Spring Phenology in Finnish Noble Hardwood Gene Conservation Collections

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Many noble hardwood species of boreal and temperate climate zones will experience increased selection pressure for adaptive traits as climate change proceeds. This will cause shifts in the timing of phenological events such as budburst in spring. Shifts in phenology may disrupt interaction between species and their environment as well as interaction within and between species. Changes in the timing of annual life cycle traits may threaten species’ current fitness and potential to adapt to future climates. The rate and magnitude of phenological shifts will increase along with increasing temperatures. To adapt to changing environmental conditions, species need to possess adaptive variation in life cycle traits. In small and isolated populations at northern margins of species distribution, variation in adaptive traits is threatened by low genetic variation, low gene flow and genetic drift. To preserve the adaptive potential of noble hardwood populations in Finland, *ex situ* gene conservation collections are established where genetic resources are conserved as living trees. To characterize the adaptive potential currently present in Finnish gene conservation collections of European maple (*A. platanoides* L.), small-leaved linden (*T. cordata* Mill.) and European white elm (*U. laevis* Pall.), the amount of current phenological diversity should be evaluated. For this purpose, budburst progress in the collections was tracked by phenotypic observations on budburst phenological phases in relation to accumulating temperature. The data did not allow for analysis of variance between origin populations; therefore, a more conservative approach was chosen where conserved genetic resources were assessed by examining budburst differentiation between groups of origin populations. The level of differentiation in budburst temperatures is used as an indicator of spring phenological diversity to determine if the conserved origins are adapted to the same spring temperature range. No significant differentiation was found in the timing of budburst between groups of origins. Therefore, the conserved origins are presumed to be adapted to the same spring temperature range within their respective species distribution in Finland. However, low phenological diversity may magnify the threats imposed to these species by climate change. Genetic studies are recommended for deeper understanding of underlying genetic diversity and adaptive potential of the stored genetic resources.

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Boreaalisen ja lauhkean ilmastovyöhykkeen jalolehtipuulajit kokevat lisääntynyttä sopeutumisominaisuuksien valintapainetta ilmastonmuutoksen edetessä. Tämä aiheuttaa muutoksia fenologisten tapahtumien kuten kevään silmunpuhkeamisen ajoituksessa. Fenologian muutokset voivat häiritä lajien ja niiden ympäristön välistä vuorovaikutusta sekä vuorovaikutusta lajien sisällä ja niiden välillä. Muutokset lajien vuotuisen elinkaaren ajoituksessa voivat uhata niiden nykyistä kelpoisuutta ja kykyä sopeutua tulevaan ilmastoon. Fenologisten siirtymien esiintyminen ja suuruusluokka lisääntyvät maapallon lämpötilan noustessa. Sopeutuakseen muuttuviin ympäristöolosuhteisiin lajeilla on oltava adaptiivista vaihtelua vuotuiseen elinkaareen liittyvissä ominaisuuksissa. Pienissä ja eristyneissä populaatioissa lajien levinneisyyden pohjoisilla reunoilla adaptiivisten ominaisuuksien vaihtelua uhkaa alhainen geneettinen vaihtelu, alhainen geenivirta ja geneettinen ajautuminen. Suomalaisten jalolehtipuupopulaatioiden sopeutumispotentiaalin säilyttämiseksi on perustettu geenivarakokoelmia joissa geenivaroja säilytetään elävissä puissa. Euroopan vaahteran (*A. platanoides* L.), metsälehmuksen (*T. cordata* Mill.) ja kynäjalavan (U. laevis Pall.) suomalaisten geenivarakokoelmien adaptiivisen potentiaalin hahmottamiseksi tulisi arvioida nykyisen fenologisen monimuotoisuuden määrää näissä kokoelmissa. Tätä varten, silmunpuhkeamista kokoelmissa seurattiin fenotyyppisillä havainnoilla silmunpuhkeamisen edistymisestä suhteessa kertyvään lämpösummaan. Aineiston perusteella ei voitu analysoida yksittäisten alkuperäpopulaatioiden välistä vaihtelua; Siksi valittiin konservatiivisempi lähestymistapa, jossa geenivarojen monimuotoisuutta arvioitiin tarkastelemalla alkuperäryhmien välistä silmunpuhkeamisen erilaistumista. Eroavaisuudet silmunpuhkeamisen lämpötiloissa toimivat kevätfenologisen monimuotoisuuden indikaattorina. Tarkoituksena on määrittää, ovatko säilytetyt alkuperät sopeutuneet samaan keväiseen lämpötilavaihteluun Suomessa. Silmunpuhkeamisen lämpötiloissa ei havaittu merkittävää eroa alkuperäryhmien välillä. Näin ollen kokoelman alkuperien oletetaan olevan sopeutuneet samaan keväiseen lämpötilavaihteluun niiden lajilevinneisyysalueella Suomessa. Vähäinen fenologinen vaihtelu voi kuitenkin lisätä ilmastonmuutoksen näille lajeille aiheuttamia paineita. Tutkittavien geenivarojen geneettisen monimuotoisuuden ja adaptiivisen potentiaalin syvemmäksi ymmärtämiseksi suositellaan geneettisiä tutkimuksia.

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1. INTRODUCTION

The study of phenology investigates the timing and expression of recurring life cycle events in relation to environmental factors (Schwartz 2013). Such as the annual migration of birds in response to the length of photoperiod, or leaf emergence and flowering of trees in response to accumulating temperatures in spring (Walther 2003, Mayor et al. 2017). Many phenological events in the annual life cycle of noble hardwood species in boreal and temperate climate zones are triggered by temperature cues. The timing of these events correlates with the annual temperature regime of the local climate (Hänninen & Kramer 2007) This synchrony between trait expression and climate is important for the successful completion of the annual life cycle and therefore important to individual as well as population fitness (Hänninen & Kramer 2007). In general, it is regarded that temperature is the key regulator of spring phenological events for forest tree species in boreal and temperate climate zones. Accumulating temperature in spring accelerates physiological processes such as cell division and development leading to budburst and growth onset (Sarvas 1972, 1974, Linkosalo et. al. 2006, Cooke et. al. 2012). In comparison to other traits, traits that regulate the timing of growth in forest trees show strongest signs of climatic adaptation (Savolainen et. al. 2007). Variation in the timing of annual life cycle traits, allows forest trees to respond to yearly fluctuation of climatic factors at their growing site while also providing adaptive potential in the face of climate change (Polgar and Primack 2011, Donnelly et. al. 2017). Phenological variation within populations increases the chances of adaptation to changing environmental conditions (Howe et al. 2003, Aitken et al. 2008). However, if a population becomes very specifically adapted to their local environment, it can lead to a loss in genetic diversity and adaptive potential in a changing environment (Savolainen et al. 2013). In field trials, locally adapted populations show higher fitness at their home site compared to non-local populations grown at the same site (Savolainen et al. 2007). Local adaptation can also be concluded from phenological clines as indicated by the correlation of phenotypic expression to an environmental gradient such as temperature (Savolainen et. al. 2007). Common garden experiments often exhibit a pattern where the timing of budburst correlates with temperatures at the natural location of the original population (Silvestro et. al. 2019, Guo et.

al. 2020, Thibault et. al. 2020). Southern genotypes typically require a greater heatsum in spring than northern genotypes to initiate growth. This is an indication of adaptive differences between populations to their local temperature conditions. (Silvestro et. al. 2019, Thibault et. al. 2020, Guo et. al. 2020)

As climate change progresses, increasing temperatures will alter species' timing of life cycle traits that respond to their seasonal temperature environment (Walther 2003). Phenological events happening early in the growing season, such as budburst, are expected to gradually take place earlier whereas late season events in autumn, although not as pronounced as the shifts in spring phenology, are expected to continuously happen later (Walther et al. 2002). This will lead to longer growing seasons for many tree species in the boreal climate zone (Hänninen & Tanino 2011). However, shifts in the timing of phenological events may disrupt interaction between species and their environment as well as interaction within and between species (Kharouba et al. 2018). The shifts in the timing of phenological traits may threaten the fitness and genetic diversity of species (Walther et al. 2002). The magnitude of phenological shifts will further grow as selection pressure toward adaptive traits increases along with temperature (Menzel 2006b). Under climate change, species must adapt their phenology to match changes in temperature conditions (Aitken et al. 2008). Adaptation to new conditions through genetic changes is possible if there is enough genetic variation in the population for selection to act on. Populations can also migrate toward more favorable conditions to track their trait optima. Phenotypic plasticity allows populations to respond to a changing environment without changes in distribution or genotypic composition (Aitken et al. 2008). However, the extent of phenotypic plasticity and range expansion is dependent on the amount of existing genetic variation (Savolainen et al. 2004, Alberto et al. 2013, Goldstein & Ehrenreich 2021). If these strategies fail, populations face a reduction in fitness that may lead to demographic declines and range contractions (Aitken et. al. 2008).

Noble hardwood species in Finland exist as small and isolated populations at the northern borders of species distribution (Rusanen et al. 2021). These distribution patterns are typically associated with low genetic variation, low gene flow, high chances of genetic drift and strong selection pressure (Savolainen et al. 2007, Kujala et al. 2017). Under climate change, these population characteristics may accentuate the threats imposed to the genetic diversity and adaptive potential of these species (Savolainen et al. 2007, Rusanen et al. 2012). A common practice for conserving genetic diversity and the adaptive potential of forest trees is the establishment of *ex situ* gene conservation collections. In these collections, genetic resources from natural populations are conserved as living trees grown at the same location (Rusanen et al. 2021). Reproductive material for conservation is collected from the natural distribution range of species in a way that can be expected to represent their natural diversity. These resources act as an important reservoir of forest genetic material that guards the diversity and adaptive potential of the conserved species (Rusanen et. al. 2021). Characterizing the current phenological diversity in Finnish gene conservation collections of European maple (*Acer platanoides L.)*, small-leaved linden (*Tilia cordata* P. Mill.) and European white elm (*Ulmus laevis* Pall.) is needed to achieve a representation of the adaptive potential of northern populations of these species as well as aid in decision making related to the conservation of these genetic resources. In this thesis, I attempt to determine whether the conserved origins are adapted to the same temperature range regarding budburst, or if their budburst phenology is differentiated within Finland. As the timing of budburst is based on adaptation to temperature conditions, trees originating from different sites should vary in the heat requirement preceding budburst if spring temperatures between sites varies enough to induce differentiation (Vitasse et. al. 2009, Liang 2019, Thibault et. al. 2020). However, in Finland, the populations of the studied species are inhabiting a rather narrow range in terms of annual temperatures and thus budburst differentiation is not expected between populations.

2. LITERATURE REVIEW

2.1 Spring phenology

2.1.1 Chilling- and heat requirement preceding budburst

During inactive periods of growth from late fall until early spring, noble hardwood species persist in a dormant state which is a mechanism that protects trees against unfavourable environmental conditions by inhibiting growth (Sarvas 1974). This inhibition allows trees to ignore minor environmental cues for growth onset and does not break until favourable conditions for growth persist for a longer time. This enables species to safely time the start of growth onset with minimal risks (Sarvas 1974). Growth inhibited by physiological restrictions is called endodormancy or rest during which trees do not grow despite the presence of favourable environmental cues. Growth inhibition by environmental restriction is called ecodormancy or quiescence during which tree growth is ready to continue in response to a favourable growth environment (Lang et al. 1987). Endodormancy is broken by filling the chilling requirement as enough time in a specific low-temperature range, typically between 0 °C and 5 °C. In addition to the chilling requirement, accumulation of time over a temperature threshold, referred to as the heat requirement, is needed to break ecodormancy. For tree species in general, temperatures above 5 °C are considered to contribute to filling the heat requirement (Sarvas 1972, 1974). The chilling requirement has been shown to influence the heat requirement so that the response to increasing temperatures in spring is dependent on the amount of chilling during dormancy (Heide 1993a, Heide 1993b). Longer exposure to chilling temperatures reduces the heat requirement and therefore the timing of budburst onset. However, this effect exists only until a certain point after which additional chilling has no reducing effect on the heat requirement. Tree species of the boreal zone typically require a relatively short chilling period (Heide 1993a, Heide 1993b). High temperatures in late summer and early autumn are shown to increase the depth of dormancy so that a greater chilling requirement needs to be met for growth inhibition to be released but also higher heat accumulation is needed in spring to initiate budburst (Heide 2003). Therefore, the temperature environment leading to and during dormancy also influences springtime phenology. Furthermore, a delayed budburst of one season may also affect budburst of the next season due to delayed effects resulting from the dependency of life cycle phases on each other (Hänninen & Tanino 2011). Between species' differences in chilling- and heat requirement temperatures are known to exist between late- and early flushing species (Fu et. al. 2015). Due to the differences in fulfilling the chilling and heat requirement, tree species respond differently to climate change induced changes (Hänninen & Tanino 2011). Responses to warming climate are expected to differ due to genetic differences within and between species as well as differences in local climate conditions. However, warming of spring temperatures is predicted to cause earlier budburst and growth onset in most boreal and temperate tree species (Hänninen & Tanino 2011). Early initiation of budburst creates an adaptive trade-off between utilization of the full potential of resources at the local site and avoidance of frost damage. While lengthening the growing season, early budburst also increases the chances of frost damage exposure (Saxe et al. 2001).

2.1.2 Frost damage

As a result of climatic warming, the threat of spring frost damage is expected to increase in tree species in boreal and temperate climate zones (Hänninen 1991). Tree organs exhibiting an early phenological response are more susceptible to frost injury than organs exhibiting later phenological development in the current climate (Linkosalo et. al. 2006). Triggering of ontogenetic development during ecodormancy in early spring, is a critical factor in exposure to frost damage as occasional frosts may still occur after growth has been initiated (Hänninen 2006). As mild temperatures in early spring become more frequent in the boreal zone due to climate warming, trees may fill their heat requirement while freezing temperatures still occur. Spring phenological events consistently take place earlier in spring and thus species are more frequently subjected to occasional periods of freezing temperatures (Hänninen 1991). Once growth has initiated, the trees are vulnerable to freezing temperatures as the protective mechanisms of dormancy are no longer in use (Hänninen 1991). Since the earlier studies on the subject, it has been debated whether climate change induced risk of frost damage is a threat in temperate and boreal regions. Hänninen (2006) emphasized the need for more species-specific experiments and that whether frost damage risks are pronounced due to climate change or not, remains open to interpretation. Some studies suggest that while the threats may not be as pronounced as first proposed, the risks of frost damage still exist (Saxe et. al. 2001).

2.2 Adaptation and phenology under climate change

2.2.1 Phenological shifts and mismatches

Phenological patterns within species typically follow climatic clines where population responses to climate correlate with the gradient of the environmental factor such as temperature. For example, northern tree populations typically burst buds later in the spring than southern populations. Also, northern populations are adapted to shorter growing seasons than populations in warmer areas (Mimura & Aitken 2007). Strong phenological clines in relation to budburst and temperature are well known in broadleaf tree genera such as *Populus* (Keller et. al. 2010) and *Quercus* (Alberto et. al. 2011). Increasing temperatures brought on by climate change are constantly advancing the timing of spring phenological events (Walther et al. 2002). Budburst has been reported to have advanced for numerous forest tree species in boreal and temperate climate zones (Linkosalo 1999). Modelling of spring phenology under climate change is predicting further lengthening of growing seasons resulting from spring phenological events continuously taking place earlier. The advance in spring phenology and thus lengthening of the growing season, are largely attributable to increased temperatures due to climatic warming (Parmesan & Yohe 2003, Menzel et. al. 2006a, Menzel et. al. 2006b). Historical records show that spring phenology in Europe has advanced on average by 6 days within the last 30 years and length of the growing season has increased by 10.8 days from 1960-1990 (Menzel & Fabian 1999) However, Linkosalo et al. (2009) note that the rate of warming in Finland is roughly twice the average rate observed in Europe and found spring events to have advanced by 3.3 – 11 days per century for boreal species (Linkosalo et al. 2009). The contradiction between these estimates could rise from the difference in the length of time series. The approximated rate of advancement also varies according to studied species, location and on the years of observations (Linkosalo et al. 2009). With climate change, global mean temperatures are expected to further increase 0.6 – 1.4 °C within the next 30 years depending on the depicted scenario of climate change (IPCC 2021). Jing et al. (2021) predict a further advance in budburst of boreal and temperate species of 10 – 15 days with a 2°C increase in temperature. They also noted that boreal and early flushing species would advance their budburst more than temperate or late flushing species (Jing et al. 2021). The increasing temperatures may also disrupt the synchronic timing of phenological events between and within many species. Correct timing of phenological events uphold the synchrony of life cycle traits within and between species (Walther et. al. 2002). Asynchrony in life cycle traits within and between species may disrupt local ecosystem dynamics by disturbing food webs and ecological niches of species (Kharouba et al. 2018). Mismatches in phenological patterns can be expected to increase as species respond differently to the changes in climate (Ovaskainen et. al. 2013, Kharouba et. al. 2018). Asynchrony will increase between interacting species that share mutual dependency over the timing of life cycle traits and respond to environmental cues at different sensitivities such as the response to temperature (Kharouba et al. 2018). For interacting species that time life cycle traits to different cues entirely, such as photoperiod and temperature, the advance in asynchrony is likely greater (Mayor et al. 2017). An example of this is the timing of leaf emergence and arrival of migratory birds. Migratory birds often respond to photoperiod to time their migration, a constant phenological cue, whereas the timing of leaf out undergoes annual variation and is directionally shifting with increasing temperature (Mayor et al. 2017). As temperature cues are happening earlier, the food provided by hatching of insects that is in synchrony with leaf emergence, may not provide sufficient nutrition for the birds that arrive later in relation to the timing of leaf emergence (Mayor et al. 2017). Hence, the timing of these life cycle traits no longer matches between the interacting species. Such events will likely have cascading effects on ecosystem communities (Kharouba et al.2018). As the timing of phenological cues no longer matches, the life cycle of mutual species involved in such networks becomes compromised (Kharouba et al. 2018). Shifts in phenological patterns of species may also lead to mismatches with the abiotic environment such as optimal growing conditions and resource availability, potentially reducing growth and reproductive success (Kharouba et al. 2018). Competition for resources such as light, water, and nutrients can also intensify as the climate warms. Resource availability can be further challenged if competitive species that are better adapted to warmer conditions expand northward into the territories of current species (Aitken et al. 2008). Climate change can also affect the distribution and behavior of pests and pathogens introducing new pest pressures or shifts in the timing of pest life cycles (Sampaio et al. 2016).

2.2.2 Challenges in adaptation

As climate change proceeds, species with high population differentiation in adaptive traits, will be subjected to strong selection (Savolainen et al. 2004). As the adaptive response to climate change largely depends on existing genetic variation, the proportion of genotypes that already possess adaptive potential for warmer climates could be expected to increase (Rehfeldt 2002, Savolainen et al. 2004). However, this could happen at the expense of genetic diversity and thus reduce adaptive potential rising from other genotypes such as those that exhibit later budburst to avoid frost damage (Bradshaw & McNeilly 1991, Saxe et al. 2001). High amounts of genetic variation, however, does not necessarily mean that species or populations will adapt to changed environmental conditions or that the rate of adaptation can keep up with the rate of increasing temperatures (Bürger & Lynch 1995, Savolainen et al. 2004). According to Bürger and Lynch (1995) the ability of populations to track their phenotypic optimum will always be imperfect no matter the level of genetic variation and that the rate of adaptive evolution will always lag from the rate of environmental change. A study on silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.) (Billington & Pelham 1991) examining the amount of genetic variation in relation to budburst timing revealed that even populations with high amounts of heritable variation for this trait would be unable to keep up the rate of adaptation with the expected increases in temperature. Similar results are reported for other adaptive traits. Savolainen et. al. (2004) examined genetic variation in relation to budset and frost hardiness of Scots pine (*Pinus sylvestris* L.) and similarly found that even in populations that exhibited high genetic variation, the rate of adaptation rising from these traits could not match the pace of warming temperatures (Savolainen et al.2004). As boundaries for the distribution of these species shift toward higher latitudes, populations currently at the northern margins could be expected to inhabit new territories that previously were unsuitable for the species (Rehfeldt et. al. 2002, Solarik et. al. 2018). However, range expansion and adaptation to the current temperature environment might be a short-lived solution to climate change and does not guarantee adaptation in the long run (Jump & Penuelas 2005). Furthermore, species cannot successfully migrate without adapting to the new conditions (Savolainen et. al. 2004). Therefore, even if temperature allows for range expansion, adaptation then depends on the diversity of traits that face a novel environment. Iverson et. al. (2008) applied a simulation model based on seed dispersal of North American tree species and revealed small chances for establishment beyond 20 km from the current habitat within a generation. Furthermore, should tree species be able to keep up with the rate of climate change, colonization would have to happen in a successive series of long distance colonization events (Iverson et al. 2008) Under colonization however, initial populations are likely very small which further threatens genetic diversity due to a bottleneck effect. Small size of initial populations may also result in high chances of inbreeding and low seed production further threatening the adaptive potential of the founding population (Joyce & Rehfeldt 2013).

2.3 Local adaptation and adaptive potential

2.3.1 Small and isolated populations

The adaptive potential of a population determines the capability of that population to adapt to changing environmental conditions in response to natural selection. The adaptive potential of a population depends on the amount of existing genetic variation and the efficiency to distribute this variation within the population through dispersal and establishment of new individuals (Alberto et al. 2013). Adaptive potential is usually high in populations that exhibit large effective population sizes and high amounts of genetic variation. Species with large population sizes can typically respond faster to natural selection than smaller populations due to larger genetic variance within populations (Alberto et al. 2013). This potential enables species to eventually evolve traits that enhance their fitness in specific environments ultimately leading to local adaptation if natural selection is strong (Savolainen et al. 2007). Large effective population sizes also show stronger signs of local adaptation than populations with small effective sizes (Savolainen et al. 2007). Locally adapted populations have evolved traits that allow maximum use of resources at their specific home environment and have higher fitness at their home site than non-local populations. Conversely, they exhibit lower fitness outside their home site. (Savolainen et al. 2007). For local adaptation to develop selection for adaptive traits needs to be strong and genotypes conferring an advantage in the current environment must have ecological space for establishment (Alberto et. al. 2013). Small populations with a fragmented distribution are especially susceptible to strong directional selection (Tóth et al. 2019). Local adaptation to the current environment however, may narrow genetic variation within populations (Savolainen et al. 2011). As certain genotypes are favored over others, the variation in traits is decreased leading to lower genetic diversity and less genetic variation for selection to act on in the future (Rehfeldt et al. 2002, Tóth et al. 2019). This tradeoff between the amount of adaptive variation and level of specialisation aims to optimize the balance in the amount of genetic variation that is enough to uphold adaptive potential while allowing a sufficient level of specialisation to bring forth a fitness advantage in the current local environment (Hereford 2009).

Local adaptation and adaptive potential may be hindered in isolated populations with small effective sizes due to low genetic variance within populations rising from pronounced effects of genetic drift and lack of gene flow (Savolainen et. al. 2007). More precisely, genetic drift increases the possibility that adaptive alleles are lost from the population and maladaptive alleles become fixed (Savolainen et al. 2004). Whereas gene flow can restore and uphold genetic variation by replenishing lost alleles or introducing alleles that were not previously present in the population (Savolainen et al.2007). The rate of local adaptation is slowed by high gene flow (Lenormand 2002) and genetic drift (Blanquart et. al. 2012) whereas it is enhanced by strong selection (Savolainen et. al. 2013). Adaptive potential on the other hand, is also reduced by genetic drift but is increased by high geneflow whereas strong selection decreases it (Alberto et al. 2013). The balance between gene flow and selection largely governs the level of local adaptation. If selection is very intense, it can lead to a decline in genetic variation (Savolainen et. al. 2007). If there is too much gene flow, allelic swamping might overwhelm selection and hinder local adaptation (Lenormand 2002). However, if gene flow is low, selection for well-adapted genotypes may be hindered as the introduction of new genetic variation to populations is restricted (Davis & Shaw 2001). In small and isolated populations gene flow is often limited (Savolainen et al. 2007) and the chances of genetic drift are high (Savolainen 2011). Such populations are also more prone to inbreeding depression (Young et. al. 1996). Therefore, the ability to respond to climatic changes and the chances for local adaptation in small and isolated populations are especially dependent on the amount of existing genetic variation within populations (Savolainen et. al. 2004, Savolainen et al. 2007).

2.3.2 Populations at the leading edge of distribution

Evolutionary forces shaping genetic variation at distribution borders typically include asymmetric gene flow from central populations to peripheral populations (Savolainen et. al. 2011) and strong directional selection due to harsher environments (Hurme et. al. 1997). Populations located at northern margins of species distribution likely experience stronger selection as harsher temperature conditions in the north increase selection pressure of adaptive traits (Kujala et. al. 2017). Studies on forest tree species indicate marginal populations to exhibit lower genetic diversity but higher genetic differentiation compared to more central populations. In other words, variation within populations tends to be smaller than variation between populations (Tollesfrud et. al. 2016, Graignic et. al. 2017, Tóth et. al. 2019). Keller et. al. (2010) found that the genetic diversity of balsam poplar decreases from central ranges of distribution toward range margins. Similar results are reported for other broadleaf species such as sugar maple (*Acer saccharum* Marshall) (Graignic et. al. 2017) and European ash (*Fraxinus excelsior* L.)(Tollesfrud et. al. 2016). Kujala et. al. (2017) suggested that large genetic variances in scots pine (*Pinus sylvestris* L.) can indicate weak selection pressure, and therefore northern populations would show less genetic variation than central populations as having a maladaptive genotype in the harsher northern conditions would likely have more drastic consequences than in the south. Therefore, genetic variation could be reduced in more northern populations due to stronger selection (Kujala et al. 2017). While local adaptation is typically evident throughout the main range of species distribution, it is known that local adaptation diminishes toward distribution borders (Savolainen et. al. 2007). Billington & Pelham (1991) and Davis & Shaw (2001) emphasized the importance of gene flow from populations inhabiting warmer areas toward northern populations to allow establishment of genotypes adapted to higher temperatures. However, Holliday et. al. (2012) noted that marginal isolated populations may benefit from the lack of gene flow from central populations as allele frequencies contributing to adaptive variation are not diluted by gene flow from central populations.

3. RESEARCH OBJECTIVES

The main objective of this thesis is to discover the amount and pattern of spring phenological diversity in Finnish noble hardwood gene conservation collections. Work done under this master's thesis is related to the objectives set by the Genetic Resources Program for Forest Trees implemented by National Resources Institute Finland (Luke) to characterize existing gene conservation collections in Finland. The obtained results are intended to aid in decision making related to conservation matters of these species and to act as background work for further evaluation of the conserved genetic resources.

Budburst differentiation was assessed in *ex situ* gene conservation collections of European maple, small-leaved linden, and European white elm in southwestern Finland to evaluate spring phenological diversity within these species. In the absence of significant variation, the origins within species are non-differentiated in budburst phenology. Whereas differentiation between origins would indicate adaptation to varying local conditions within Finland and a possible trade-off between avoidance of frost damage and the advantage provided by an early season start to growth. However, the differences in annual heatsum between origin locations are rather small. Therefore, the overall thermal range is likely too small to induce differentiation in budburst timing between origins. This thesis hypothesizes that there is no differentiation within species in the temperature required for budburst phenological phases. Therefore, the genetic resources of each respective species conserved in these collections are presumed to be adapted to the same spring temperature range within their distribution ranges in Finland. Because trees in gene conservation collections are grown in common environmental conditions at a single site, variation rising solely from environmental factors is mitigated (Luquez et al. 2008). Therefore, variation observed in budburst is considered as an indication of variation rising from genetic differences and differences in the interaction effect between genes and the environment (Luquez et. al. 2008, Thibault et. al. 2020).

4. MATERIALS AND METHODS

4.1 Gene conservation collections of noble hardwood species

Gene conservation collections of noble hardwoods examined in this thesis are part of genetic resource collections of forest trees maintained by the National Resources Institute Finland (Luke). The *ex-situ* collections for European maple (*Acer platanoides* L.), small-leaved linden (*Tilia cordata* Mill.) and European white elm (*Ulmus laevis* Pall.) observed in this study are located in the boreal climate zone. The collection site is in southwestern Finland, at latitude 60.46°, longitude 22.75° in Preitilä, Paimio. Annual heatsum at the location is ~ 1300 day degrees (dd). The day degree values reported for the collection site and the locations of origin are based on mean annual temperature accumulation between years 1961-1990 (Finnish Meteorological institute, FMI). Day degrees (dd) are calculated over a time period as the amount of accumulating temperature degrees (°C) that exceed a daily average of 5 °C. Trees in the collections are growing in natural soil and areas are maintained regularly by culling invasive species and by field management to maintain homogenous soil and water conditions. Collections of European maple and small-leaved linden were established in 1998 whereas the collection of European white elm was established in 2001. The collections have been expanded since establishment by increasing the number of origins. European maple in 1999, 2000, 2002. Small-leaved linden in 1999, 2000, 2002, 2003, 2004 and European white-elm in 2002 and 2007. Collections have been restocked to make up for perished material by replenishing the number of trees for some origins. The collection for small-leaved linden has been restocked in 2006 and European white-elm in 2006 and 2007 (Personal correspondence: Leena Yrjänä, Finnish National Resources Institute, Luke).

At the time of establishment, trees of European maple were originally planted in growing squares to accommodate 10 trees belonging to the same half-sib family. However, since planting, the number of trees per square has decreased and at the time of observations made for this thesis the number of trees varied between 1 to 10 per square. Trees of small-leaved linden and European white elm are planted as single trees in individual squares. Square sizes are 5 x 7 m for all species. Forest reproductive material chosen for establishment of each collection is gathered from geographical ranges that represent the natural distribution of these species in Finland (Rusanen et. al. 2021) (Figure 1). In this geographical frame, the annual heatsums of origin locations range between ~1180-1360 dd for European maple, ~1210-1350 dd for European white elm and between ~1050-1360 dd for small-leaved linden.

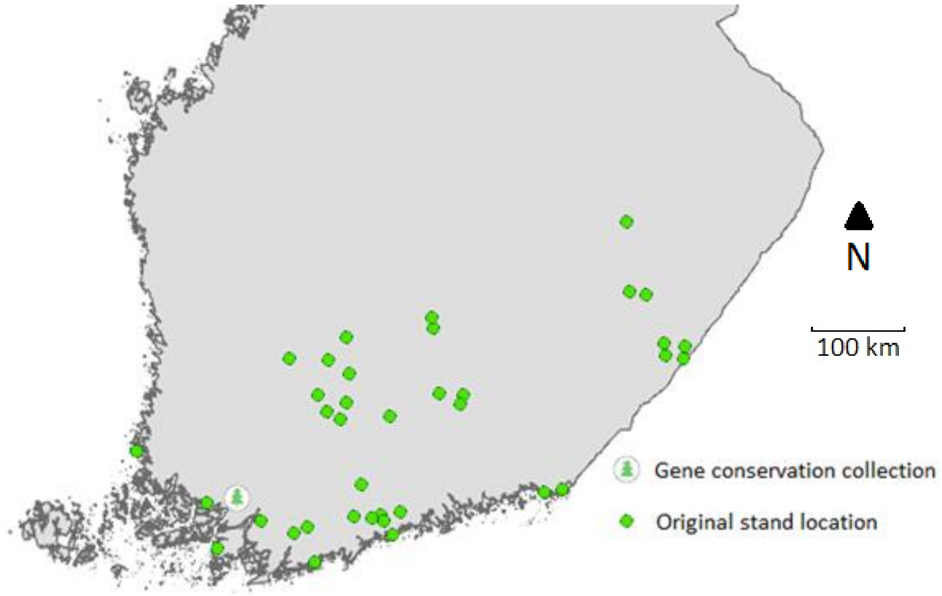

Figure 1A. Location of origins and the gene conservation collection of European maple.

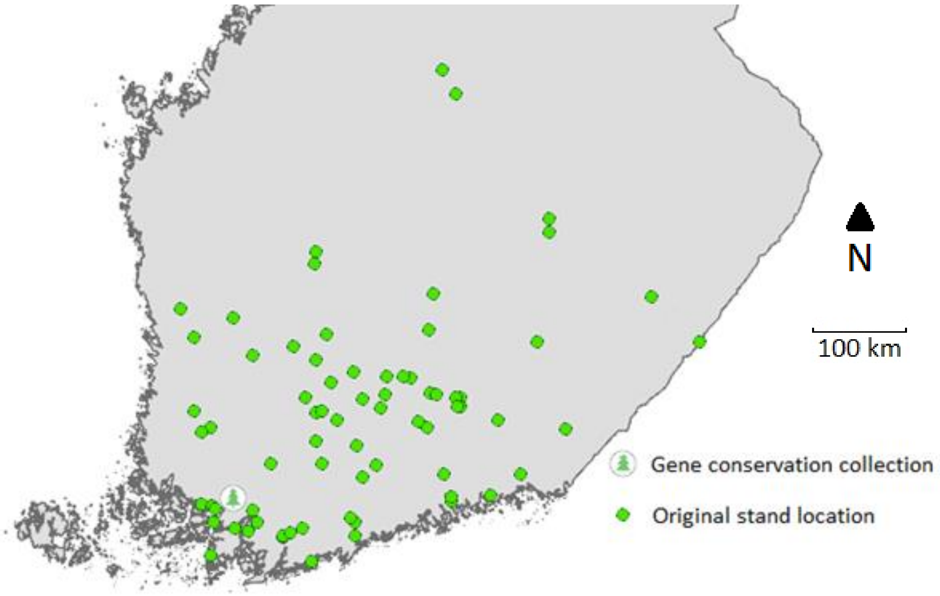

Figure 1B. Location of origins and the gene conservation collection of small-leaved linden.

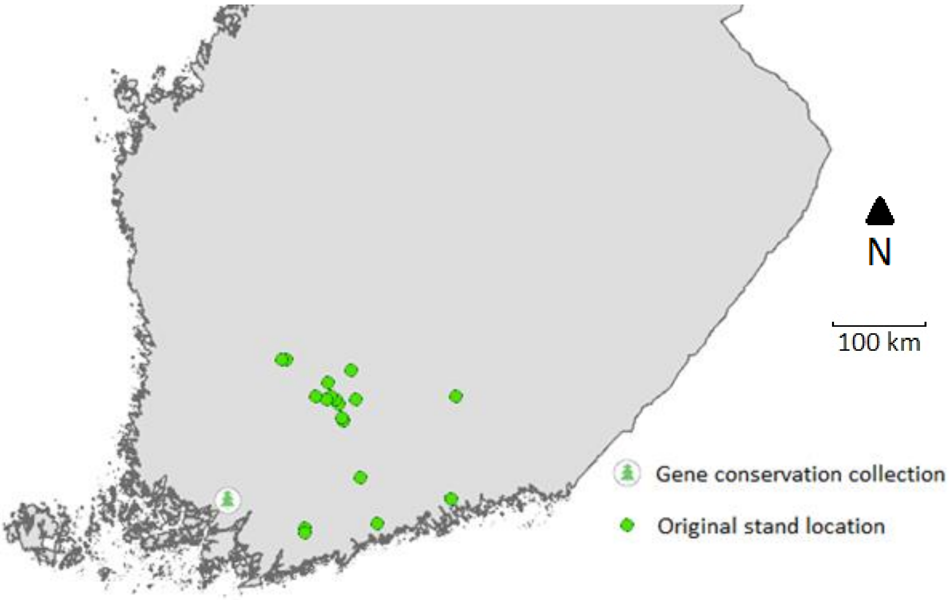

Figure 1C. Location of origins and the gene conservation collection of European white elm.

The gene conservation collection of European maple is composed of half-sib families. These half-sib families consist of trees that have been grown from seed collected from the same mother tree at the natural origin location. Due to open pollination, individuals within half-sib families have the same maternal genes whereas the rest of their genetic composition may vary. The gene conservation collections of small-leaved linden and European white elm are composed of clones. Trees of the same clone are considered genetically identical. Individuals of small-leaved linden and European white elm have been grafted onto common rootstock to better accommodate growth in the collection. In this thesis, half-sib families and clones are also referred to as subgroups. Because conserved resources at the collection originate from a representative range of the species natural distribution in Finland, observed variation in budburst phenology is considered to represent spring phenological diversity of the species in Finland.

4.2 Phenotypic observations on spring phenology

Data used for this thesis was gathered in spring 2021 for all species at the collection site (Table 1).

Table 1. Composition of gathered data from noble hardwood gene conservation collections. Subgroups of European maple are half-sib families whereas subgroups of small-leaved linden and European white elm are clones.

Species	Origins	Subgroups (total)	Trees (total)	Subgroups within single origin (range)	Trees within single subgroup (range)
European maple	32	143	428	1-10	2-43
Small-leaved linden	77	256	320	1-9	1-14
European white elm	19	118	218	1-11	2-39

For European maple, many trees were left out of observations due to observation difficulties of very tall trees. Phenotypic observations on budburst phenology of these trees could not be made in a uniform manner with the rest of the collection. The same reason applied for trees of European maple that were under 150 cm in height. These individuals were left out of observations as the growth habit of small trees tended to exhibit a morphology with only a few branches and an elongated main branch. This meant that 284 trees of European maple were not included in the observations. For small-leaved linden and European white elm all trees that exhibited budburst were included. Therefore, two individuals of European white elm from a single origin were left out from observations. One individual of small-leaved linden was left out due to stunted growth and diseased appearance.

Progress of budburst was tracked by observing the phase of budburst and accumulated temperature sum (dd) for every observation date. For this purpose, budburst phenological phases were classified for each species into classes 1-5 (Figure 4). Phase 1 represents the dormant state before budburst is initiated. Accumulating temperature sum (dd) was calculated onwards from the first day of April 2021.

Figure 4A. European maple budburst phases: (1) buds are compact and dormant, (2) buds are swollen and folded, (3) protective bud scales begin to unfold and leaf tips begin to show, (4) petiole has emerged, and leaf tips are visible but folded, (5) leaf is not fully grown but is unfolded so that "bottom" of leaf blade is visible.

Figure 4B. Small-leaved linden budburst phases: (1) buds are compact and dormant, (2) buds are swollen and folded, (3) protective bud scales begin to unfold and leaf tips begin to show, (4) first leaves are emerging and appear as rosettes, (5) petioles and whole leaves are visible but not fully grown.

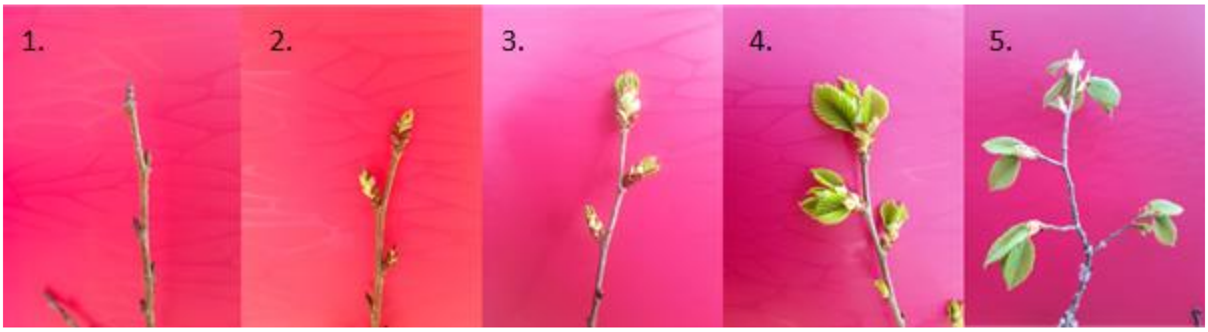

Figure 4C. European white elm budburst phases: (1) buds are compact and dormant, (2) buds are swollen, and scales have retracted, (3) leaf tips are visible, (4) leaves are visible and unfolding, (5) petiole is visible, first leaves are not fully grown but unfolded so that the "base" of the leaf blade is visible.

Temperature data was obtained from the Finnish Meteorological Institute (FMI). Temperature information is based on kriging interpolation (Venäläinen et. al. 2005). Temperature data for the year 2021 was obtained using a 1 km x 1 km grid. The annual temperature sum at the collection site, and at origin locations are based on a 10 km x 10 km grid. The latter are based on yearly averages between 1961-1990 to account for the age of the mother trees and their environment at the time of establishment as a 1km x 1km scale was not available prior to 1990. Observations were made daily once budburst had started by evaluating the phase of budburst on majority of buds at mid-height of each tree leaving out apical buds and lowest branches. Observations were carried out from the start of the growing season when signs of budburst started to appear until the day that every tree included in the observations for each collection had reached the final phase of budburst.

4.3 Statistical analysis

Origins were arranged into three or four groups according to the range of annual heatsum at origin locations for the given species (Table 2). To assess differentiation in budburst between groups, average day degree values for reaching each budburst phase (2-5) were used to perform analysis of variance (ANOVA) and post-hoc test based on least significant difference (LSD). At phase 1 buds are in a dormant state and therefore this phase was left out of analysis.

Table 2. Grouping of origins according to annual heatsum at natural origin locations. EM = European maple, SL = small-leaved linden, EW = European white elm. Subgroups within origins of European maple are half-sib families whereas subgroups within origins of small-leaved linden and European white elm are clones.

Species	Natural range (dd, annual)	Group	Group range (dd, annual)	Origins	Subgroups	Trees
EM	1177 - 1357	1	<1250	11	62	210
		2	1250 - 1300	8	45	128
		3	>1300	13	36	90
SL	1044 - 1357	1	<1200	10	19	22
		2	1200 - 1250	26	102	128
		3	1250 - 1300	16	49	64
		4	>1300	25	86	106
EW	1214 - 1348	1	1200 - 1250	6	47	93
		2	1250 - 1300	9	55	102
		3	1300 - 1350	4	16	23

ANOVA and LSD post-hoc tests were performed by SPSS statistical package (IBM SPSS Statistics for Windows, v. 27, IBM Corp., NY, USA). In the case of non-normally distributed parameters, Box-Cox transformation was utilized to meet assumptions of normality before running analyses (Sakia 1992).

5. RESULTS

A comparison between groups is shown in table 4. For European maple and small-leaved linden there was no significant difference in the temperature sum required for reaching any of the budburst phases. For small-leaved linden phase 2, the comparison between groups was not possible due to almost complete lack of variation and thus the values could not be transformed to meet assumptions of normality and would not meet the requirements for analysis of variance. The only significant difference found between groups was for European white elm to reach phase 2. Post hoc test based on least significant difference (LSD) revealed group 3 to significantly differ ($p<0.05$) from groups 1 and 2. Origins within group 3 initiated budburst earlier than groups 1 and 2. The average temperature sum for group 3 to reach phase 2 was only 6 and 9 dd less compared to that of groups 1 and 2 respectively.

Table 4. For each species, the mean temperature sum (dd) for reaching budburst phases and analysis of variance (ANOVA) between origin groups. Significant differences between groups are marked by an asterisk.

Species			Phase 2	Phase 3	Phase 4	Phase 5
European maple						
	Mean temperature sum (dd)					
		Group 1	20	57	78	100
		Group 2	19	58	78	101
		Group 3	18	57	78	99
	ANOVA					
		F statistic	0,768	0,044	0,011	0,263
		Sig.	0,473	0,957	0,989	0,77
Small-leaved linden						
	Mean temperature sum (dd)					
		Group 1	20	92	109	121
		Group 2	20	90	110	121
		Group 3	16	86	107	117

		Group 4	19	88	106	117
	ANOVA					
		F statistic		0,532	0,608	0,727
		Sig.		0,662	0,612	0,539
European white elm						
	Mean temperature sum (dd)					
		Group 1	26	47	70	90
		Group 2	29	49	71	91
		Group 3	20	49	69	88
	ANOVA					
		F statistic	3,847	0,179	0,054	0,302
		Sig.	0,043*	0,838	0,947	0,744

6. DISCUSSION

6.1 Origin populations

The ideal approach to assess the phenological diversity of these collections would have been to observe within and between population variation in budburst temperatures of individual origins. However, the large differences in the number of subgroups and the number of trees between origins would make statistical approaches unreliable. As such, the data did not allow for analysis of variation between origins. To avoid statistical uncertainty rising from unbalanced data, a rather conservative approach was chosen where budburst differentiation was assessed between groups of origins instead of individual origins. The expected uncertainty for small-leaved linden and European white elm may be less pronounced due to subgroups being composed of clones. Whereas half-sib families of European maple are expected to possess larger genetic variation. Furthermore, grouping by local temperature instead of geographical location at such a small scale, can mean that origins within some groups may be geographically closer to origins of another group than origins within the group that they belong to. Thus, gene flow could be shared between origins belonging to different groups. Furthermore, some origins may be geographically more isolated than others. The differences in the level of geographic isolation and gene flow between origins could diminish the distinctness of defined research groups. Some of the natural origin populations could be subpopulations of a metapopulation spanning across defined research group borders. This could mask variation between groups and act as a confounding factor. However, even though not ideal, this way of grouping seemed like the best option statistically as grouping by geography or analyzing variation between individual origins would have unbalanced the setting further and produced more limitations for the options of analysis. Furthermore, as this thesis examines pre-established gene conservation collections, there was no possibility to affect the composition of genotypes or design of the experimental setting.

6.2 Budburst differentiation among populations of noble hardwood species

Population differentiation studies on budburst phenology of noble hardwood species are few for populations located on the leading edge of distribution. However, Lobo et. al (2018) found a high degree of phenological variation between fragmented populations of small-leaved linden in Denmark in the timing of budburst in a common garden experiment. Furthermore, when populations were grouped geographically, eco-groups were found to be significantly differentiated in budburst temperatures. Lobo et. al (2018) conclude the differences in budburst of eco-groups to be an indication of adaptive differentiation due to temperature rather than geographical distance. In fact, differentiation by environment is often more effective for inducing local adaptation than differentiation by geographical distance (Sexton et al. 2013, Tiffin & Ross-Ibarra 2014). Likewise, Rungis & Krivmane (2021) report moderate levels of genetic differentiation between Latvian populations of small-leaved linden. Based on these studies, budburst differentiation could have been expected also for the populations of small-leaved linden studied in this thesis. However, it is known that population differentiation and local adaptation of populations can decrease toward the leading edge of distribution (Savolainen et al. 2007).

Most studies on budburst phenology of these noble hardwood species in northern climates emphasize the lack of differentiation for this trait. Eriksson et al. (2003) found unexpectedly low variation in budburst of European maple populations from Norway and Sweden despite genetic differentiation shown by allozyme studies. The study utilized two trial locations hosting populations from different northern climates. A coastal population from Ås, Norway, an inland population from Lillehammer, Norway, and a population from the Öland island in Sweden. At the southern trial location in Årslev, Denmark, the Öland population showed slight differentiation from the Norwegian populations in budburst. However, at the northern trial location in Ås, Norway, no differences were observed in budburst between the populations likewise to the findings in this thesis (Eriksson et. al 2003). Similar results are also reported for European white elm. Whiteley et al. (2003) examined phenological differentiation of budburst in a field trial between populations of European white elm and found no significant differentiation between the northernmost populations despite being separated by large geographical distance and originating from contrasting environments. The other source population is located on the southeast coast of Sweden on the Baltic Sea on Öland island. While the other source population is located several hundred kilometers inland in Moscow Russia. Whiteley et al. (2003) concluded the variability in budburst of European white elm to be low among and within the studied populations which would support the results obtained in this thesis. Whiteley et al. (2003) noted however that late spring in the trial site compared to the natural population locations could reduce observed variation. This could be due to phenotypic plasticity or genotype x environment interaction. The same could also be concluded from the findings of Eriksson et al. 2003 where budburst differentiation was not observed at the northern trial location despite slight levels of differentiation found in the southern trial location.

6.3 Budburst phenology in Finnish gene conservation collections

Spring phenological diversity of the studied species seems to be low as no significant differentiation was observed in any budburst phase for any of the species in this thesis apart from one exception. Phase 2 of European white elm showed group 3 to be significantly different from groups 1 and 2. Overall, the differences between groups for reaching any of the phenological phases were small and all of them were under 10 dd. For context, the mean temperature in May in southwestern Finland between the years 1991 – 2021 was 10 °C (FMI). Thus, on an average day in May the heatsum would accumulate by 5 dd and even the largest observed difference in a single phenological phase (9 dd) could be filled within two average temperature days. Also, the origins within group 3 come from the warmest areas of the species distribution in Finland and reached phase 2 earlier than the other groups. This is contradictory to the well documented phenomenon that populations from warmer regions typically exhibit budburst at a higher heatsum than populations from colder regions (Silvestro et. al. 2019, Thibault et. al. 2020, Guo et. al. 2020). Furthermore, the lack of differentiation in later phenological phases would also support the conclusion that, even though statistically significant, the very small difference most likely is not of adaptive relevance.

The groups of origins in this thesis show no differentiation in the timing of budburst when grown at the collection site. Most likely because the thermal range within the distribution of these species in Finland is not large enough to induce divergent selection pressure that would bring forth differentiation in budburst (Kawecki & Ebert 2004). Therefore, these populations are not phenologically differentiated in budburst temperatures at this thermal scale as was hypothesized. Based on the obtained results, the groups of origins seem to be adapted to the same spring temperature range within species respective distribution ranges in Finland. Despite low phenotypic variation, the species likely harbour some level of genetic variation related to budburst as it is a quantitative trait. In the absence of local adaptation, phenotypic plasticity could mask small genetic differences in certain traits between origins when grown in the same environment (Villellas et al. 2020). In fact, it is reported that populations of forest trees firstly respond to environmental pressure by phenotypic plasticity (Valladares et al. 2014, Mata et al. 2022) and that this is especially relevant for temperature related traits such as budburst (Aitken & Whitlock 2013). Therefore, the effects of phenotypic plasticity could mask the effects of underlying genetic variation on budburst in this study. Due to their sessile nature, forest trees can typically tolerate a wide range of climatic conditions and exhibit high phenotypic plasticity (Alberto et al. 2013). Furthermore, it is known that the initial response to climate change in forest trees is by phenotypic plasticity (Savolainen et al. 2011). Studying adaptation under climate change is challenged by the difficulty of differentiating the effects of local adaptation and phenotypic plasticity due to the lack of genetic evidence (Savolainen et al. 2013). Timing of spring phenology in the studied origins could therefore be influenced by phenotypic plasticity that allows genotypes to deviate from their phenotypic optimum.

6.4 Implications for natural populations

Limited phenological variation can reduce a species' ability to adapt to changing temperature conditions (Wolkovich et al. 2017). Selection toward adaptation may be hindered due to low variation of adaptive alleles in the population. Over time, this can lead to a loss of genetic diversity within populations, making them more vulnerable to environmental changes (Savolainen et al. 2007). Climate change may require northern populations of noble hardwoods to shift their ranges northward to track suitable conditions. However, low phenological variation may induce challenges in dispersal and establishment in new areas, which can result in range contractions and population declines (Aitken et al. 2008, Joyce & Rehfeldt 2013). Also, adaptation through natural selection will be more challenging for populations with low variation in adaptive traits (Savolainen et al. 2007) However, as more southern populations expand northward, genetic diversity could increase in northern regions for these species (Holliday et al. 2012, Solarik et al. 2018). This could increase adaptive potential for future climates but may reduce local adaptation and fitness in the current northern climate. However, Jing et al. (2009) predict that spring phenology between temperate and boreal zones is not likely to converge which would exert more adaptive pressure for these noble hardwood species at the leading edge of species distribution.

6.5 Conclusions

The major shortcoming of a single site study is that the underlying genetic effects on phenotypic expression cannot be separated from the interaction effect between genes and the environment. Replicated genetic studies over multiple sites could give more insight into the genetic diversity underlying the adaptive potential of these species under climate change. However, the conserved genetic resources can be expected to represent natural phenological diversity of these species in Finland, and thus safeguard their current adaptive potential for a warming climate. As the conserved origins within species are adapted to the same spring temperature range in Finland, their genetic resources could be utilized for different purposes covering the distribution areas of respective species. Such as, forest restoration or assisted gene flow to uphold the diversity and adaptive potential of natural populations and possibly alleviate climate change induced pressure on adaptation.

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