comai, 2005; Otto, 2007), triploid aspen individuals are sometimes fertile and produce viable offspring (fig. S29 in Goessen *et al.*, 2022). Triploids are also often fertile in the closely related *P. tremula* (Johnsson, 1940).

Environmental and genetic drivers of phenology (Question 3)

The effect of cytotype was strongest on maximum greenness and otherwise negligible for other aspects of phenology. The apparent null effect probably arises because the spatial distribution of cytotype on the landscape is correlated with the spatial distribution of other environmental factors. In this region, triploids are more common on lower-elevation south-facing slopes (Blonder *et al.*, 2021a,b). Thus, the phenology models are interpreting effects of cytotype as effects of the environment. Similar results for an apparently negligible effect of cytotype on flowering date but a larger effect on growth have been found in *Artemisia tridentata* (Richardson *et al.*, 2017).

There was a strong effect of the environment, both in the current year and in prior years. We found evidence that these lags extend for at least three prior years and that each phenological metric is sensitive to different climate variables at different temporal lags. Such lags are acknowledged for growth and mortality in aspen (Peltier *et al.*, 2021) and other species (Peltier *et al.*, 2016; Trugman *et al.*, 2018). This study now shows that lagged environmental effects also influence phenology, in addition to the current-year climate effects that are widely known for aspen (Meier *et al.*, 2015) and for other species (Cooper *et al.*, 2011; Zheng *et al.*, 2022).

Lagged environmental effects were strongest for prior-year snowmelt dates. Later snowmelt in prior years led to shorter growing seasons, later greenup dates, earlier greendown dates, and lower maximum greenness in following years. Maximum temperatures also were important, with greater values in prior years leading to higher maximum greenness, later greenup date, and later greendown date, while the opposite was true for current-year temperature. Finally, greater minimum soil moisture (reduced drought) in prior and current years led to greater maximum greenness, greater growing season length, earlier greenup date, and later greendown date.

The soil moisture results may indicate a temporal integration of hydraulic stress. As droughts increase over multiple years, accumulated hydraulic damage likely increases (Peltier *et al.*, 2016; Kannenberg *et al.*, 2020). In turn, phenology becomes progressively more conservative, as individuals have less capacity to hydraulically support flushed leaves.

The snowmelt results can be interpreted similarly, with earlier snowmelt potentially associated with increased drought. However, we observed longer legacies for snowmelt than for soil moisture. This in turn may reflect the difficulty of modeling relevant root-zone soil moisture estimates across all sites, rather than a real biological effect.

The temperature results are challenging to interpret. The increases in greenness and greendown date driven by prior-year maximum temperatures suggest that prior conditions either cause more prior-year carbon gain and enable more aggressive phenology in future years; or alternatively suggest that temperature-related stress in prior years cause less prior-year carbon gain that induces more risky phenology in future years to offset carbon deficits, perhaps via some unknown mechanisms regulating homeostasis of NSC pools or hydraulic damage (Trugman *et al.*, 2018). The second hypothesis seems more compelling, given that the current-year effects are generally opposite in direction to the prior-year effects and seem stress-associated – that is, higher maximum current-year temperatures decrease greenness, increase growing season length, and drive earlier greenup date. The only

Table 1 Summary of spatial data products used in this study.

Data product	Unit	Provenance	Original resolution	Years of coverage
Aspen cover	Dimensionless	NEON AOP hyperspectral imagery + RAD-Seq genomic data	1 m	2018
Canopy height	m	NEON AOP LiDAR	1 m	2018
Cytotype, fraction triploid	Dimensionless	NEON AOP hyperspectral imagery + RAD-Seq genomic data	1 m	2018
Topography, elevation	m	DOE LiDAR	1 m	2015
Topography, slope	°	DOE LiDAR	1 m	2015
Topography, cosine aspect	Dimensionless	DOE LiDAR	1 m	2015
Maximum temperature	°C	gridMET	4 km	2013–2019
Snowmelt date	Day of year	MODIS	500 m	2013–2019
Soil moisture low value (drought extremity)	Fraction	Parflow-CLM	1 km	2013–2019
Soil moisture median no. of consecutive days below 20% moisture (drought duration)	d	Parflow-CLM	1 km	2013–2019
Phenology, greenup date	Day of year	Landsat-8 + Sentinel-2	30 m	2016–2019
Phenology, greendown date	Day of year	Landsat-8 + Sentinel-2	30 m	2016–2019
Phenology, growing season length	d	Landsat-8 + Sentinel-2	30 m	2016–2019
Phenology, maximum greenness	Dimensionless	Landsat-8 + Sentinel-2	30 m	2016–2019

The acronym 'doy' indicates day of year since 1 January.

challenge to this viewpoint is that greendown date actually decreases slightly with higher current-year temperatures. However, prior studies of *Populus* have shown that there are positive genetic correlations between productivity and fall phenology (McKown *et al.*, 2014; Soolanayakanahally *et al.*, 2015), as well as high temperatures delaying next-year bud formation (Strømme *et al.*, 2017). These patterns are consistent with the second hypothesis.

The observed effects of canopy height on phenology are inconsistent with recent hypotheses for structural overshoots in response to stress (Trugman *et al.*, 2018). This theory suggests that taller trees may have disproportionately higher xylem repair costs, such that they are at greater risk of carbon deficit from producing too many leaves in future years after accumulating hydraulic damage in prior years. As such, taller trees should potentially have shorter growing seasons. Instead, we found that greater canopy height was associated with higher maximum greenness and longer growing season length. This is more consistent with larger trees having greater tolerance for hydraulic and carbon risk, as well as perhaps higher respiration costs (more stem tissue) requiring longer growth to maintain the same carbon balance. In support, a common garden study (Ding *et al.*, 2020) also found that taller trees are associated with earlier greenup and later greendown. Consistent with this, taller trees also could have greater leaf area index and thus higher remotely sensed greenness, or also deeper roots and access to more consistent soil moisture to support higher growing season length.

Our results are potentially inconsistent with the hypothesis (Zani *et al.*, 2020; Norby, 2021) that greater growing season productivity drives early leaf senescence. We were not able to directly estimate productivity, but found that early greendown date was associated with factors that are likely associated with lower productivity (higher current-year temperature and lower current-year soil moisture). Our tentative rejection of this hypothesis rather agrees with other multispecies remote sensing analyses showing no linkage between growing season productivity and greendown date (Lu & Keenan, 2022). An alternate possibility is that greenup date influences greendown date, perhaps to drive a relatively invariant growing season length despite environmental variation (Keenan & Richardson, 2015), an idea supported by the limited interannual variation in growing season length we observed.

Conclusion

This observational study characterized the environmental and genetic drivers of phenology in quaking aspen spanning 391 km^2 of Colorado and covering 4 yr. Within this domain, we first showed that phenological patterns in this species are the outcome of spatial environmental and genetic mosaics, and can vary by multiple weeks in response to these factors. Second, we showed that the proportion of phenology variance explained by genetics is high and also that phenotypic plasticity enables phenological variation of similar magnitudes. And third, we showed that phenology in 1 yr can be shifted by days to weeks by drought, heat, and snow events occurring in prior years, indicating that lagged environmental effects occur for phenology. These results may be contingent on the genetic and environmental diversity observed in the study area, especially given the drought conditions that characterized the study period. Nevertheless, they provide a snapshot of the diversity of phenological responses possible in this iconic species.

Acknowledgements

The Rocky Mountain Biological Laboratory assisted with United States Forest Service research permits and land access. Hyperspectral and LiDAR data used in this research were obtained through the NEON Assignable Assets program by the United States Department of Energy's Watershed Function Scientific Focus

Area. Machine learning was supported by Microsoft's AI 4 EARTH program. BWB was supported by USDA NIFA award no. 2022-67019-36366. RC was supported by NSF BIO postdoctoral fellowship no. 2011030. KDC was supported by NSF EAR postdoctoral fellowship no. 1725788. PGB was supported by the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration. Carol A. Rowe and Karen Mock also contributed to DNA sequence assembly and analysis. ME-A and SH are supported by the Carnegie Institution for Science. Leander Anderegg, Karen Mock, and Bryce Richardson provided feedback on a draft manuscript. Donal S. O'Leary provided snowmelt date analyses.

Competing interests

None declared.

Author contributions

BWB conceived the study. BWB, KDC, EC, RMC-dH, and CAR carried out fieldwork. PGB, MM, and HT contributed remote sensing analyses. ME-A, SH, and JAW contributed genetic analyses. BWB and SH contributed statistical analyses. All authors contributed to writing the manuscript.

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Data availability

Data products are summarized in Table 1. Reprojected spatial data products sufficient to replicate analyses, along with code to replicate all statistical analyses and figures, are available at https://github.com/bblonder/aspen_phenology_remote_sensing and archived on Zenodo at Blonder (2023). Genetic data have been previously deposited (Blonder *et al.*, 2020).

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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 Regional map with inset showing the study extent and plot-based datasets.

Fig. S2 Variation in topographic and genetic predictors within the study extent.

Fig. S3 Variation in environmental variables across 2013–2019 within the study extent.

Fig. S4 Variation in phenology across 2016–2019 within aspen forests in the study extent, as illustrated for maximum greenness.

Fig. S5 Variation in phenology across 2016–2019 within aspen forests in the study extent, as illustrated for greenup date.

Fig. S6 Variation in phenology across 2016–2019 within aspen forests in the study extent, as illustrated for greendown date.

Fig. S7 As in Fig. 3, but with sites containing ≥ 30% fractional aspen cover.

Fig. S8 As in Fig. 3, but with sites containing ≥ 70% fractional aspen cover.

Fig. S9 As in Fig. 4, but with sites containing ≥ 30% fractional aspen cover.

Fig. S10 As in Fig. 4, but with sites containing ≥ 50% fractional aspen cover.

Fig. S11 As in Fig. 4, but with sites containing 70% fractional aspen cover.

Fig. S12 Performance of random forest model ensembles for each phenology response variable.

Fig. S13 Predictor importance in random forest model ensembles for each phenology response variable.

Fig. S14 As in Fig. 5, but with all predictors shown.

Fig. S15 As in Fig. 5, but with sites containing ≥ 30% fractional aspen cover.

Fig. S16 As in Fig. 5, but with sites containing ≥ 70% fractional aspen cover.

Fig. S17 As in Fig. S14, but with sites containing ≥ 30% fractional aspen cover.

Fig. S18 As in Fig. S14, but with sites containing ≥ 70% fractional aspen cover.

Fig. S19 As in Fig. S12, but with sites containing ≥ 30% fractional aspen cover.

Fig. S20 As in Fig. S12, but with sites containing ≥ 70% fractional aspen cover.

Fig. S21 As in Fig. S13, but with sites containing ≥ 30% fractional aspen cover.

Fig. S22 As in Fig. S13, but with sites containing ≥ 70% fractional aspen cover.

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