ORIGINAL RESEARCH

Differences among six woody perennials native to Northern Europe in their level of genetic differentiation and adaptive potential at fine local scale

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Abstract

The ability of perennial species to adapt their phenology to present and future temperature conditions is important for their ability to retain high fitness compared to other competing plant species, pests, and pathogens. Many transplanting studies with forest tree species have previously reported substantial genetic differentiation among populations within their native range. However, the question of "how local is local" is still highly debated in conservation biology because studies on genetic patterns of variation within and among populations at the local scale are limited and scattered. In this study, we compare the level of genetic differentiation among populations of six different perennial plant species based on their variation in spring flushing. We assess the level of additive genetic variation present within the local population. For all six species, we find significant differentiation among populations from sites with mean annual temperature ranging between 7.4°C and 8.4°C. The observed variation can only be partly explained by the climate at the site of origin. Most clear relationship between early flushing and higher average spring temperature is observed for the three wind-pollinated species in the study, while the relations are much less clear for the three insect-pollinated species. This supports that pollination system can influence the balance between genetic drift and natural selection and thereby influence the level of local adaptation in long-lived species. On the positive side, we find that the native populations of woody plant species have maintained high levels of additive genetic variation in spring phenology, although this also differs substantially among the six studied species.

KEYWORDS
climate change, life-history traits, local adaptation, phenology, quantitative genetics

1 | INTRODUCTION

Phenology is important for fitness of perennial species. Higher spring temperatures are expected to prolong the growing seasons due to earlier bud burst (Dragoni et al., 2011; Menzel & Fabian, 1999; Menzel et al., 2006; Richardson et al., 2010, 2013; Vitasse, Delzon, Dufrêne, et al., 2009), while lack of synchrony between phenology and occurrence of spring frost events increases risk of damage to early flushing plants (Duputié, Rutschmann, Ronce, & Chuine, 2015). Yet again, raised temperatures with no frost events and changes in daily

temperature fluctuations could also result in later bud burst and influence time between bud development and growth cessation (Chmura et al., 2011; Rohde, Bastien, & Boerjan, 2011; Way, 2011). Several studies have documented substantial variation among populations in their phenology reflecting their geographic origin (Alberto, Derory, et al., 2013; Chuine & Beaubien, 2001; Salmela, Cavers, Cottrell, Iason, & Ennos, 2013). It is therefore fair to assume that genetic variation within species combined with divergent selection has played an important role for the ability of many tree species to thrive in a large distribution range across strong environmental gradients (Brousseau et al., 2016; Kawecki & Ebert, 2004; Pluess et al., 2016). However, species differ in their response and spring and autumn phenology may show different patterns. A study based on 59 tree species from similar climatic clines thus showed a relative clear pattern with respect to bud set in autumn but less clear pattern in spring bud burst (Alberto, Aitken, et al., 2013). The expectation of local adaptation often leads restoration and conservation programs to focus on gene pools at the local scale rather than the regional scale (Stanturf et al., 2015), but only few studies have actually assessed the variation at the local scale (within ~100 km).

Fast climate change calls for high phenotypic plasticity (Valladares et al., 2014) and genetic variation in relevant traits in plant populations (Franks, Weber, & Aitken, 2014) in order to ensure continued adaptation. Many tree species have revealed phenotypic plasticity and demonstrated their ability to thrive and reproduce when transferred to new areas (Kjær, Lobo, & Myking, 2014; Lascoux, Glémin, & Savolainen, 2016; Myking, Rusanen, Steffenrem, Kjær, & Jansson, 2016; Rehfeldt, Ying, Spittlehouse, & Hamilton, 1999). However, there are indications of declining plasticity in phenology in spring with continued global warming (Fu et al., 2015) and increased frequency of warm periods in the early spring may lead to frost backlashes (Jönsson & Bärring, 2011). The temperatures are likely to change in the future, but the seasonal variations in day length remain the same. Species will therefore respond differently as regards phenology to temperature changes depending on the species-specific importance of day length versus heat sum in controlling flushing time (Alberto, Aitken, et al., 2013; Caffarra & Donnelly, 2011; Fu et al., 2015; Vitasse, Delzon, Bresson, Michalet, & Kremer, 2009; Vitasse, Delzon, Dufrêne, et al., 2009).

Variation among individual genotypes in their phenology triggers selection at the population level in favor of the new conditions, if the variation is in fitness traits and expressed with moderate or high heritability (Alberto, Aitken, et al., 2013; Falconer & Mackay, 1996). Genetic differentiation within and among population is therefore important for the species ability to adapt to climate change (Kubisch, Holt, Poethke, & Fronhofer, 2014). However, natural selection is not the only evolutionary force, because the combined actions of natural selection, gene flow and genetic drift drive the level of genetic variation within and among populations in fitness traits (Nadeau, Meirmans, Aitken, Ritland, & Isabel, 2016). The realized patterns therefore depend on effective population sizes, force of natural selection and limitations in pollen and seed dispersal (Aitken & Whitlock, 2013; Savolainen, Pyhäjärvi, & Knürr, 2007). Natural selection tends to increase the level of genetic variation among populations, but reduce the variation within

populations leading to local adaptation. Fragmentation into small populations can create drift that also increase variation among population variation, but without supporting local adaptation. Small populations can rather reduce fitness due to inbreeding, at least in outcrossing species. Pollination and seed dispersal across the landscape among populations will counteract the fragmentation, but seed and pollen dispersal across environmental gradients will also counteract natural selection and thereby reduce the ability of populations to adapt to specific local conditions (Kubisch et al., 2014). The level of gene flow is expected to differ among woody species depending on occurrence, landscape features, mating system, pollen, and seed dispersal vectors. We therefore expect that the degree of local differentiation—and the degree to which this reflect local adaptation—will differ among woody plant species depending on their major life-history traits.

1.1 | Objectives of this study

On the above background, the objectives of this study were to use data on spring phenology to

1. Assess the degree to which woody perennials reveal fine-scale genetic differences among geographically and climatic close (differences in mean annual temperature around 1°C) populations, and test whether differences (if any) influence the fitness revealing signs of local adaptation.
2. Estimate levels of genetic variation within and among populations in spring phenology and compare the levels of population differentiation (Q_{ST}) and additive genetic variation (V_A) among species.

Based on the results, we discuss the implications for dynamic conservation of genetic resources and the ability of woody plant species to adapt to future climate conditions.

2 | MATERIALS AND METHODS

Six broadleaved species in Denmark were studied, of which three are insect pollinated—Common dogwood (*Cornus sanguinea* L.), European Crab Apple (*Malus sylvestris* (L.) Mill.) and Glaucous Dog Rose (*Rosa dumalis* (Bechst) Boulay), while three are wind pollinated—Downy birch (*Betula pubescens* Ehrh.), Common Hazel (*Corylus avellana* L.) and Sessile oak (*Quercus petraea* (Matt.) Liebl.). We used data from progeny trials with offspring from open pollinated trees based on seed collected from putative natural populations across Denmark (Figure 1). Description of the field trials, location of populations, and climate information at the population site and information on families within each population are provided in Appendix S1.

2.1 | Assessment of phenotypic data and estimation of variance components

We used spring phenology assessed as bud burst scores using a scale from 0 to 8, where class 0 was closed winter buds and class 8 was fully
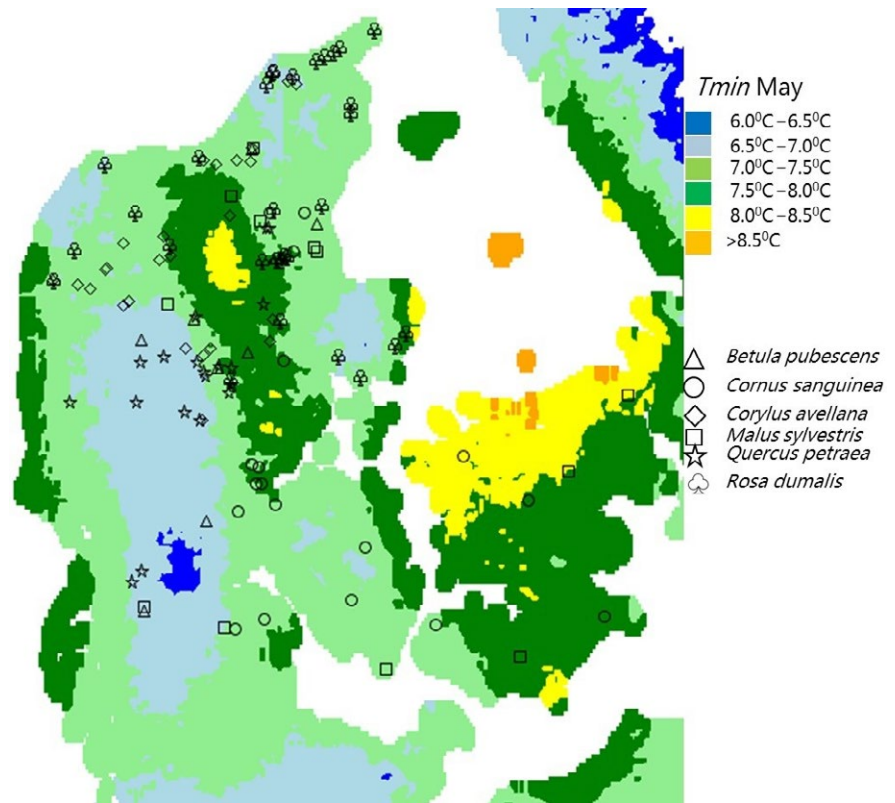
FIGURE 1 Locations of original populations for the different species in the study are spread across different climate zones (here represented by temperature minimum in May [T_{min}])

opened leaves. The time of assessment was adapted for each species so that the trees in the trials were approximately in the middle of their flushing time (average values of bud burst score between 3 and 5). This was to ensure maximum variation among individuals in their bud burst ranking. Bud burst for *B. pubescens*, *C. sanguinea*, *M. sylvestris*, and *R. dumalis* were assessed in spring 2010, while assessments of *C. avellana* and *Q. petraea* were from spring 2013. Height was measured in the year of phenology assessment except for *R. dumalis*.

All analyzes were made per species, because they were tested in different common garden trials. The variation among trees in their phenological stage was separated into variance and covariance components reflecting genetic differences among and within populations as implemented in the software ASReml (Gilmour, Gogel, Cullis, & Thompson, 2009) from the model described below.

Population values were predicted by application of mixed model (1), and genetic variance component corresponding to each of the random effects in the model was estimated from the analysis;

$$Y_{ijkl} = \mu + B_i + P_j + \lambda_{ij} + f_{k(j)} + \tau_{ikj} + \varepsilon_{ijkl} \qquad (1)$$

where Y_{ijkl} is the trait measured for tree *l*, μ is the overall mean of the trait, B_i is the fixed block effect, P_j is the fixed population effect, λ_{ij} is the fixed population by block interaction, $f_{k(j)}$ is the random effect of family within population, τ_{ijk} is the random effect of plot *k*, and ε_{ijkl} is the residual. Q_{ST} values were estimated from model (1) as well having population effects as random.

The significance of the genetic variances of traits within sites and genotype by environment interaction across sites was tested using the log likelihood ratio (Gilmour et al., 2009). The significance

of the populations was tested using the Satterthwaite approximation (Satterthwaite, 1946) in the procedure GLM in the statistical software program SAS (SAS Institute Inc. 2011).

2.2 | Quantitative genetic analysis

We estimated the additive genetic variance (V_A) as $4\sigma_f^2$ and narrow sense heritability within sites as; $\hat{h}^2 = 4\sigma_f^2/(\sigma_f^2 + \sigma_\tau^2 + \sigma_\varepsilon^2)$, where σ_f^2 is the estimated family variance, σ_τ^2 is the estimated plot variance, and σ_ε^2 is the estimated within plot variance. The phenotypic variance was estimated as $\hat{V}_P = \sigma_f^2 + \sigma_\tau^2 + \sigma_\varepsilon^2$. Families were considered to represent groups of half-sibs. This assumption will overestimate the additive genetic variance, heritability, and expected response to selection, if the offspring within progeny groups on average are more related than half-sibs (Falconer & Mackay, 1996). While the assumption of half-sibs may provide a good fit for most trees species (Kjær, McKinney, Nielsen, Hansen, & Hansen, 2012; Larsen & Kjær, 2009), the situation may be more complicated for *R. dumalis*, where polyploidy may be present as the variance between families is biased due to a fraction of the dominance genetic variance, even if the families consist of pure half-sibs (Roberts, Gladis, & Brumme, 2009).

The degree of differentiation among populations was estimated as the Q_{ST} values (Spitze, 1993) using the formula;

$$\hat{Q}_{ST} = \hat{V}_{POP}/(\hat{V}_{POP} + 2\hat{V}_A)$$

where $\hat{V}_{POP}$ is the estimated variance between populations, and $\hat{V}_A$ is the estimated additive genetic variance.
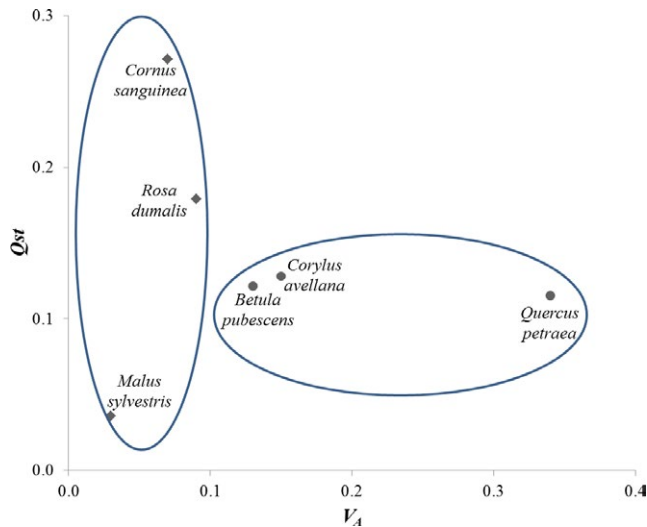
FIGURE 2 Difference among species in genetic variation within populations (V_A = additive genetic variance) and population differentiation (Q_{ST} value) for bud burst ◆ insect-pollinated species, ● wind-pollinated species

Variance components, narrow sense heritability estimates, Q_{ST} estimates, and their standard errors were estimated from model (1) using the software ASReml (Gilmour et al., 2009).

2.3 | Support to the hypothesis of location adaptation

Climate data for the locations of origins were estimated with the ClimateEU v4.63 software package following the methodology described by (Hamann, Wang, Spittlehouse, & Murdock, 2013). Weighted regression analysis to test relationship between phenology of populations and climate at the population sites was carried out using procedure REG in SAS (SAS Institute Inc. 2011) having the predicted values of populations as dependent variable and climate variables as explanatory variables. One divided by the error variance for the predicted population values were used as weights. The climate variables tested for bud burst were monthly minimum temperatures and differences between monthly maximum and minimum temperatures in March,

April, May and June, because these data are expected to provide good proxies for the risk of frost occurring after flushing. A backward selection approach (5% level) was used for the selection of climate variables that could best explain the variation in flushing time. The relationship between geographic distances and difference in average budburst was tested by a Mantel test as implemented in R version 3.2.2 (Dray & Dufour, 2007).

2.4 | Fitness effects of spring phenology

Genetic correlations between bud burst and height growth were estimated based on individual tree data to assess whether the height as a proxy for realized fitness in the present climate varied with the phenology. Genetic correlations could be estimated between survival and phenology based on plot means, because the plants were grown in family plots in all trials (see Appendix S1). Genetic correlations were estimated according to Falconer and Mackay (1996) as $\hat{r}_g = \frac{\sigma_{fxy}}{\sqrt{\sigma_{fx}^2 \sigma_{fy}^2}}$, where σ_{fxy} is the family (within population) covariance between trait x and y, σ_{fx}^2 is the family (within population) variance for trait x, and σ_{fy}^2 is the family (within population) variance for trait y.

3 | RESULTS

3.1 | Presence of genetic variation within and among populations in bud burst

Genetic variation both within and among populations was significant in all six species, but with large differences among the species. The additive genetic variance for bud burst (V_A) thus ranged from 0.07 in _C. sanguinea_ to 0.34 in _Q. petraea_ (Table 1), while the level of differentiation among populations (Q_{ST}) ranged from 0.04 in _M. sylvestris_ to 0.27 in _C. sanguinea_ (Table 2). The three wind-pollinated species _B. pubescens_, _C. avellana_ and _Q. petraea_ showed the highest level of additive variance, while the insect-pollinated species _C. sanguinea_ and _R. dumalis_ showed the highest level of population differentiation as measured by the Q_{ST} (Figure 2).

TABLE 1 Family variation and genetic parameters for bud burst in different species

Species	Trait	Mean	_p_-Value family	V_A	V_P	h^2	SE
Betula pubescens	Bud burst 24 April 2010	3.27	.003	0.15	0.30	0.50	0.12
Cornus sanguinea	Bud burst 6 May 2010	3.81	<.0001	0.07	0.27	0.24	0.11
Corylus avellana	Budburst 24 April 2013	3.09	<.0001	0.13	0.27	0.47	0.09
Malus sylvestris	Bud burst 28 April 2010	4.05	<.0001	0.03	0.10	0.31	0.08
Quercus petraea	Bud burst 24 May 2013	4.64	<.0001	0.34	0.58	0.58	0.26
Rosa dumalis	Bud burst 26 April 2010	3.58	<.0001	0.09	0.21	0.41	0.14

V_A = additive genetic variance, V_P = p variance, h^2 = narrow sense heritability, SE = standard error for h^2.

TABLE 2 Level of population differentiation for bud burst in different species

Species	Trait	Q_{st}	SE	p-Value population	Max. pop. diff.[a]
Betula pubescens	Bud burst 24 April 2010	0.13	0.08	<.001	0.71
Cornus sanguinea	Bud burst 6 May 2010	0.27	0.14	<.001	0.88
Corylus avellana	Budburst 24 April 2013	0.12	0.05	<.0001	0.86
Malus sylvestris	Bud burst 28 April 2010	0.04	0.03	.02	0.28
Quercus petraea	Bud burst 24 May 2013	0.13	0.09	.0003	2.45
Rosa dumalis	Bud burst 26 April 2010	0.18	0.09	<.001	1.52

[a]Maximum difference between populations in bud burst score.

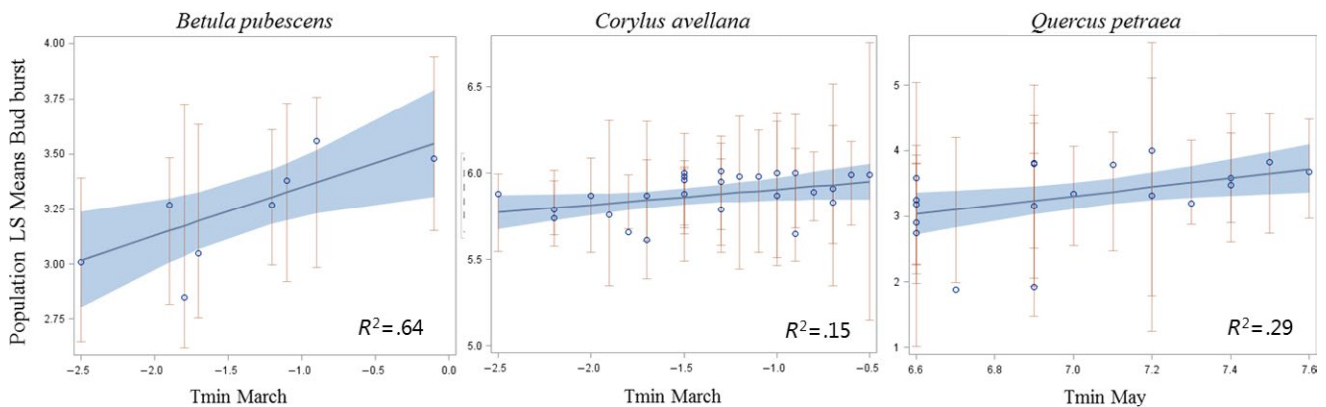QST = quantitative genetic differentiation among populations in bud burst, SE = standard error for QST

FIGURE 3 Weighted regression between population means of bud burst score and T_{min} (temperature minimum) of different months in spring at original population site in different species

3.2 | Relationship of phenology with growth, fitness, distance between populations and climate at original population site

The regression between bud burst of populations and population site minimum temperatures in March were significant ($p < .05$), or close to significant ($p = .05$) for *B. pubescens* and *C. avellana* (Figure 3), and the regression between bud burst of populations and population site minimum temperatures in May was close to significant ($p = .05$) for *Q. petraea* (Figure 3). This corresponds to the general time for bud burst, where *B. pubescens* and *C. avellana* flush before *Q. petraea* in Denmark and indicate that spring frost event has played an important role in developing the pattern of variation among population in these species. Similar significant correlations were not observed in any of the three insect-pollinated species. Mantel tests for correlation between geographic distance and difference in bud burst at population were nonsignificant in all the species (Figure 4). Survival was not significantly correlated to bud burst data in any of the species evaluated, and a significant additive genetic correlation between height and budburst was only observed in *B. pubescens* (rg = 0.35; SE 0.23).

4 | DISCUSSION

Our results show that fine-scale local genetic differentiation in fitness traits such as phenology indeed can be present and to a larger extent than previously anticipated in the Danish gene conservation strategy (Graudal, Kjær, & Canger, 1995). However, the analysis across the six different species did not provide unique patterns of local adaptation, as the absence of significant regression between bud burst time and spring temperature in the three insect-pollinated species suggests that the variation among population is not always simply reflecting local adaptation. It is rather more likely a result of natural selection and neutral processes simultaneously acting as predicted by theory (Nadeau et al., 2016).The fact that populations in all studied six species were significantly differentiated in their spring phenology suggests that local populations of woody perennials more often than not are genetically differentiated, and this can be the case even if they only are separated by 10 to 35 km (Figure 4) and growing in areas with low variation in altitude and where the spring temperature varies only between 1°C and 2.5°C (Figure 3). This finding should be compared to the prediction of Northern Europe becoming 2–4°C warmer by
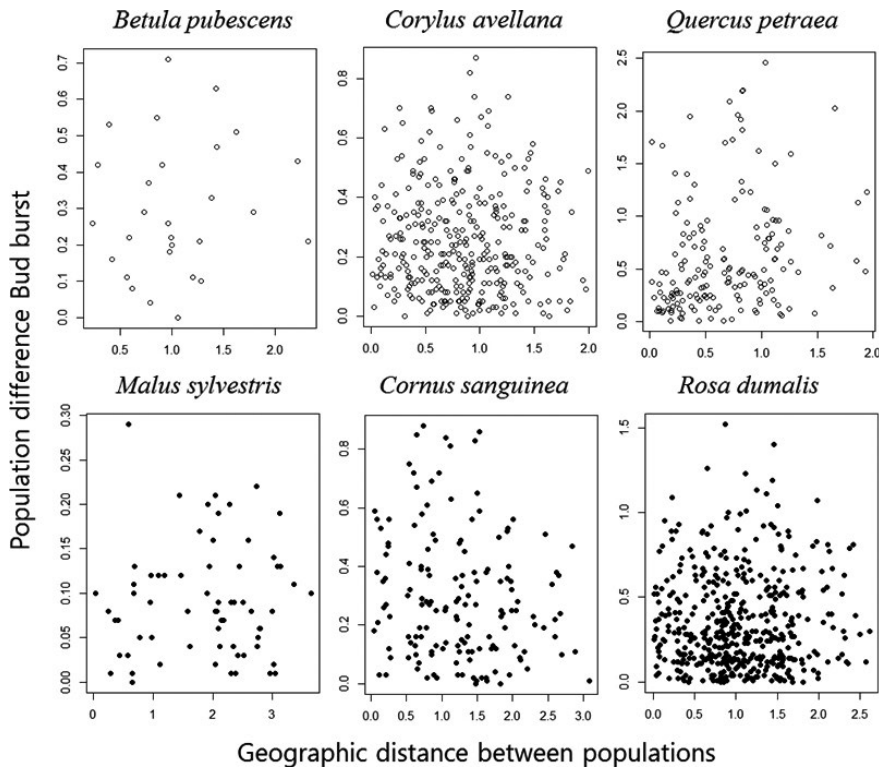
FIGURE 4 Population genetic differentiation in bud burst show no relationship with geographic distance (in 100 km) between them in different species

the year 2100 (Nikulin, Kjellström, Hansson, Strandberg, & Ullerstig, 2011). However, the observed high level of additive genetic variation in the studied species indicates that the native populations of woody species do possess substantial evolutionary potential. Divergent natural selection is possible within few generations, if the timing of phenology creates large differences in fitness. This is supported by the fact that the observed patterns in our study for *B. pubescens*, *C. avellana*, and *Q. petraea* (Figure 3) are at least partly due to past natural selection in favor of local adaption.

The presence of local adaptation and evolutionary potential in woody species are often attributed to their life-history traits including pollination mechanisms and associated genetic processes, and it is therefore interesting that the patterns were different for the wind- and insect-pollinated species in our study. The result that the three wind-pollinated, continuous distributed species (*B. pubescens*, *C. avellana*, *Q. petraea)* were characterized by having high levels of genetic variation within populations (right circle of Figure 2) supports the general assumption that wind-pollinated, widely distributed species have allocated genetic variation within populations to a larger extent than insect-pollinated species with small and scattered populations (Petit & Hampe, 2006; Smith & Donoghue, 2008). The results correspond to the expectation that long-distance gene flow through wind pollination will maintain connectivity among populations (lowering Q_{ST}) and counteract loss of genetic variation (maintaining high h^2) within populations (Sork, 2016). High levels of gene flow among populations across landscapes have been reported in the wind-pollinated *Quercus* (Gerber et al., 2014). The insect-pollinated *C. sanguinea* was in the opposite corner of *Q. petraea* in Figure 2, reflecting a much higher proportion of genetic variation located among populations. *C. sanguinea* in general

occurs in scattered and small populations in Denmark (Ødum, 1968). Seed dispersal by birds (Krüsi & Debussche, 1988) may generate long-distance gene flow across the landscape, but the observed level and distribution of genetic diversity in the present study point toward genetic drift rather than natural selection as important driver behind the observed population differentiation. The true difference between *Q. petraea* and *C. sanguinea* in level of genetic variation may be even higher, because the assumption of half-sib relationship within seed from single tree collection is likely to create a bias toward overestimation of V_A in the rose as discussed in M&M above. *M. sylvestris* is again a different case in Figure 2, with low level of genetic differentiation but also relatively low additive genetic variance. Pollinating insects have been reported to visit primarily trees in immediate vicinity, but long-distance pollination events are also likely to occur (Larsen & Kjær, 2009; Reim et al., 2015) and seeds moved by birds and deer that feed on the wild apples also create long-distance gene flow (Larsen & Kjær, 2009). Studies based on neutral SSR markers revealed low level of population differences within Denmark (F_{ST} = 0.03) (Larsen, Asmussen, Coart, Olrik, & Kjær, 2006), which is very close to the estimated Q_{ST} = 0.04 for flushing time in the present. Therefore, the observed variation among populations in *M. sylvestris* may have been generated by random processes without significant effects of divergent selection for flushing time.

The studied populations have in general maintained a high level of genetic variation and thereby possess a high ability to adapt to new conditions if exposed to natural selection in favor of—or against—early flushing. The question is how fast species can adapt under the anticipated rapid increase in temperature over the next 100 years, and how important such changes are for the fitness of

the species. In *B. pubescens*, the maximum difference in minimum temperature in March of the populations tested in this study was 2.4°C (Figure 3). Even with a conservative estimate of a generation time of 50 years (Hynynen et al., 2010), the results suggest that *B. pubescens* possesses the resilience at the population level to change phenology corresponding to the predicted increase in temperature. A similar conclusion can probably be drawn for *Q. petraea* also (Figure 3). For many of the other studied species, the generation time is possibly less than 20 years (e.g., *R. dumalis*, *M. sylvestris*, *C. avellana*, and *C. sanguinea*), and for these species, it therefore also seems reasonable to assume that populations can evolve in spring phenology with a speed that can match the predicted increase in temperature. The response will only be realized if early bud burst has substantial influence on fitness (survival and reproduction) or because of directional selection implemented in domestication programmes. The estimates in the present study refer to the latter situation, because the heritability was estimated in managed progeny test and the realized heritability in natural population may be substantially lower due to higher environmental and developmental heterogeneity. In Denmark, all major woody plant species are included in domestication programs based on breeding seed orchards in order to support effective and rapid selection for continued fitness (Kjær et al., 2009).

Our finding on genetic differences among geographically close populations (cf. Table 2) supports that selectively divergent phenotypes can be maintained even if there are options for gene flow among these populations as suggested by Aitken and Bemmels (2016). Kremer et al. (2012) argue that the positive effect of gene flow on adaptation of trees to climate change is larger than the negative effect due to the introduction of genetic variation in adaptive traits. Species are different with respect to their genetic differentiation within and among their populations as revealed in our study. Assisted migration for genetic enrichment can serve as a supplement to genetic management based on native populations; especially, the species associated with small population sizes and limited gene flow. Populations at the low latitudinal limit of the species range have in general maintained high biological diversity over time as shown for, for example, *Abies alba* (Bergmann, Gregorius, & Larsen, 1990; Larsen, 1986) and southern populations should therefore be considered as a source of gene pool for assisted migration of genotypes under the future climate predictions (Hampe & Petit, 2005). The populations in the present study are close to the northern distribution range of the species (except for *B. pubescens*) and genetic diversity may therefore be lower compared to populations closer to the refugial areas. But we still observed substantial level of genetic variation in the fitness-related trait. For species occurring in extremely small fragmented populations assisted migration at a more localized landscape level may still be desirable to counteract inbreeding within these populations due to random drift. Adaptive potential of a species is not only determined by the presence of genetic variation within and among populations as studied here, but also by the presence of phenotypic plasticity in adaptive traits (Nicotra et al., 2010). Final conclusions on the adaptive potential of

species to climate change will therefore also depend on the role of plasticity in local adaptation in the studied species.

DATA ACCESSIBILITY

Data for this study is available at University of Copenhagen—Electronic Research Data Archive (UCPH-ERDA).

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Albin Lobo was responsible for the design of study, analysis and interpretation of data, and writing of the manuscript. Jon Kehlet Hansen was responsible for supervision of the work, design of study, and writing and approving of manuscript. Lars Nørgaard Hansen was responsible for field data collection, and revising and approving the final version of the manuscript before submission. Erik Dahl Kjær was responsible for overall supervision of the study, conception and design of work, data interpretation, and writing and final approval of the manuscript.

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