ACCLIMATION AND MIGRATION POTENTIAL OF A BOREAL FOREST TREE, BALSAM POPLAR (*POPULUS BALSAMIFERA* L.) IN A CHANGING CLIMATE

A

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Abstract

In the North American boreal forest, 21st century climate change is projected to result in longer growing seasons, increased forest productivity, and northward expansions or shifts in species ranges. These projected impacts are largely based on observations across natural temperature gradients, e.g., latitude or altitude, or correlations between current species' distributions and modern climate envelopes. These approaches, although valuable, do not consider biological capacities important in a species' ability to cope with novel environments through physiological or phenological acclimation. Within a single species, adaptation to local environments may cause some populations to respond differently to climate change than others. Acclimation (phenotypic plasticity) is often treated as a separate phenomenon from local adaptation, but the latter may determine the range of acclimation responses or thresholds. To more accurately predict how boreal tree species will respond to a directionally changing climate, it is necessary to experimentally examine the effects of warming on the growth and physiology of individual species and how those effects differ across a species' range.

This research investigated how tree growth responses to increasing temperature are influenced by differences in adaptation and acclimation across the latitudinal range of the North American boreal forest tree, *Populus balsamifera* L. (balsam poplar). Warming experiments, both in the greenhouse and in the field, indicated that growth of balsam poplar trees from a broad latitudinal gradient responds positively to increased growing temperatures, with increases in height growth ranging from 27-69 % in response to 3-8 °C average warming. Genotypes from southern populations grew consistently taller in

both field and greenhouse experiments. The field experiment enabled investigation into the effects of warming and source latitude on balsam poplar phenology; both experimentally warmed and southern individuals grew larger and exhibited longer growing seasons (more days of active growth). Lastly, I describe a theoretical/methodological framework for exploring the role of epigenetics in acclimation (plasticity) and adaptation to changing environments. The results from these experiments are integrated with information on adaptive gradients in balsam poplar to predict both the *in situ* responses of balsam poplar to increased temperatures, and the potential for northward range shifts in the species.

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Chapter 1

General Introduction

The boreal forest is the northernmost forested ecosystem and is one of the most extensive biomes in the world. It contains approximately one-third of global carbon stocks (McGuire et al. 2010) and impacts of global climate change in this region will have wide reaching implications (Chapin et al. 2010). In North America, the boreal forest covers over 600 million hectares of Canada and Alaska. The boreal zone is characterized by cold winters and short summers, and climate extremes are typical, with growing season temperatures ranging from below freezing to 30 °C (Hinzman et al. 2006). Much of the boreal forest is underlain with discontinuous permafrost which plays a large role in determining species composition, and nutrient and hydrological cycles (Hinzman et al. 2006). Cold air and soil temperatures are important abiotic factors that influence boreal species' range limits (Van Cleve et al. 1991, Lin et al. 2010).

The northwest boreal forests of Alaska and Canada have experienced a mean annual temperature increase of 1.4 °C during the past 100 years, twice the global average of 0.8 °C (Wendler and Shulski 2009). This change has resulted in an increase in the potential growing season , as measured as average consecutive days above freezing, in the northwest boreal forest by 45-50% in the last 50 - 100 years (Juday et al. 2005, Wendler and Shulski 2009). This recent warming trend has resulted in increased forest productivity, earlier spring green up, and later fall browning, as observed from remotely-sensed imagery (Nemani et al. 2003, Kimball et al. 2006, Robin et al. 2008). However,

recent studies have also shown a negative trend in productivity, presumably due to an increase in temperature-induced drought stress (Goetz et al. 2005, McGuire et al. 2010, Beck et al. 2011), but also possibly due to differing resolutions of datasets and whether or not they capture greening trends following wildfires (Alcaraz-Segura et al. 2010). These trends vary regionally, however, and there are indications that not all boreal tree taxa are responding positively to higher temperatures (Barber et al. 2000, Wilmking et al. 2004). Downscaled climate models project an additional 3-7 °C warming for this region by the end of the 21st century (Walsh et al. 2008). This warming is widely expected to result in continued lengthening of growing seasons, increased productivity, and northward expansions or shifts in species ranges tracking their thermal niches (Rupp et al. 2001, Euskirchen et al. 2006, Euskirchen et al. 2009).

Projections of temperature effects on tree growth and phenology are largely based on observations collected across large temperature gradients, such as latitude and altitude. Relationships of growth or phenology to temperature are based on empirical physiological or phenological measurements and current, site-based temperature regimes. These landscape-scale correlative efforts, substituting space for time, have formed the basis of our understanding of forest-climate dynamics. Likewise, species distribution modeling uses correlations among species presence-absence data and current climate envelopes to generalize a species' fundamental niche (Pearson and Dawson 2003). These models assume that climate is the primary factor determining species' ranges; by creating maps of future projected climate niches, species distribution models provide hypotheses of future distributions based on climate suitability (Pearson and Dawson 2003).

These approaches have been extremely valuable in generating hypotheses of climate change impacts on forest ecosystems; however, they lack biologically-relevant information such as interactions between adaptation to local climates or photoperiods and responses to increased temperature (Reinhardt et al. 2011). It is necessary to combine species-specific mechanistic information (i.e., physiological, microevolutionary) with the above-mentioned techniques to identify vulnerability and inform conservation practices in a changing climate (Dawson et al. 2011). For example, boreal tree species often exhibit strong latitudinal clines in phenology and growth in response to local photoperiod/temperature regimes (Aitken et al. 2008). These clines are likely a result of adaptive strategies that balance the tradeoff between maximizing the number of days of active growth versus avoidance of cold injury by properly timing spring and fall phenology (Loehle 1998, Saxe et al. 2001, Green 2005). Adaptation to a northern photoperiod (local adaptation to short growing seasons) can potentially limit northern genotypes from taking advantage of increasing growing-season lengths in high-latitude environments. This could greatly affect the overall growth and carbon sequestration of northern forests and could result in differences between projected and realized temperature-induced increases in forest productivity. Similarly, local adaptation to photoperiod, which does not change with climate, could inhibit the northward migration of southern genotypes by creating a phenological mismatch; southern genotypes that set bud when day lengths shorten to 12-13 hours would still be growing when the expected first frost occurs at northern latitudes. Southern genotypes may grow better in northern latitudes at warmer temperatures, yet it is unclear whether northward migration will be

limited by or benefit from latitudinal differences in local adaptation under future climate scenarios.

The types of latitudinal clines described above have formed relatively recently, since the Last Glacial Maximum 18 - 21 k years ago, and have presumably reformed repeatedly with Pleistocene glacial oscillations (Davis and Shaw 2001, Petit et al. 2004, Levsen et al. 2012). Although fossil evidence suggests that tree species were mainly tracking their climatic niches across landscapes (McLachlan et al. 2005), the importance of adaptation associated with large-scale migration may be underestimated (Olson et al. in press). For example, during the course of transcontinental migrations, species would have been exposed to drastically differing photoperiod regimes. The magnitude and rate of change of projected future climate warming in boreal latitudes, however, may outpace the rate of adaptation necessary for trees to persist *in situ* or migrate across latitude (Davis et al. 2005, Savolainen et al. 2011).

As long-lived, sedentary organisms, trees generally display high levels of phenotypic plasticity and thus are likely to acclimate to changing conditions long enough for adaptation to novel environments to occur (Hamrick 2004), even if fitness is temporarily reduced. This could result in an adaptational lag (Aitken et al. 2008) in that local populations are no longer the best suited for local environments. In the example of increasing temperature effects on boreal-forest trees, northern populations with conservative growth strategies may be limited in their capacity to respond to increasing temperatures, due to short growing seasons, and would require either adaptation to the new photoperiod/temperature regime in northern populations or migration from 'pre-

adapted' southern genotypes or alleles into northern environments. In another example, temperatures may surpass the optimum for physiological processes in northern genotypes, and growth may decline. Some species will be able to acclimate to the new temperatures, allowing for time for adaptation to occur, whereas other species may reach upper temperature thresholds and become locally extinct (Pelini et al. 2009).

To better understand the influences of local adaptation in the temperature-acclimation responses of boreal-forest trees, it is necessary to test the effects of increasing temperature on genotypes sampled from large latitudinal gradients in both field and greenhouse experiments (Aitken et al. 2008). Here I describe a framework for investigating how tree growth responses to warming are influenced by differences across a latitudinal gradient in the northwest boreal forests of North America. My candidate species, balsam poplar (*Populus balsamifera* L.), and its sister species black cottonwood (*P. trichocarpa* Torr & A. Gray), have a long history of common garden experiments that demonstrate local adaptation to photoperiodic/temperature regimes for phenological timing, particularly growth cessation and bud set (Pauley and Perry 1954, Howe et al. 1996, Soolanayakanahally et al. 2012, Olson et al. in press), and local adaptation results in a strong latitudinal cline in phenology and growth related to decreasing growing season length with increasing latitude.

Black cottonwood is the model organism for tree genomics and physiology (Tuskan et al. 2006), which has facilitated quantitative and association genetic analyses into the important traits and genes associated with this adaptive latitudinal cline in balsam poplar. Components of the *CONSTANS/FLOWERING TERMINAL* regulon, which in

Arabidopsis controls the phenology of growth and flowering and is influenced by both photoperiod and temperature (Koornneef et al. 1991), have been identified as being important in bud flush and bud set in balsam poplar (Keller et al. 2012, Olson et al. in press). Historical demographic studies in balsam poplar, using both genetic and niche modeling techniques, predict that balsam poplar populations were displaced from their current locations into the Rocky Mountain region of the United States during the Last Glacial Maximum (Levsen et al. 2012). Recolonization of Canada and Alaska occurred since the Last Glacial Maximum within the last 10-15k years (Breen et al. 2012, Keller et al. 2012, Levsen et al. 2012). Although fossil evidence indicates that a Beringian population of balsam poplar may have been present at the height of the last ice age, these populations were likely overwhelmed by migration from the south (Breen et al. 2012). Thus, the current cline in phenology is likely to have been lost and re-formed several times during the glacial periods of the Pleistocene.

The clonal growth habit of *Populus* has been an important facet of research in this genus as it allows for genotypic replication among experimental treatments. For studies described in this dissertation, cuttings were collected from across the North American distribution of balsam poplar as part of the Agriculture Canada Balsam Poplar (AgCanBaP) collection of the Agriculture and Agrifood Canada, Agroforestry Division (Fig. 1.1); this collection was supplemented with cuttings collected from Alaskan populations in Galena, Nome and Cottonwood (Olson et al. in press). Replicate common gardens have been planted in Indian Head, Saskatchewan, Vancouver, British Columbia, and Fairbanks, Alaska. Combined, these gardens provide information concerning the

roles of adaptation and acclimation near the northern and southern extremes of the species' range (Keller et al. 2011, Soolanayakanahally et al. 2012, Olson et al. in press). Cuttings taken from these gardens have provided the material for experiments on how source environment impacts physiological and morphological traits in balsam poplar (Soolanayakanahally et al. 2009, Silim et al. 2010). Moreover, all of these experiments are using the same genotypes, which allows for broad-scale comparison across environmental gradients and experimental treatments.

In Chapter 2 of this dissertation, I describe the results from an experimental warming experiment in which balsam poplar trees from a latitudinal transect in the species' western range were grown in growth chambers under two temperature regimes. Identical genotypes were placed in growth chambers of different temperatures so phenotypic differences between treatments should be due to warming effects, not random differences in genetic composition. In the high-temperature growth chamber, trees were grown at 29/19 °C (day/night) temperatures, based on upper-end estimates of warming projected for the northwest boreal forest (IPCC 2007, Walsh et al. 2008). The low-temperature growth chamber was set to 21/9 °C, temperatures based on 40-year climate normals in Fairbanks, Alaska, USA (http://akclimate.org/Climate/Normals/index.html). This experiment was designed to examine the effects of increased temperature on the growth and physiology of balsam poplar trees from across the latitudinal gradient. The use of growth chambers allowed for the control of the magnitude of warming while holding other environmental variables constant (e.g., soil type, soil moisture, nutrients).

Chapter 3 presents results from a passive warming experiment in a common-garden setting in Fairbanks, Alaska, using many of the same genotypes included in the growth chamber study. This experiment was conducted in a more natural setting but the magnitude of the warming treatment was less, and because we used passive warming, the temperature treatment varied both diurnally and seasonally. This experiment had the additional environmental component natural changes in photoperiod throughout the season. This work tested the effects of warming and source environment on growth, physiology, and phenology of balsam poplar trees collected from a latitudinal transect in the northwest boreal forest.

In Chapter 4, I present a theoretical discussion of the role of epigenetics (meiotically and mitotically stable alterations in gene expression that are not based on DNA sequence changes) in plant adaptation and phenotypic plasticity. It has been suggested that epigenetics, through effects on seedling growth, phenology and cold tolerance, can inflate estimations of population differentiation and possibly play a role in the adaptive response of boreal trees to climate change (Aitken et al. 2008). Studies in Norway spruce, *Picea abies* (L.) H. Karst, have shown correlations between epigenetic markers and phenotypic change in the timing of bud set and cold hardiness (Kvaalen and Johnsen 2008). In a changing climate, epigenetic mechanisms may allow greater amplitudes of phenotypic plasticity, or increase the acclimation capacity of sedentary organisms such as trees, but landscape-scale information on patterns in DNA methylation in natural populations is lacking. To assess if DNA methylation plays a role in tree acclimation to climate, it is necessary to compare within- and among-population

methylation variation from across the species' range. Moreover, methylation may play a role in the phenotypic responses of trees to increased ambient temperature. My warming experiments provide an opportunity to compare methylation profiles between identical genotypes growing under two different temperature regimes. A detailed laboratory protocol for examining the potential role of one epigenetic mechanism, DNA methylation, on population differentiation and temperature responses in balsam poplar is described in Appendix 1.

Chapter 5 presents a summary and integration of the research presented in this dissertation. The primary objectives of this dissertation were to 1) examine the effects of temperature warming on growth, cold tolerance, physiological processes, and phenology in balsam poplar, or to test for a plastic response in balsam poplar phenotype to temperature and length of growing season increases; 2) examine how those temperature responses vary across a latitudinal transect, or to test for genetic differences among populations, and 3) determine if adaptation to local environments influences the ability of balsam poplar to acclimate *in situ* or migrate by looking for a genotype by plasticity interaction.

References

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications **1**:95-111.

Alcaraz-Segura, D., E. Chuvieco, H. E. Epstein, E. S. Kasischke, and A. Trishchenko. 2010. Debating the greening vs. browning of the North American boreal forest: differences between satellite datasets. Global Change Biology **16**:760-770.

Barber, V. A., G. P. Juday, and B. P. Finney. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature **405**:668-673.

Beck, P. S. A., G. P. Juday, C. Alix, V. A. Barber, S. E. Winslow, E. E. Sousa, P. Heiser, J. D. Herriges, and S. J. Goetz. 2011. Changes in forest productivity across Alaska consistent with biome shift. Ecology Letters **14**:373-379.

Breen, A. L., D. F. Murray, and M. S. Olson. 2012. Genetic consequences of glacial survival: the late Quaternary history of balsam poplar (*Populus balsamifera* L.) in North America. Journal of Biogeography **39**:918-928.

Chapin, F. S., A. D. McGuire, R. W. Ruess, T. N. Hollingsworth, M. C. Mack, J. F. Johnstone, E. S. Kasischke, E. S. Euskirchen, J. B. Jones, M. T. Jorgenson, K. Kielland, G. P. Kofinas, M. R. Turetsky, J. Yarie, A. H. Lloyd, and D. L. Taylor. 2010. Resilience of Alaska's boreal forest to climatic change. This article is one of a selection of papers from The Dynamics of Change in Alaska's Boreal Forests: Resilience and Vulnerability in Response to Climate Warming. Canadian Journal of Forest Research **40**:1360-1370.

Davis, M. B. and R. G. Shaw. 2001. Range shifts and adaptive responses to Quaternary climate change. Science **292**:673-679.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology **86**:1704-1714.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. Science **332**:53-58.

Euskirchen, E. S., A. D. McGuire, F. S. Chapin, S. Yi, and C. C. Thompson. 2009. Changes in vegetation in northern Alaska under scenarios of climate change, 2003–2100: implications for climate feedbacks. Ecological Applications **19**:1022-1043.

Euskirchen, E. S., A. D. McGuire, D. W. Kicklighter, Q. Zhuang, J. S. Clein, R. J. Dargaville, D. G. Dye, J. S. Kimball, K. C. McDonald, J. M. Melillo, V. E. Romanovsky, and N. V. Smith. 2006. Importance of recent shifts in soil thermal dynamics on growing season length, productivity, and carbon sequestration in terrestrial high-latitude ecosystems. Global Change Biology **12**:731-750.

Goetz, S. J., A. G. Bunn, G. J. Fiske, and R. A. Houghton. 2005. Satellite-observed photosynthetic trends across boreal North America associated with climate and fire disturbance. Proceedings of the National Academy of Sciences of the United States of America **102**:13521-13525.

Green, D. S. 2005. Adaptive strategies in seedlings of three co-occurring, ecologically distinct northern coniferous tree species across an elevational gradient. Canadian Journal of Forest Research **35**:910-917.

Hamrick, J. L. 2004. Response of forest trees to global environmental changes. Forest Ecology and Management **197**:323-335.

Hinzman, L. D., L. A. Viereck, P. C. Adams, V. E. Romanovsky, and K. Yoshikawa. 2006. Climate and Permafrost Dynamics of the Alaskan Boreal Forest. Pages 39-61 *in* F. S. Chapin III, M. W. Oswood, K. V. Cleve, L. A. Viereck, and D. L. Verbyla, editors. Alaska's Changing Boreal Forest. Oxford University Press, New York.

Howe, G. T., G. Gardner, W. P. Hackett, and G. R. Furnier. 1996. Phytochrome control of short-day-induced bud set in black cottonwood. Physiologia Plantarum **97**:95-103.

IPCC. 2007. Climate Change 2007: The Physical Science Basis: Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *IN* S. Soloman, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Cambridge University Press, Cambridge, UK.

Juday, G. P., V. Barber, P. Duffy, H. W. Linderholm, S. R. Rupp, S. Sparrow, E. Vaganov, and J. Yarie. 2005. Forest, Land Management, and Agriculture. Pages 782-854 *in* C. Symon, L. Arris, and B. Heal, editors. Arctic Climate Impact Assessment. Cambridge University Press, New York.

Keller, S. R., N. Levsen, M. S. Olson, and P. Tiffin. 2012. Local adaptation in the flowering time gene network of balsam poplar, *Populus balsamifera* L. Molecular Biology and Evolution.

Keller, S. R., R. Y. Soolanayakanahally, R. D. Guy, S. N. Silim, M. S. Olson, and P. Tiffin. 2011. Climate-driven local adaptation of ecophysiology and phenology in balsam poplar, *Populus balsamifera* L. (Salicaceae). American Journal of Botany **98**:99-108.

Kimball, J. S., K. C. McDonald, and M. Zhao. 2006. Spring Thaw and Its Effect on Terrestrial Vegetation Productivity in the Western Arctic Observed from Satellite Microwave and Optical Remote Sensing. Earth Interactions **10**:1-22.

Koornneef, M., C. J. Hanhart, and J. H. Veen. 1991. A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. Molecular and General Genetics MGG **229**:57-66.

Kvaalen, H. and Ø. Johnsen. 2008. Timing of bud set in Picea abies is regulated by a memory of temperature during zygotic and somatic embryogenesis. New Phytologist **177**:49-59.

Levsen, N. D., P. Tiffin, and M. S. Olson. 2012. Pleistocene Speciation in the Genus *Populus* (Salicaceae). Systematic Biology.

Lin, D., J. Xia, and S. Wan. 2010. Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. New Phytologist **188**:187-198.

Loehle, C. 1998. Height growth rate tradeoffs determine northern and southern range limits for trees. Journal of Biogeography **25**:735-742.

McGuire, A. D., R. W. Ruess, A. Lloyd, J. Yarie, J. S. Clein, and G. P. Juday. 2010. Vulnerability of white spruce tree growth in interior Alaska in response to climate variability: dendrochronological, demographic, and experimental perspectives. Canadian Journal of Forest Research **40**:1197-1209.

McLachlan, J. S., J. S. Clark, and P. S. Manos. 2005. Molecular indicators of tree migration capacity under rapid climate change Ecology **86**:2088-2098.

Nemani, R. R., C. D. Keeling, H. Hashimoto, W. M. Jolly, S. C. Piper, C. J. Tucker, R. B. Myneni, and S. W. Running. 2003. Climate-Driven Increases in Global Terrestrial Net Primary Production from 1982 to 1999. Science **300**:1560-1563.

Olson, M. S., N. Levsen, R. Y. Soolanayakanahally, R. D. Guy, W. R. Schroeder, S. R. Keller, and P. Tiffin. in press. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology.

Pauley, S. S. and T. O. Perry. 1954. Ecotypic variation of the photoperiodic response in *Populus*. Journal of Arnold Arboretum **35**:167-188.

Pearson, R. G. and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? Global Ecology and Biogeography **12**:361-371.

Pelini, S. L., J. D. K. Dzurisin, K. M. Prior, C. M. Williams, T. D. Marsico, B. J. Sinclair, and J. J. Hellmann. 2009. Translocation experiments with butterflies reveal limits to enhancement of poleward populations under climate change. Proceedings of the National Academy of Sciences **106**:11160-11165.

Petit, R. J., R. Bialozyt, P. Garnier-Géré, and A. Hampe. 2004. Ecology and genetics of tree invasions: from recent introductions to Quaternary migrations. Forest Ecology and Management **197**:117-137.

Reinhardt, K., C. Castanha, M. J. Germino, and L. M. Kueppers. 2011. Ecophysiological variation in two provenances of *Pinus flexilis* seedlings across an elevation gradient from forest to alpine. Tree Physiology **31**:615-625.

Robin, J., R. Dubayah, E. Sparrow, and E. Levine. 2008. Monitoring start of season in Alaska with GLOBE, AVHRR, and MODIS data. J. Geophys. Res. **113**:G01017.

Rupp, T. S., F. S. Chapin, and A. Starfield. 2001. Modeling the Influence of Topographic Barriers on Treeline Advance at the Forest-Tundra Ecotone in Northwestern Alaska. Climatic Change **48**:399-416.

Savolainen, O., S. T. Kujala, C. Sokol, T. Pyhäjärvi, K. Avia, T. Knürr, K. Kärkkäinen, and S. Hicks. 2011. Adaptive Potential of Northernmost Tree Populations to Climate Change, with Emphasis on Scots Pine (Pinus sylvestris L.). Journal of Heredity **102**:526-536.

Saxe, H., M. G. R. Cannell, Ø. Johnsen, M. G. Ryan, and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. New Phytologist **149**:369-399.

Silim, S., N. Ryan, and D. Kubien. 2010. Temperature responses of photosynthesis and respiration in *Populus balsamifera* L.: acclimation versus adaptation. Photosynthesis Research **104**:19-30.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, E. C. Drewes, and W. R. Schroeder. 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment **32**:1821-1832.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, and M. Song. 2012. Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment:no-no.

Tuskan, G. A. and S. DiFazio and S. Jansson and J. Bohlmann and I. Grigoriev and U. Hellsten and N. Putnam and S. Ralph and S. Rombauts and A. Salamov and J. Schein and L. Sterck and A. Aerts and R. R. Bhalerao and R. P. Bhalerao and D. Blaudez and W. Boerjan and A. Brun and A. Brunner and V. Busov and M. Campbell and J. Carlson and M. Chalot and J. Chapman and G.-L. Chen and D. Cooper and P. M. Coutinho and J. Couturier and S. Covert and Q. Cronk and R. Cunningham and J. Davis and S. Degroeve and A. Déjardin and C. dePamphilis and J. Detter and B. Dirks and I. Dubchak and S. Duplessis and J. Ehlting and B. Ellis and K. Gendler and D. Goodstein and M. Gribskov and J. Grimwood and A. Groover and L. Gunter and B. Hamberger and B. Heinze and Y. Helariutta and B. Henrissat and D. Holligan and R. Holt and W. Huang and N. Islam-Faridi and S. Jones and M. Jones-Rhoades and R. Jorgensen and C. Joshi and J. Kangasjärvi and J. Karlsson and C. Kelleher and R. Kirkpatrick and M. Kirst and A. Kohler and U. Kalluri and F. Larimer and J. Leebens-Mack and J.-C. Leplé and P. Locascio and Y. Lou and S. Lucas and F. Martin and B. Montanini and C. Napoli and D. R. Nelson and C. Nelson and K. Nieminen and O. Nilsson and V. Pereda and G. Peter and R. Philippe and G. Pilate and A. Poliakov and J. Razumovskaya and P. Richardson and C. Rinaldi and K. Ritland and P. Rouzé and D. Ryaboy and J. Schmutz and J. Schrader and B. Segerman and H. Shin and A. Siddiqui and F. Sterky and A. Terry and C.-J. Tsai and E. Uberbacher and P. Unneberg and J. Vahala and K. Wall and S. Wessler and G. Yang and T. Yin and C. Douglas and M. Marra and G. Sandberg and Y. Van de Peer and D. Rokhsar. 2006. The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray). Science **313**:1596-1604.

Van Cleve, K., F. S. C. III, C. T. Dyrness, and L. A. Viereck. 1991. Element cycling in taiga forests: State-factor control. BioScience **41**:78-88.

Walsh, J. E., W. L. Chapman, V. Romanovsky, J. H. Christensen, and M. Stendel. 2008. Global climate model performance over Alaska and Greenland. Journal of Climatology **21**:6156-6174.

Wendler, G. and M. Shulski. 2009. A century of climate change for Fairbanks, Alaska. Arctic **62**:295-300.

Wilmking, M., G. P. Juday, V. A. Barber, and H. S. J. Zald. 2004. Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds. Global Change Biology **10**:1724-1736.

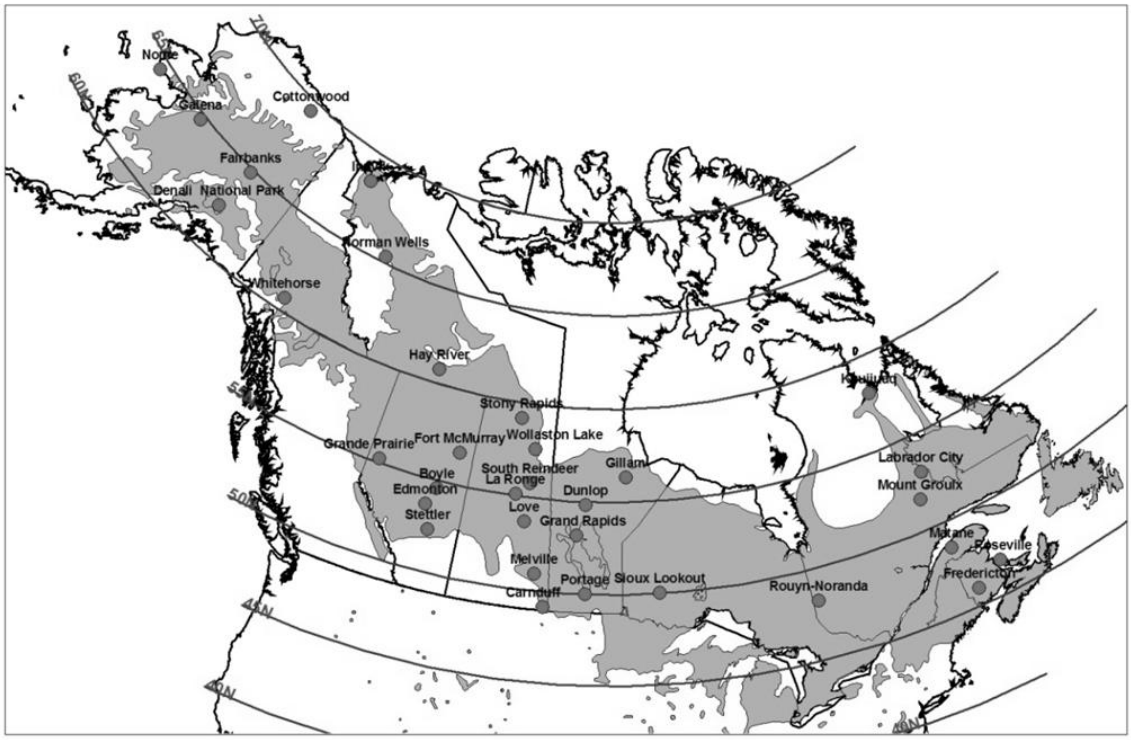

Figure 1.1 The locations of the populations sampled for inclusion in the Agriculture Canada Balsam Poplar collection, with additional populations (Cottonwood, Galena, Nome) collected for the experiment described in Olson et al. (in press). The distribution of balsam poplar (*Populus balsamifera* L.) is shown in gray.

Chapter 2

Latitudinal variation in growth responses to experimental warming in the boreal forest tree, balsam poplar (*Populus balsamifera* L.)[1]

Abstract

The effects of projected 21st Century climate change on boreal tree growth may differ across a species' range due to genetic and environmental variation. Experimental manipulations are necessary for a mechanistic understanding of how the interactions between genotype and plasticity contribute to patterns in growth responses in response to warming within in boreal tree species. Balsam poplar (*Populus balsamifera* L.) trees from across a latitudinal transect were grown in growth chambers under two temperature regimes: high temperature, 29/19 °C (day/night), and low temperature, 21/9 °C. As a result of experimental warming, high-temperature trees grew 27% taller relative to low temperature trees, but diameter increment was 32% lower for warmed trees. Plastic responses of tree growth to increased ambient temperatures were consistent in all populations across the latitudinal gradient; however, genotypes from southern source populations grew consistently taller than northern counterparts. This indicates that intraspecific variation across a species' range is an important determinant of growth responses to temperature. Photosynthetic rate per unit leaf area was lower in the high-temperature treatment in genotypes from all populations, even though an increase in stomatal index showed a temperature-acclimation response to increased growth temperatures. Cold tolerance, as measured by electrolyte leakage, was higher in

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genotypes from the north regardless of growth temperature, but also exhibited plasticity with temperature; warmed trees were on average less cold tolerant than those grown in lower ambient temperatures. There were no effects of warming or source environment on foliar nitrogen concentration. Balsam poplar trees from across the species' latitudinal range respond positively to increased temperature, in terms of height growth, indicating that plasticity to growth temperature is a generalized response. Genotypic differences are also an important determinant of both growth and cold tolerance at both experimental temperatures, suggesting that adaptation to local climates influences growth patterns in balsam poplar.

Introduction

The boreal forest is the northernmost forested biome, and in North America it covers over 600 million hectares in Alaska and Canada. Boreal species' distributional limits are often determined by temperature. There is a strong correlation between mean January temperatures and boreal tree distributions (Saxe et al. 2001), and cool growing-season temperatures at high latitudes are a critical factor in limiting plant growth (Lin et al. 2010). Projected 21st Century climate warming is widely expected to increase productivity across the boreal biome (Rupp et al. 2001, Reich and Oleksyn 2008, Beck et al. 2011) and result in northward expansion of species' ranges (Hamann and Wang 2006, McKenney et al. 2007). But increases in productivity and growth may not be consistent among plant functional types, e.g., coniferous versus deciduous taxa (Way and Oren 2010); or within species (Saxe et al. 2001, Wilmking et al. 2004). Across a species'

range, there may be differing biological capacity to respond to increases in temperature as a result of adaptation to local environments (Aitken et al. 2008), which is rarely considered in efforts to predict species' responses to climate change (Dawson et al. 2011). For example, populations near their northern range limits are often growing below their thermal optima and thus are expected to increase biomass accumulation in response to higher ambient temperatures (Grace et al. 2002, Danby and Hik 2007, Ghannoum and Way 2011). In some species, however, high-latitude populations may be unable to take advantage of increased temperatures due to genetically-determined short growing seasons, or investment in physiological, phenological, or structural cold-tolerance traits that come at a cost to growth (Way and Oren 2010). In this case, adaptation to northern environments influences the amplitude of potential acclimation responses to increased growth temperature. Thus, a species' ability to respond *in situ* to increased growing temperature is a function of both phenotypic plasticity (acclimation potential) and genotype. In order to better predict how boreal tree species will respond to climate warming, it is necessary to understand the roles of within-species genetic and plastic variation in growth responses to temperature.

Although temperature effects on tree growth clearly involve interactions with other abiotic and biotic factors, such as nutrient and water availability and associations with mycorrhizal fungi, studying direct effects of temperature on tree growth is useful for developing a mechanistic perspective of species responses to climate change. At a basic level, higher temperatures increase the rate of enzymatic activity until an upper-temperature threshold is reached, thus rates of physiological processes, such as

photosynthesis and respiration, often increase with modest warming (Saxe et al. 2001). Photosynthetic capacity often increases with latitude, (Körner 1989, Guy and Gornall 2007, Soolanayakanahally et al. 2009) despite thermal optima for high-latitude plants being over 10 °C lower than temperate counterparts (Lambers et al. 1998). This may be explained by the general trend of increasing foliar nitrogen concentration with latitude (Chapin and Shaver 1985, Reich and Oleksyn 2004) as a function of leaf life span and either leaf thickness and/or decreasing area (Körner 1989, Reich et al. 1992). If soil nutrient availability and uptake is equal, both thicker leaves and smaller leaves typical of cold environments, would accumulate higher foliar nitrogen concentration per unit mass or per unit area than conspecifics at lower latitudes (Reich et al. 1998, Lambers et al. 1998). Optimum temperatures for photosynthesis are generally close to the growing temperatures of local climates, and higher temperatures often result in short-term declines in photosynthetic rates, although acclimation is also common (Lambers et al. 1998, Sage and Kubien 2008). Differences in photosynthetic acclimation capacity for cool-adapted populations (Ow et al. 2008) may contribute to different growth responses across a species' range (Way and Sage 2008b, Silim et al. 2010).

At the structural level, growth responses to temperature can include shifts from below-ground to above-ground biomass allocation, increased height relative to diameter growth, and increased leaf production (Tjoelker et al. 1998, Day et al. 2005, Way and Oren 2010). When genotypes are transferred into warmer environments, new leaves that emerge in higher temperatures often exhibit changes in leaf anatomy, including structural changes in thylakoid membranes and stroma (Walters 2005), foliar nitrogen content (Way

and Sage 2008a) and number and size of stomata (Ceulemans et al. 1995). These examples of temperature-induced phenotypic plasticity may result in changes in whole-plant carbon balance in response to warming.

Across a species range, differences in growth responses to higher temperature may be ultimately caused by differences in cold tolerance rather than direct effects of temperature on photosynthesis and respiration (Körner 1991). Cold tolerance traits, important for survival in northern environments, may come at the expense of growth, resulting in a tradeoff (Loehle 1998). There are many cold tolerance mechanisms that could explain this phenomenon, many of which come at a carbon or nutrient cost. Conservative growth strategies that reduce the risk of frost damage in the spring and fall can result in reduced growth by limiting the number of active growing days per year (Sakai and Weiser 1973, Savolainen et al. 2004, Olson et al. in press). Physiological and structural investments include non-soluble carbohydrates that lower freezing temperatures (Guy 1990), increased lignin and pectin in cell walls to fortify against damage from cellular ice-crystal formation (Hausman et al. 2000), and increased photo-protective pigments that protect against free radicals created by photooxidation (Lambers et al. 1998). Little is known about the plasticity in cold-tolerance traits, but if trees can shift resource allocations from cold tolerance towards growth with temperature cues, northern genotypes could have a broader range in growth plasticity, allowing them to take advantage of future, warmer climates.

In this study, we conduct a warming experiment to examine the effects of growth temperature (plasticity) and source environment (genotype) on the morphology,

photosynthesis, leaf characteristics, and cold tolerance on a widespread North American boreal-forest tree, balsam poplar (*Populus balsamifera* L.). Identical genotypes, grown under two temperature regimes, were chosen from source provenances spanning a large latitudinal gradient (Fig. 2.1). Photoperiod was held constant under 20 hours day/4 hours night in order to isolate temperature influences of temperature on growth and cold tolerance from interactive influences of changing photoperiod. We hypothesized that balsam poplar trees collected from southern populations would display higher growth and show increased acclimation capacity compared to northern trees in the high-temperature treatment relative to the low-temperature treatment. We also expected that there would be an interaction between growth temperature and source environment regarding cold tolerance; specifically, we expected higher cold tolerance in genotypes from northern populations and that trees grown under high temperatures would have lower cold tolerance than those grown under low temperatures.

Materials and methods

Plant material and growth chambers

In 2009 and 2010, *ca.* 300 dormant cuttings of balsam poplar, representing 150 genotypes (two cuttings from each individual), were rooted and grown in a greenhouse environment in 167 ml plastic conetainers. Soil medium was equal parts perlite, vermiculite and coconut coir. Genotypes were selected from 17 populations across 15 degrees of latitude in the western range of balsam poplar (Fig. 2.1). We refer to trees rooted in 2009 as year 1, and trees rooted in 2010 as year 2. Year 1 trees were grown in

greenhouse conditions during their first growing season and placed outside with pots buried in a raised bed to overwinter. Prior to the start of the experiment in 2010, year 1 trees were placed in the greenhouse for four weeks before being placed in the growth chambers. Year 2 trees were rooted for four weeks in a greenhouse before being placed in growth chambers. Trees were supplied both from the AgCanBaP collection of the Agriculture and Agri-Food Canada (AAFC), Agroforestry Division, Indian Head, Canada, and from trees collected for the experiment described by Olson *et al.* (Olson et al. in press).

To control for genetic differences in growth-temperature responses, one of the two identical genotypes was grown in a high-temperature growth chamber (HT 29/19 °C day/night; Conviron CMP 3244 and CMP 3246) and the other in low-temperature growth chamber (LT 21/9 °C day/night) for 10 weeks. LT temperature settings were based on 40-year averages in Fairbanks, Alaska (64.8 °N) for June, July, and August (http://akclimate.org/Climate/Normals/index.html), and the HT settings were chosen to represent higher-end (business as usual) estimates of expected climate warming at this latitude by the end of this century (IPCC 2007). Light levels were set at 500 µmol m^{-2} sec^{-1} irradiance, relative humidity was 55%, and photoperiod was 20 hours light and 4 hours dark for the entire experiment in both growth chambers. Every other day, trees were watered to saturation, fertilized with a liquid solution (17-5-17 NPK), and the position of trees in the growth chambers was rotated. Mortality was low in both growth chambers: ten trees died in the HT treatment, and six trees died the LT growth chamber. In the HT chamber, 11 trees (~ 7 %) set bud and ceased to grow before the end of the experiment,

compared to 13 trees (~9%) in the LT chamber. There was no strong relationship between latitude of origin and probability of bud set (logistic regression, $p = 0.08$) nor date of bud set (linear regression, $r^2 = 0.09$; $p = 0.10$). All individuals that set bud were excluded from analyses.

Growth and photosynthesis

Three growth response variables were measured on all trees before placement in the growth chambers and at the end of the 10-week experiment: height from soil level to apical meristem, stem diameter 3 cm above the soil, and number of fully extended leaves. Growth increment was calculated as the difference between the final measurements and initial measurements for each growth variable.

Average stomatal index (SI) was measured twice for a subset of 20 trees, 10 from each growth chamber, representing 10 populations chosen to maximize latitudinal variation (Table 2.1). Prior to placement in growth chambers, leaf impressions were made of the abaxial (underside) of the third and seventh leaves from the apex using clear fingernail polish and cellophane tape. At week 8 of the experiment, the same leaves were re-measured along with the third leaf from the apical meristem, which emerged after placement in the growth chambers. Leaf impressions were fixed onto microscope slides and 5 digital photographs were taken of different portions of the impression, avoiding leaf veins and margins. The number of stomata and epidermal cells were counted for each photograph, and stomata and epidermal cell counts were averaged per leaf prior to analysis. Average stomatal index was calculated as:

$$SI_{ave} = \frac{s}{s+e} \times 100$$

where *s* is the number of stomata and *e* is the number of epidermal cells, excluding guard cells (Ceulmans et al. 1995).

Instantaneous photosynthetic rate per unit leaf area (A_a) was measured on a subset of 50 trees (25 HT and 25 LT); percent foliar nitrogen (N) and carbon (C) were quantified on leaves from 62 (28 HT and 34 LT) trees, respectively. Photosynthetic measurements were taken at week 8 of the experiment using a LI-6400XT Portable Photosynthesis System (LI-COR Environmental, Lincoln, Nebraska) equipped with a 6400-40 Leaf Chamber Fluorometer with integrated LED light source. Photosynthesis was measured on the third leaf from the apex on all trees to minimize differences in leaf age and morphology. The block temperature was set to 23 °C and light levels inside the leaf chamber were matched to that of the growth chambers (500 µmol m^{-2} sec^{-1}). Leaf tissue for N and C content analysis was collected by removing one whole leaf per tree at week 10 of the experiment. The leaves were dried, ground and weighed before being analyzed in a LECO truSpec C/N determinator (LECO Coorporation, St. Joseph, Michigan) by the Forest Soils Ecology Lab (University of Alaska Fairbanks).

Cold tolerance

To test for differences in chilling or freezing tolerance, we measured electrolyte leakage for leaf samples collected from genotypes growing in each of the two growth

temperatures, which were chosen to maximize the latitudinal gradient (Li et al. 2004, Friedman et al. 2008). Electrolyte leakage is measured as the conductivity, or electrolyte concentration, of a solution resulting from damage to the cell membrane, as may occur during chilling, freezing, or other types of stress (Murray et al. 1989); increased tissue damage results in increased conductivity. Electrolyte leakage was measured for 6.0 mm leaf disks at 0 °C for 22 genotype pairs (one tree from each of the two growth chambers) from 11 populations and at -5 °C for 68 genotype pairs from 16 populations. Leaf disks were collected using a hole punch from one mature, fully developed leaf located near the middle of the tree. Punches were taken near the base of the leaf, avoiding veins or leaf margins, and were immediately placed in separate 10 x 13 mm glass test tubes. Tubes with leaf disks were placed in a NesLab circulating cold bath (Portsmouth, New Hampshire) at 16 °C, which was programmed to decrease 3 °C per hour until 0 °C, where it was maintained for 20 minutes, and then cooled at the same rate to -5 °C. When the cold bath reached 0 °C, a subset of tubes was removed from the bath and stored at 4 ºC overnight. To the remaining samples, a small ice chip was added to each tube to initiate ice nucleation and avoid supercooling. At -5 °C, the remaining tubes were removed and stored at 4 ºC overnight. The following day 5 ml of ddH_2O was added to each test tube, and samples were agitated for one hour at room temperature before conductivity of the solution was measured for each sample in randomized order (Oakton Instrument Con6/TDS6; Vernon Hills, Illinois). Following a second overnight agitation at room temperature, the test tubes were loosely capped and autoclaved to ensure 100 % cellular damage and conductivity measurements were repeated. Percentage of freeze-induced

electrolyte leakage (EL) was calculated as:

$$EL = \frac{l_0}{l_1} \times 100$$

where l_0 was conductivity after the first measure and l_1 was conductivity after autoclaving.

The 0 ℃ and -5 °C temperatures were chosen to represent cold periods at high latitudes which trees may naturally experience during the growing season. Over the last 107 years at 64.8 °N in Fairbanks, Alaska, low temperatures of 0 °C/ -5 °C have been observed on average 9/1 days per month in May, 2/2 days in June, 0/0 days in July, 2/1 days in August, and 3/0 days in September (National Weather Service, Fairbanks, xmclimat database). Given their regular occurrence in most years, damaging growing-season freeze events may have a strong influence on plant distributional limits at northern latitudes (Rehfeldt et al. 2001, Vitt et al. 2010).

Geo-climatic data

Geo-climatic variables for each source population are listed in Table 2.1. Thirty-year climate normals (1971 - 2000) for weather stations located near source locations for sample populations were obtained from Environment Canada (http://climate.weatheroffice.gc.ca/climate_normals/index_e.html) and from the Alaska Climate Research Center (http://akclimate.org/Climate/Normals/index.html). Geo-climate variables were chosen to be consistent with definitions of Soolanayakanahally et al.

(2009) and include latitude (LAT; °N), longitude (LON; °W), elevation (ELV; m a.s.l.), frost-free days, or the average number of days with the minimum temperature above freezing (FFD; days), mean annual air temperature (MAT; °C), mean annual summer air temperature (MST; June, July, August; °C), mean temperature of the coldest month (MTCM; °C), mean temperature of the warmest month (MTWM; °C), mean annual precipitation (MAP; mm), and mean summer precipitation (MSP; mm). Continentality (CONT) which is defined as the difference between MTWM and MTCM is a proxy of the effects of large land masses on temperature (Guy and Holowachuk 2001); annual dryness index (ADI) and summer dryness index (SDI) were calculated following the equations in Guy & Holowachuk (2001), which relates saturation of vapor pressure (as a proxy for potential evapotranspiration) to precipitation and temperature (annually or seasonally). Cumulative growing degree days (cGDD) were also included and calculated as:

$$\sum\left(\frac{T_{max} - T_{min}}{2} - T_{base}\right)$$

where T_{max} and T_{min} are maximum and minimum normal temperatures for each day during the growing season, and T_{base} is the threshold temperature under which plants are not expected to grow; here, T_{base} was 5 °C (Hinzman et al. 2006).

Analyses

Although we were primarily concerned with the latitudinal gradient and the inverse trend with mean annual temperature with respect to latitude, source provenances for the populations chosen for this study also vary longitudinally. Moreover, along with the latitudinal gradient in our study, there were differences among populations in seasonal temperatures, precipitation, and elevation. To account for the various components of variation among source environments to which genotypes in this study may be adapted, we performed a principal components analysis (PCA) on the geo-climatic variables in Table 2.1.

To determine the effects growth temperature and association between source environment and growth of trees, we first performed a MANCOVA, followed by univariate ANCOVA if the MANCOVA provided a significant result (protected ANCOVA; Scheiner 2001). The three growth parameters, height, diameter and leaf number, were included as dependent variables. Independent variables included growth temperature treatment and the two dominant principal components of the geo-climatic environment, PC1, and PC2. Tree age and initial height, diameter, and leaf count (as measured before the start of the experiment) were included as covariates. Trees with missing values for any response variable were discarded prior to analysis. Significance of the effects of independent variables was tested using Roy's greatest root. Prior to analysis, the dependent variables were transformed to multivariate normality using a box-cox multivariate transformation (Weisberg 2005).

Univariate ANCOVA tests were calculated for each growth response variable,

using linear mixed effects (lme) models. For each response variable, the most parameter-rich lme model (full model) considered was:

$$y_{ijkl} = \mu + \tau_i + aPC1_j + bPC2_j + \gamma_k + \delta i_l + (\tau PC1)_{ij} + (\tau PC2)_{ij} + (\tau\gamma)_{ik} + (PC1PC2)_j + (PC1\gamma)_{jk} + (PC2\gamma)_{jk} + (\tau PC1\gamma)_{ijk} + (\tau PC2\gamma)_{ijk} + (\tau PC1PC2)_{ij} + (PC1PC2\gamma)jk + (\tau PC1PC2\gamma)ijkk + pj + gl + \varepsilon ijkl,$$

$$p \sim N(0, \sigma^2{}_p)_j, g \sim N(0, \sigma^2{}_g)_l$$

where y_{ijkl} is the response variable measured for the i^{th} growth chamber (HT or LT), j^{th} population, for the k^{th} age cohort, and the l^{th} individual tree. Fixed effects included: growth temperature (τ), the two dominant principal components (PC1 and PC2), tree age (γ), initial measurement (i) with coefficient δ, e.g., initial height or initial diameter. The significance of initial measurements (i) was not tested and was included in all models. Random effects include population (p) and genotype (g), which are normally distributed with a mean of 0. In order to select the best model for each response variable, model selection was conducted. First, the significance of random effects was evaluated. Non-significant random effects were removed prior to evaluation of fixed effects. Starting from the full model, an interaction term or a single independent variable was dropped in a hierarchical fashion, and the likelihoods of two models with and without the term were compared with a likelihood ratio test: -2 times the differences in the two log likelihoods ($-2 \Delta\ lnL$), and the significance of this difference was determined by parametric bootstrapping with 5000 iterations (Faraway 2010). If the fit of the model, assessed by

likelihoods, was not significantly improved by the additional term ($\alpha = 0.05$), the term was dropped for all subsequent model selection procedures. All appropriate model combinations were compared.

Stomatal index was analyzed using mixed-effects repeated measures ANOVA. Due to the small sample size, the latitudinal gradient was represented by two discrete groups, north and south. North was defined as all genotypes originating from latitudes higher than 60 °N (median latitude). Fixed effects included growth temperature, measurement time (Time 1 = before and Time 2 = after experiment), and latitude group (north and south), as well as all two-way and three-way interaction terms. Random effects included genotype and individual within genotype; the latter effect accounts for the repeated measures. In order to assess whether the temperature treatments influenced the stomatal index, we conducted two separate analyses: (i) comparison of the same leaves at different times (Time 1 and Time 2) and (ii) comparisons of leaves formed prior to the experimental warming vs. those that formed in the growth chamber.

Foliar N and C:N, A_a, and electrolyte leakage were analyzed with similar linear mixed effects models as those for the three growth-response variables outlined above. Electrolyte data were analyzed separately for 0 °C and -5 °C using ANCOVA linear mixed effects models. For these dependent variables, fixed effects included growth temperature, principal components PC1 and PC2, tree age (year of rooting), and the interaction terms.

All statistical analyses were completed in R (v. 2.15; R Development Core Team, 20011), and the linear mixed effects models utilized the *lme4* R package (Bates and

Maechler 2009). Normality and homogeneity of variance was determined for each variable, and variables were transformed via box-cox transformations as needed.

Results

Variation in source-provenance environment

The first two principal components explained 93% of variation in the geo-climate data set, thus PC1 and PC2 were selected to represent the environmental gradients across the populations in the linear mixed effects models. Loadings (eigen vectors) and correlations of the original geo-climatic variables for the first two PCs are displayed in Table 2.2. PC1 largely represents latitude, mean summer precipitation and mean annual air temperature, which account for 64% of the total variation. PC2 is largely a measure of continentality and accounted for 29% of the total variation in the geo-climatic data.

The effects of increased temperature on growth and ecophysiology

Balsam poplar trees grew an average of 27% taller in the high temperature treatment than the low temperature treatment (Table 2.4; mean ± SEM for HT height growth 6.00 ± 0.42 cm, N = 136; LT 4.71 ± 0.40 cm, N=138). Conversely, relative stem diameters of trees in the LT growth chambers were 32% larger than their counterparts in the HT treatment (Table 2.4; mean diameter HT = 0.38 ± 0.03 cm; LT = 0.50 ± 0.05 cm). The difference in allometry between the two temperature treatments suggests that there is temperature-induced change in allocation to growth. There was no significant pattern in the change in number of leaves between temperature treatments (Table 2.4; mean leaf

increment HT = 7.75 ± 0.54; LT = 7.05 ± 0.45). Overall growth, as determined by the MANCOVA, was influenced by growth temperature (Table 2.3). Significant growth differences between temperature treatments are shown on Figure 2.2.

Increased growth temperature caused a significant increase in average stomatal index of leaves. SI of leaves formed inside the HT treatment growth chamber was 21% higher compared to leaves formed in the greenhouse, but there was no significant difference in the LT treatment (Fig 2.3b; Table 2.4). This response indicates that an increase in number of stomata relative to epidermal cells is an acclimation response to increased growth temperatures. More stomata facilitate CO_2 diffusion into the leaf but also allow for higher rates of evapotranspiration. Further, when the same leaves were compared before and after the growth-chamber treatments, no significant differences were found; SI for the 3^{rd} and 7^{th} leaves did not differ significantly from one another and were analyzed together (Fig 2.3a; Table 2.4; HT stomatal index mean = 0.15 ± 0.01; LT mean = 0.15 ± 0.01). This means that once a leaf develops, there is little plasticity in stomata with respect to temperature cues.

Higher growth temperature did not result in an increase in instantaneous photosynthetic rate. Photosynthesis per unit leaf area had a significant interaction between tree age (year of rooting) and PC2 (log-likelihood = 4.83, p = 0.04), thus the two age groups (year 1 and year 2) were analyzed separately. Year 1 trees, which had grown the previous year and overwintered, had significantly higher instantaneous photosynthetic rates in the LT treatment than in the HT treatment (Fig. 2.5a; Table 2.4; mean A_a HT = 5.58 ± 1.3 μmol $m^{-2}sec^{-1}$, LT = 13.19 ± 2.05 μmol $m^{-2}sec^{-1}$) but year 2 trees, which were

rooted in the year of the growth-chamber experiment, showed no significant trend across treatments (Figure 5b; Table 2.4**;** mean A_a HT = 6.39 ± 1.34 µmol $m^{-2}sec^{-1}$, LT = 7.88 ± 1.06 µmol $m^{-2}sec^{-1}$). The higher height growth exhibited by high-temperature trees was thus not a direct effect of increased photosynthesis. Moreover, growth chamber temperature did not influence foliar N concentration or carbon to nitrogen ratio (Table 2.4), indicating that growth temperature did not influence nitrogen use efficiency.

Growing temperature significantly affected electrolyte leakage when tissue samples were cooled to both 0 °C and -5 °C (Table 2.4). When leaves were cooled to 0 °C, electrolyte leakage was 40% higher in HT trees than the same genotypes at LT (mean % EL 0 °C HT = 45.09 ± 12.30, LT = 32.30 ± 10.81). When cooled to -5 °C, electrolyte leakage increased in both treatments. Although leaves in HT exhibited only 14% more electrolyte leakage after cooling to -5 °C than those in LT, the difference between leaves in the two treatments remained significant (mean % electrolyte leakage at -5 °C HT = 52.13 ± 16.55, LT = 45.57 ± 17.32). The plasticity in cold tolerance suggests that temperature cues can influence cold hardiness.

Influence of source environment on growth and ecophysiology

As indicated by the MANCOVA, both growth temperature and source environment influenced overall growth patterns (Table 2.3). The importance of source environment is highlighted by a significant effect of the interaction between PC1 and PC2 on growth factors. Additionally, there was a significant interaction between tree age and PC1, suggesting that year 1 and year 2 grew differently (Table 2.3). When the three

growth components were analyzed separately, PC1, which is mainly associated with latitude, mean summer precipitation and mean annual temperature, significantly influenced relative height growth (Table 2.4). Trees from the southern range of balsam poplar grew taller than trees originating from northern latitudes in both temperature treatments (Fig 2.2a). Relative diameter growth and leaf increment had no significant relationship with source environment (Fig. 2.2b,c).

Environment of the source population (PC1 & PC2) was not correlated with change in average stomatal index in either temperature treatment (Table 2.4), indicating that an increase in stomatal index is a typical response to increased growing temperatures in both warm- and cool-adapted genotypes.

Growth chamber temperature did not influence foliar N concentration, nor was there a trend between environment of source population (PC1 & PC2) and foliar N (Fig. 2.4a); however, C to N ratio was significantly associated with PC2 (log-likelihood ratio = 6.07, $p = 0.02$; Fig 2.4a,b), with genotypes from provenances with more mild summers and winters (high value of PC2) having lower C:N. We did not find the expected trend of increasing leaf N with latitude, however, source environment did influence C:N.

PC2 also significantly influenced photosynthesis, but the effects were different between trees rooted in 2009 versus 2010. Year 1 trees, which had grown the previous year and overwintered, had significantly higher instantaneous photosynthetic rates in the LT treatment than in the HT treatment (Fig 2.5a; Table 2.4; mean A_a HT = 5.58 ± 1.3 µmol $m^{-2}sec^{-1}$, LT = 13.19 ± 2.05 µmol $m^{-2}sec^{-1}$) but year 2 trees, which were rooted in the year of the growth-chamber experiment, showed no significant trend across

treatments (Figure 5b; Table 2.4**;** mean A_a HT = 6.39 ± 1.34 µmol $m^{-2}sec^{-1}$, LT = 7.88 ± 1.06 µmol $m^{-2}sec^{-1}$). Thus, the increase in height growth observed in high temperature trees was not a direct result of higher instantaneous photosynthetic rate.

Source environment had no effect on electrolyte leakage measured at 0 °C (Fig. 2.6a; Table 2.4), but at -5 °C, both tree age, and the environment of the source population (PC1) were significantly associated with electrolyte leakage. In general, southern genotypes had greater electrolyte leakage than northern genotypes (Fig. 2.6b; Table 2.4) and the trees rooted in 2009 (year 1) had 11% higher electrolyte leakage than trees rooted in 2010 when cooled to -5 °C (year 2; Fig. 2.6b).

Discussion

Warming effects

Balsam poplar trees exhibit phenotypic plasticity in response to increased growth temperatures. The trees grown at 29/19 °C were 27% taller than those grown at 21/9 °C. This is consistent with other warming experiments on deciduous boreal trees (Lin et al. 2010, Way and Oren 2010, Way et al. 2012), as well as with the observation that young trees that develop under warmer temperatures tend to have different allometries compared to those grown at cooler temperatures. A meta-analysis of temperature effects on tree growth showed that, although height, diameter, numbers of leaves and biomass are functions of tree growth, they do not all respond in tandem within a functional group (Way and Oren 2010). Integrating across 120 experiments on deciduous taxa, Way and Oren (2010) predict an average 3.4-fold increase in height growth, a 1.5-fold increase in

diameter growth, and a 1.7-fold increase in biomass in response to a 10 °C increase in temperature. At the juvenile stage, increases in height growth relative to diameter growth may result in a lifetime fitness increase given the intense competition for light gaps in forest understories (King 1981, Schwinning and Weiner 1998, Falster and Westoby 2003, King 2011), although over longer time periods, taller, skinnier trunks, as found in our study, could impact stem hydraulics leading to higher susceptibility of cavitation (Way et al. 2012) and stem breaking due to ice and snow loads (King 2011).

The increased height growth in the warmer growth chambers was not a direct result of increased photosynthetic rate; warm acclimation either had no effect or reduced levels of instantaneous photosynthesis. These results are consistent with those of Silim et al. (2010) who measured photosynthesis in northern and southern balsam poplar trees grown at 19 °C and 27 °C. In the lower temperature, trees exhibited higher rates of electron transport, and slightly higher maximum capacities of photosynthesis and RuBisCo. Photosynthesis in trees from both temperature treatments were limited by RuBisCo activity from 17 - 37 °C, regardless of source location, which suggests that there is little capacity for acclimation of photosynthetic rates to increased temperatures in balsam poplar (Silim et al. 2010). Limited photosynthetic acclimation to increased temperature has been found in other poplar species (Ow et al. 2008, Silim et al. 2010, Centritto et al. 2011) and other plant types (Yamori et al. 2005, Way and Sage 2008a, Tjoelker et al. 2009). The decline in photosynthesis in HT plants occurred despite an increase in stomatal index, which in other studies on poplar is correlated with increased stomatal conductance (Reich and Lassoie 1984, Ceulemans et al. 1988, Pearce et al.

2006, but see Centritto et al. 2011). Increased stomatal density provides more conduits for CO_2 diffusion into the leaf, and increased CO_2 availability for photosynthesis. Thus photosynthesis in balsam poplar at HT is likely more limited by ribulose-1,5-bisphosphate consumption by RuBisCo than CO_2 (Silim et al. 2010).

Photosynthesis is only one of several factors that control plant growth which include biomass partitioning, defenses, and respiration (Körner 1991). Autotrophic respiration generally increases at higher rates in response to increases in growth temperature than photosynthesis but acclimation responses are also generally higher than in photosynthesis (Atkin and Tjoelker 2003). In poplar, acclimation capacity in autotrophic respiration within single genotypes has been clearly documented to be higher than acclimation in photosynthesis (Ow et al. 2008, Silim et al. 2010). Like photosynthesis acclimation, high-latitude/altitude genotypes compared to low-latitude/altitude genotypes exhibit similar acclimation potential in respiration (Larigauderie and Körner 1995, Silim et al. 2010). If the high temperature trees in this study have a reduced autotrophic respiration to photosynthesis ratio, a positive leaf-level carbon balance could be achieved with lower photosynthetic rates.

Influence of source environment

If adaptation to northern climates (conservative growth strategies) constrained genotypes' responses to warming, we would have expected to see an interaction between growth temperature and PC1, or more specifically, southern genotypes would have increased growth relative to northern genotypes in the HT versus LT treatment. Instead,

our results show no interaction between source environment and acclimation capacity and that the plasticity in response to increased growth temperature appeared to be a generalized response across genotypes. Trees from southern provenances (as indicated by PC1) grew taller in both growth chamber temperatures. Therefore, we predict that, as the climate warms at higher latitudes, southern genotypes may replace northern genotypes, if height growth is indicative of selective advantage.

In contrast to our results, Soolanayakanahally et al. (2009) found that balsam poplar genotypes from southern populations, many of which were the same as studied here, showed less height growth than northern genotypes when grown in a greenhouse environment with 21-hr photoperiod and no nutrient limitation. Seasonal trends in growth patterns (phenology) may explain the opposite trends as the trees used for the experiment by Soolanayakanahally et al. (2009) were rooted under the experimental photoperiod, and those utilized here were grown or rooted in a greenhouse environment prior to placement in the growth chambers. Critical day length cues for seasonal timing of growth cessation and initiation of cold hardiness can be reached within five weeks in balsam poplar (Soolanayakanahally et al. 2012), which could have started a natural growth cessation process in northern genotypes relative to southern genotypes in our experiment. The growth environment, greenhouse versus growth chambers, also may have influenced the different outcomes of the two studies. Although both studies showed a clear effect of source latitude on poplar growth in controlled growth environments, the contrasting nature of the source-population effect in the two studies suggests that environmental conditions, in addition to those that were explicitly controlled in these two experiments,

are likely to strongly influence competitive outcomes among genotypes in the field, as genotypes change their distribution in response to climate change.

Cold tolerance

Plasticity in cold tolerance induced by growing temperature was observed when balsam poplar leaf disks were chilled to 0°C and -5°C, suggests that growing temperatures can affect cold tolerance. This has ecological implications as it suggests that northern genotypes may be able to shift growth strategies from those that favor cold tolerance to those that favor height growth. More investigation is required before it can be demonstrated that growth temperature induced a shift in the height growth/cold tolerance tradeoff, however these results indicate that further investigation is warranted. In addition, when we exposed leaf tissue to -5 °C, there was a positive relationship with cold tolerance and PC1 (correlate of latitudinal environment) with genotypes from higher latitudes showing the least damage, a trend consistent with the findings for several temperate and boreal trees (Loehle 1998, Repo et al. 2000, Li et al. 2004, Friedman et al. 2008). It should be noted, however, that there are alternative explanations for the measured differences in electrolyte leakage besides cold injury. For example, differences in leaf thickness or water content between leaves from genotypes derived from the north and south, or stress responses instigated by the growth chamber treatments could have also influenced electrolyte leakage.

Few studies have quantified plasticity in cellular damage caused by cold temperatures due solely to growing plants at different temperatures. Plasticity in xylem-

vessel diameter was observed in response to warming in *Salix pulchra* Cham. (Gorsuch and Oberbauer 2002). Smaller diameter vessels are an important trait to prevent cavitation in cold environments but may also restrict plant growth. *Salix pulchra* grown under increased temperatures exhibited increased xylem diameters, which can lead to greater nutrient transport and gas exchange (Sperry et al. 2008, Way et al. 2012). This plasticity can result in higher growth potential but also leaves plants more susceptible to growing-season frosts due to cavitation damage (Gorsuch and Oberbauer 2002). Decreased cold tolerance in warmer growth temperatures is of high ecological significance. Even though average summer temperatures are expected to increase with projected climate warming, cold weather extremes during the growing season are projected to become more common (IPCC 2007), which may make trees more susceptible to cold injury in a future, warmer environment. Extensive literature exists that describes the effects of increased growth temperatures on seasonal timing of cold hardiness traits, such as timing of growth cessation and bud set in the fall, and bud flush in the spring (Sakai and Weiser 1973, Savolainen et al. 2004, Nedlo et al. 2009, Gömöry et al. 2010, Rohde et al. 2011), however more direct tests of temperature on cold tolerance that are independent of phenology are needed.

In summary, our data and the literature are consistent with both adaptation and acclimation to warm temperatures resulting in a reduction in cold tolerance. If climate warming is associated with both warmer average temperatures and greater temperature extremes, low levels of cold tolerance could be an important trait limiting the success of southern genotypes in a warmer north. In field trials using the same genotypes (Chapter

2), experimentally-warmed trees delayed growth cessation and bud set compared to trees grown at ambient temperatures, perhaps in part related to a shift in allocation toward height growth over cold hardiness.

Implications for boreal forest ecology and species composition

The increased growth of balsam poplar trees under higher growing temperatures, including the shift in growth strategy towards increasing height growth, may increase competitive ability in a future, warmer environment. This response contrasts with dominant boreal coniferous tree species, black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss), which, in response to recent warming, appear to be approaching upper temperature thresholds for increasing growth response (Wilmking et al. 2004, Way and Sage 2008a, McGuire et al. 2010, Juday and Alix 2012). Temperature-induced drought stress is one possible mechanism for spruce growth decline (Barber et al. 2000), but so is evidence that the respiration to photosynthesis ratio increases under high temperatures in black spruce (Way and Sage 2008a). The observed growth decline in spruce, coupled with observed and projected increases in wildfire extent and severity (Kasischke and Turetsky 2006, Kasischke et al. 2010) could lead to landscape-scale biome conversion from coniferous-dominated to deciduous-dominated forest (Johnstone et al. 2010, Barrett et al. 2011). Our data support this prediction by suggesting that hardwoods may benefit by increased growth in future warmer climates. Empirical observations are already indicating that coniferous taxa may be declining in some places in the boreal biome (Beck et al. 2011). Deciduous tree seedlings dominate

after severe fires in what were previously black spruce habitats, as part of the natural succession pathway (Johnstone et al. 2010), but increases in growing-season temperature may disrupt the successional return to old-growth spruce forests and may continue to favor a deciduous-dominated landscape. The boreal forest is one of the most extensive biomes in the world and contains approximately one-third of the global carbon stock (McGuire et al. 2010). Potential consequences for a forest-type conversion range from shifts in carbon storage and albedo, changes in fire dynamics, to cascading impacts on wildlife habitat and ecosystem services. Reducing the uncertainty in land cover change projections, therefore, is of great interest to the scientific and land management communities.

Although we found that balsam poplar trees collected from across a broad environmental gradient responded positively to warming, the lower relative height growth of trees derived from northern compared to southern populations may indicate that gene flow or migration from more southern populations may increase fitness of populations near the northern range limit in a warmer climate, to the extent that height growth contributes to fitness. Given the lower cold tolerance of southern genotypes, however, growing season frosts may limit the successful colonization of southern genotypes at northern latitudes. The decrease in cold tolerance of all populations at higher growth temperatures suggests that treeline advance may still be limited by low temperatures, even in an on average warmer environment.

References

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications **1**:95-111.

Atkin, O. K. and M. G. Tjoelker. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science **8**:343-351.

Barber, V. A., G. P. Juday, and B. P. Finney. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature **405**:668-673.

Barrett, K., A. D. McGuire, E. E. Hoy, and E. S. Kasischke. 2011. Potential shifts in dominant forest cover in interior Alaska driven by variations in fire severity. Ecological Applications **21**:2380-2396.

Bates, D. M. and M. Maechler. 2009. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-32.

Beck, P. S. A., G. P. Juday, C. Alix, V. A. Barber, S. E. Winslow, E. E. Sousa, P. Heiser, J. D. Herriges, and S. J. Goetz. 2011. Changes in forest productivity across Alaska consistent with biome shift. Ecology Letters **14**:373-379.

Centritto, M., F. Brilli, R. Fodale, and F. Loreto. 2011. Different sensitivity of isoprene emission, respiration and photosynthesis to high growth temperature coupled with drought stress in black poplar (*Populus nigra*) saplings. Tree Physiology **31**:275-286.

Ceulemans, R., I. Impens, and R. Imler. 1988. Stomatal conductance and stomatal behavior in *Populus* clones and hybrids. Canadian Journal of Botany **66**:1404-1414.

Ceulemans, R., L. Van Praet, and X. N. Jiang. 1995. Effects of CO_2 enrichment, leaf position and clone on stomatal index and epidermal cell density in poplar (*Populus*). New Phytologist **131**:99-107.

Chapin, F. S. and G. R. Shaver. 1985. Individualistic growth response of tundra plant species to environmental manipulations in the field. Ecology **66**:564-576.

Danby, R. K. and D. S. Hik. 2007. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. Global Change Biology **13**:437-451.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology **86**:1704-1714.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. Science **332**:53-58.

Day, M. E., J. L. Schedlbauer, W. H. Livingston, M. S. Greenwood, A. S. White, and J. C. Brissette. 2005. Influence of seedbed, light environment, and elevated night temperature on growth and carbon allocation in pitch pine (*Pinus rigida*) and jack pine (*Pinus banksiana*) seedlings. Forest Ecology and Management **205**:59-71.

Falster, D. S. and M. Westoby. 2003. Plant height and evolutionary games. Trends in Ecology & Evolution **18**:337-343.

Faraway, J. J. 2010. Changes to the Mixed Effects Models chapters in ELM.URL: http://www.maths.bath.ac.uk/~jjf23/ELM/mixchange.pdf.

Friedman, J. M., J. E. Roelle, J. F. Gaskin, A. E. Pepper, and J. R. Manhart. 2008. Latitudinal variation in cold hardiness in introduced *Tamarix* and native *Populus*. Evolutionary Applications **1**:598-607.

Ghannoum, O. and D. A. Way. 2011. On the role of ecological adaptation and geographic distribution in the response of trees to climate change. Tree Physiology **31**:1273-1276.

Gömöry, D., E. Foffová, J. Kmeť, R. Longauer, and I. Romšáková. 2010. Norway spruce (*Picea abies* [L.] Karst.) provenance variation in autumn cold hardiness: adaptation or acclimation? Acta Biologica Cracoviensia Series Botanica **52**:42-49.

Gorsuch, D. M. and S. F. Oberbauer. 2002. Effects of mid-season frost and elevated growing season temperature on stomatal conductance and specific xylem conductivity of the arctic shrub, *Salix pulchra*. Tree Physiology **22**:1027-1034.

Grace, J., F. Berninger, and L. Nagy. 2002. Impacts of Climate Change on the Tree Line. Annals of Botany **90**:537-544.

Guy, C. L. 1990. Cold Accelimation and Freezing Stress Tolerance: Role of Protein Metabolism. Annual Review of Plant Physiology and Plant Molecular Biology **41**:187-223.

Guy, R. D. and J. L. Gornall. 2007. Geographic variation in ecophysiological traits of black cottonwood (*Populus trichocarpa*). Canadian Journal of Botany **85**:1202-1213.

Guy, R. D. and D. L. Holowachuk. 2001. Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. ex Loud.: relationship to environment, climate of origin, and growth potential. Canadian Journal of Botany **79**:274-283.

Hamann, A. and T. Wang. 2006. Potential Effects Of Climate Change On Ecosystem And Tree Speces Distribution in British Columbia. Ecology **87**:2773-2786.

Hausman, J. F., D. Evers, H. Thiellement, and L. Jouve. 2000. Compared responses of poplar cuttings and in vitro raised shoots to short-term chilling treatments. Plant Cell Reports **19**:954-960.

Hinzman, L. D., L. A. Viereck, P. C. Adams, V. E. Romanovsky, and K. Yoshikawa. 2006. Climate and Permafrost Dynamics of the Alaskan Boreal Forest. Pages 39-61 *in* F. S. Chapin III, M. W. Oswood, K. V. Cleve, L. A. Viereck, and D. A. Verbyla, editors. Alaska's Changing Boreal Forest. Oxford University Press, Oxford.

IPCC. 2007. Climate Change 2007: The Physical Science Basis: Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change *in* S. Soloman, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Cambridge University Press, Cambridge, UK.

Johnstone, J. F., T. N. Hollingsworth, F. S. Chapin, and M. C. Mack. 2010. Changes in fire regime break the legacy lock on successional trajectories in Alaskan boreal forest. Global Change Biology **16**:1281-1295.

Juday, G. P. and C. Alix. 2012. Consistent negative temperature sensitivity and positive influence of precipitation on growth of floodplain *Picea glauca* in Interior Alaska. Canadian Journal of Forest Research **42**:561-573.

Kasischke, E. S. and M. R. Turetsky. 2006. Recent changes in the fire regime across the North American boreal region; Spatial and temporal patterns of burning across Canada and Alaska. Geophys. Res. Lett. **33**:L09703.

Kasischke, E. S., D. L. Verbyla, T. S. Rupp, A. D. v. i. d. McGuire, K. A. Murphy, R. Jandt, J. L. Barnes, E. E. Hoy, P. A. Duffy, M. Calef, and M. R. Turetsky. 2010. Alaskas changing fire regime implications for the vulnerability of its boreal forests. Canadian Journal of Forest Research **40**:1313-1324.

King, D. 1981. Tree dimensions: Maximizing the rate of height growth in dense stands. Oecologia **51**:351-356.

King, D. A. 2011. Size-Related Changes in Tree Proportions and Their Potential Influence on the Course of Height Growth Size- and Age-Related Changes in Tree Structure and Function. Pages 165-191 *in* F. C. C. Meinzer, B. Lachenbruch, and T. E. E. Dawson, editors. Springer Netherlands.

Körner, C. 1989. The Nutritional Status of Plants from High Altitudes. A Worldwide Comparison. Oecologia **81**:379-391.

Körner, C. 1991. Some often overlooked plant characteristics as determinants of plant growth: a reconsideration. Functional Ecology. **5**:162-173.

Lambers, H., F. S. C. III, and T. L. Pons. 1998. Plant Physiological Ecology. Springer, New York.

Larigauderie, A. and C. Körner. 1995. Acclimation of Leaf Dark Respiration to Temperature in Alpine and Lowland Plant Species. Annals of Botany **76**:245-252.

Li, C., O. Junttila, P. Heino, and E. Tapio Palva. 2004. Low temperature sensing in silver birch (*Betula pendula* Roth) ecotypes. Plant Science **167**:165-171.

Lin, D., J. Xia, and S. Wan. 2010. Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. New Phytologist **188**:187-198.

Loehle, C. 1998. Height growth rate tradeoffs determine northern and southern range limits for trees. Journal of Biogeography **25**:735-742.

McGuire, A. D., R. W. Ruess, A. Lloyd, J. Yarie, J. S. Clein, and G. P. Juday. 2010. Vulnerability of white spruce tree growth in interior Alaska in response to climate variability: dendrochronological, demographic, and experimental perspectives. Canadian Journal of Forest Research **40**:1197-1209.

McKenney, D. W., J. H. Pedlar, K. Lawrence, K. Campbell, and M. F. Hutchinson. 2007. Potential Impacts of Climate Change on the Distribution of North American Trees. BioScience **57**:939-948.

Murray, M. B., J. N. Cape, and D. Fowler. 1989. Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytologist **113**:307-311.

Nedlo, J., T. Martin, J. Vose, and R. Teskey. 2009. Growing season temperatures limit growth of loblolly pine (*Pinus taeda* L.) seedlings across a wide geographic transect. Trees - Structure and Function **23**:751-759.

Olson, M. S., N. Levsen, R. Y. Soolanayakanahally, R. D. Guy, W. R. Schroeder, S. R. Keller, and P. Tiffin. in press. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology.

Ow, L. F., K. L. Griffin, D. Whitehead, A. S. Walcroft, and M. H. Turnbull. 2008. Thermal acclimation of leaf respiration but not photosynthesis in *Populus deltoides*×*nigra*. New Phytologist **178**:123-134.

Pearce, D. W., S. Millard, D. F. Bray, and S. B. Rood. 2006. Stomatal characteristics of riparian poplar species in a semi-arid environment. Tree Physiology **26**:211-218.

R Development Team, R. D. C. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rehfeldt, G., W. Wykoff, and C. Ying. 2001. Physiologic Plasticity, Evolution, and Impacts of a Changing Climate on Pinus Contorta. Climatic Change **50**:355-376.

Reich, P. B. and J. P. Lassoie. 1984. Effects of low level O3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant, Cell & Environment **7**:661-668.

Reich, P. B. and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America **101**:11001-11006.

Reich, P. B. and J. Oleksyn. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. Ecology Letters **11**:588-597.

Reich, P. B., M. B. Walters, and D. S. Ellsworth. 1992. Leaf Life-Span in Relation to Leaf, Plant, and Stand Characteristics among Diverse Ecosystems. Ecological Monographs **62**:365-392.

Repo, T., G. Zhang, A. Ryyppö, R. Rikala, and M. Vuorinen. 2000. The relation between growth cessation and frost hardening in Scots pines of different origins. Trees - Structure and Function **14**:456-464.

Rohde, A., C. Bastien, and W. Boerjan. 2011. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. Tree Physiology **31**:472-482.

Rupp, T. S., F. S. Chapin, and A. Starfield. 2001. Modeling the Influence of Topographic Barriers on Treeline Advance at the Forest-Tundra Ecotone in Northwestern Alaska. Climatic Change **48**:399-416.

Sage, R. F. and D. S. Kubien. 2008. The temperature response of C3 and C4 photosynthesis. Plant, Cell & Environment **30**:1086-1106.

Sakai, A. and C. J. Weiser. 1973. Freezing resistance of trees in North America with reference to tree regions. Ecology **54**:111.

Savolainen, O., F. Bokma, R. García-Gil, P. Komulainen, and T. Repo. 2004. Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of Pinus sylvestris to climatic changes. Forest Ecology and Management **197**:79-89.

Saxe, H., M. G. R. Cannell, Ø. Johnsen, M. G. Ryan, and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. New Phytologist **149**:369-399.

Scheiner, S. M. 2001. MANOVA: Multiple Response Variables and Multispecies Interactions. Pages 99-115 *in* S. M. Scheiner and J. Gurevitch, editors. Design and Analysis of Ecological Experiments. Oxford University Press, New York.

Schwinning, S. and J. Weiner. 1998. Mechanisms determining the degree of size asymmetry in competition among plants. Oecologia **113**:447-455.

Silim, S., N. Ryan, and D. Kubien. 2010. Temperature responses of photosynthesis and respiration in *Populus balsamifera* L.: acclimation versus adaptation. Photosynthesis Research **104**:19-30.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, E. C. Drewes, and W. R. Schroeder. 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (Populus balsamifera L.). Plant, Cell & Environment **32**:1821-1832.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, and M. Song. 2012. Timing of photoperiodic competency causes phenological mismatch in balsam poplar (Populus balsamifera L.). Plant, Cell & Environment:1-12.

Sperry, J. S., F. C. Meinzer, and K. A. McCulloh. 2008. Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. Plant, Cell & Environment **31**:632-645.

Tjoelker, M. G., J. Oleksyn, G. Lorenc-Plucinska, and P. B. Reich. 2009. Acclimation of respiratory temperature responses in northern and southern populations of Pinus banksiana. New Phytologist **181**:218-229.

Tjoelker, M. G., J. Oleksyn, and P. B. Reich. 1998. Temperature and ontogeny mediate growth response to elevated CO2 in seedlings of five boreal tree species. New Phytologist **140**:197-210.

Vitt, P., K. Havens, A. T. Kramer, D. Sollenberger, and E. Yates. 2010. Assisted migration of plants: Changes in latitudes, changes in attitudes. Biological Conservation **143**:18-27.

Walters, R. G. 2005. Towards an understanding of photosynthetic acclimation. Journal of Experimental Botany **56**:435-447.

Way, D. A., J.-C. Domec, and R. B. Jackson. 2012. Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. Plant, Cell & Environment:no-no.

Way, D. A. and R. Oren. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. Tree Physiology **30**:669-688.

Way, D. A. and R. F. Sage. 2008a. Elevated growth temperatures reduce the carbon gain of black spruce (*Picea mariana* (Mill.) B.S.P.). Global Change Biology **14**:624-636.

Way, D. A. and R. F. Sage. 2008b. Thermal acclimation of photosynthesis in black spruce (*Picea mariana* (Mill.) B.S.P.). Plant, Cell & Environment **31**:1250-1262.

Weisberg, S. 2005. Applied Linear Regression. John Wiley & Sons, Hoboken, New Jersey.

Wilmking, M., G. P. Juday, V. A. Barber, and H. S. J. Zald. 2004. Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds. Global Change Biology **10**:1724-1736.

Yamori, W., K. O. Noguchi, and I. Terashima. 2005. Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. Plant, Cell & Environment **28**:536-547.

Tables

Table 2.1 Geo-climatic data of source populations included in this study: latitude (LAT), longitude (LON), elevation (ELV), frost free days (FFD), mean annual air temperature (MAT), mean summer (June, July, August) air temperature (MST), mean air temperature warmest month (MTWM), mean air temperature coldest month (MTCM), mean annual precipitation (MAP), mean summer precipitation (MSP), growing degree days (GGD), continentality (CONT), annual dryness index (ADI), summer dryness index (SDI).

Source populations	LAT (°N)	LON (°W)	ELEV (m)	FFD	MAT (°C)	MST (°C)	MTCM (°C)	MTWM (°C)	MAP (mm)	MSP (mm)	GDD (°5 C)	CONT	ADI	SDI
Fairbanks[α]	64.82	147.87	248	142	-2.4	15.4	-22.2	16.9	274.6	137.4	1286	39.1	1.89	14.15
Galena	64.71	156.73	74	124	-3.7	12.7	-21.3	14.2	532.0	171	902	35.5	0.88	9.57
Nome[α]	64.56	165.34	75	129	-2.5	10.1	-14.9	11.2	427.0	160	570	26.1	1.20	8.40
Denali National Park	63.87	149.02	594	122	-3.2	11.8	-22.3	16.1	235.0	159	1245	38.4	2.08	11.62
Hay River[α]	60.80	115.78	168	144	-2.9	14.3	-23.1	15.9	320.4	125.2	1093	39	1.56	14.57
Whitehorse[α]	60.70	135.33	770	138	-0.7	12.8	-18.4	14.8	267.4	111.1	895	33.2	2.19	15.30
Stony Rapids†[α]	59.23	105.72	306	153	-0.7	13.0	-20.4	16.9	452.0	293.0	na	37.3	1.30	6.64
Fort McMurray	56.92	111.50	338	157	0.7	15.6	-18.8	16.8	455.5	228.8	1376	35.6	1.43	8.44
Gillam[α]	56.35	94.63	126	129	-4.2	13.5	-25.8	15.3	499.4	212.9	970	41.1	0.91	8.25
La Ronge[α]	55.15	105.26	379	237	-0.1	15.8	-20.4	17.2	483.8	215.8	1323	37.6	1.27	9.18
Grande Prairie	54.75	118.63	769	167	1.9	15.0	-15.0	15.9	446.6	208.7	3023	30.9	1.59	8.74
B**o**yle*	54.60	112.89	649	165	2.1	15.2	-14.9	16.2	503.7	258.8	1370	31.1	1.43	7.19
Dunlop*	54.51	99.90	755	125	-3.2	42.5	-24.9	15.8	348.2	227.7	1059	40.7	1.40	7.96
Edmonton[α]	53.31	113.58	723	273	2.4	15.0	-13.5	15.9	482.7	252.9	1360	29.4	1.52	7.21
Grand Rapids	53.16	99.28	223	232	0.8	17.1	-19.7	18.6	473.7	214.4	1508	38.3	1.38	10.10
Stettler[α]	52.35	112.73	795	161	3.0	15.4	-12.6	16.4	481.1	239.6	1430	29	1.59	7.86
Melville*	50.94	102.74	549	163	1.6	16.7	-17.9	17.8	346.4	215.7	1553	35.7	2.00	9.54

*Climate stations within 300 km of source populations; †geo-climate data from Soolanayakanahally et al. (2009);
[α]Populations chosen for stomatal index comparison.

Table 2.2 Loadings for each geo-climatic variable on the two primary principal components, PC1 and PC2, and correlations of each original geo-climate variable on PC1 and PC2.

	PCA Loadings		Correlations	
Geo-climatic variables	PC1	PC2	PC1	PC2
LAT	-0.39	-0.18	-0.90	-0.31
LON	-0.29	-0.35	-0.66	-0.60
ELEV	0.22	0.11	0.52	0.19
FFD	0.29	0.00	0.68	0.00
MAT	0.37	-0.09	0.86	-0.15
MST	0.08	0.34	0.18	0.58
MTCM	0.26	-0.38	0.60	-0.65
MTWM	0.20	0.42	0.47	0.72
MAP	0.25	-0.25	0.59	-0.43
MSP	0.38	0.00	0.89	0.01
GDD5	0.24	0.09	0.57	0.15
CONT	-0.15	0.49	-0.36	0.84
ADI	-0.04	0.19	-0.10	0.32
SDI	-0.30	0.18	-0.70	0.30

Table 2.3 MANCOVA results for three growth response variables: relative tree height growth, relative diameter growth, and leaf number increment. Significance of independent variables was determined using Roy's greatest root. Independent variables included: growth temperature, tree age (Age; rooted in 2009 or 2010), and the two dominant principal components representing source environment of the populations (PC1 and PC2).

Independent Variable	DF	Value	F	num DF	den DF	P
Growth temperature	1	0.047348	3.9299	3	249	**<0.01**
Age	1	0.008398	0.697	3	249	0.55
PC1	1	0.017877	1.4838	3	249	0.22
PC2	1	0.027038	2.2442	3	249	0.08
Growth temperature x Age	1	0.002862	0.2376	3	249	0.87
Growth temperature x PC1	1	0.013685	1.1359	3	249	0.34
Growth temperature xPC2	1	0.013145	1.091	3	249	0.35
Age x PC1	1	0.053306	4.4244	3	249	**<0.01**
Age x PC2	1	0.021717	1.8025	3	249	0.15
PC1 x PC2	1	0.042766	3.5496	3	249	**0.02**
Growth temperature x Age x PC1	1	0.005986	0.4968	3	249	0.68
Growth temperature x Age x PC2	1	0.014276	1.1849	3	249	0.32
Growth temperature x PC1 x PC2	1	0.000079	0.0065	3	249	1.00
Age x PC1 x PC2	1	0.011196	0.9292	3	249	0.43
Growth temperature x Age x PC1 x PC2	1	0.002956	0.2454	3	249	0.86

Table 2.4 Significant fixed and random effects for each response variable, representing the best-fit linear mixed effects model. PC1 and PC2 are the first two principal components representing source environment of populations; Time refers to measurements before and after growth chamber experiment; Age is the tree age cohort included in the study (rooted in 2009 or 2010); Group refers to latitudinal grouping of north and south; initial height, initial diameter are the respective measurements at the beginning of the experiment.

Response variable	Best-fit model	Significant fixed effects	Log likelihood ratio[a]/t-value*	p-value	Significant random effects	Log likelihood ratio[a]	p-value
Height growth	Growth temperature + PC1 + initial	Growth temperature	9.45	<0.01	Population	26.88	< 0.0001
	Height + Population + Genotype	PC1	5.09	0.04	Genotype	10.98	<0.01
Diameter growth	Growth temperature + initial diameter	Growth temperature	2.2*	0.03	none	n/a	n/a
Leaf number	null model + Population + Genotype	none	n/a	n/a	Population	7.62	< 0.0001
					Genotype	5.09	0.02
Stomatal index pre-existing leaves	null model + Population + Genotype	none	n/a	n/a	none	n/a	n/a
Stomatal index	Growth temperature + Time +	Growth temperature	4.44*	0.04	none	n/a	n/a
new leaves	Growth temperature x Time	Time	7.18*	0.01			
		Growth temperature:Time	3.85*	0.04			
Foliar N	null model + Population	none	n/a	n/a	Population	2.45	<0.001
Foliar C:N	PC2 + Population	PC2	6.07	0.02	Population	1.34	<0.01
A_a (Year 1)	Growth temperature + Population	Growth temperature	10.01	<0.01	Population	4.83	0.04
A_a (Year 2)	null model + Population	none	n/a	<0.01	Population	10.83	0.04
Electrolyte leakage 0 °C	Growth temperature	Growth temperature	7.34*	0.01	none	n/a	n/a
Electrolyte	T Growth temperature + Age + PC1	Growth temperature	4.93*	0.03	none	n/a	n/a
Leakage -5 °C		PC1	5.16*	0.02			
		Age	8.91*	<0.01			

[a]log-likelihood ratio calculated as - 2 Δ ln likelihood; *t-value for significant fixed effects for models without random effects

Figures

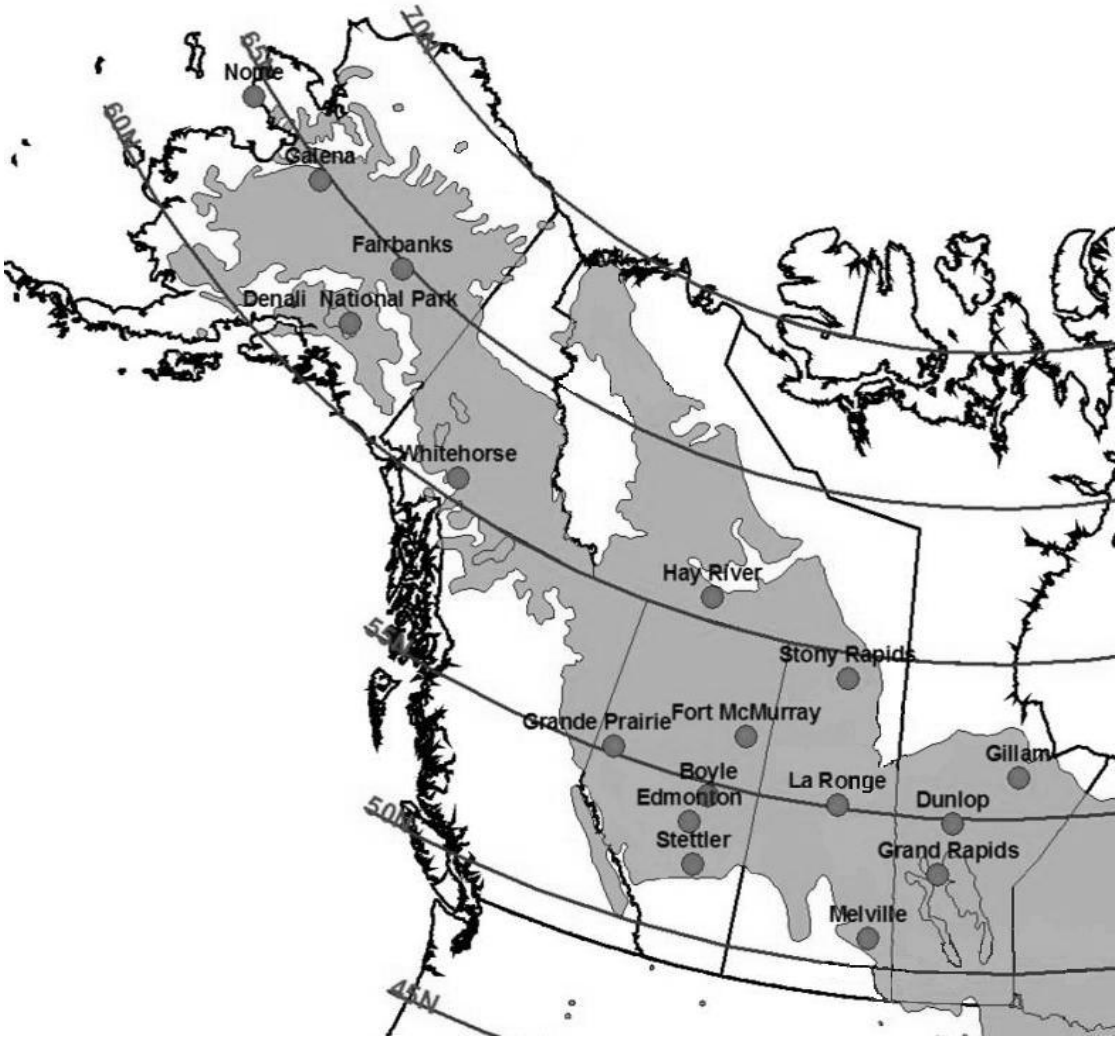

Figure 2.1 Locations of the 17 source populations from which cuttings were originally collected for this study are indicated as dots, with the western range of balsam poplar shaded in gray.

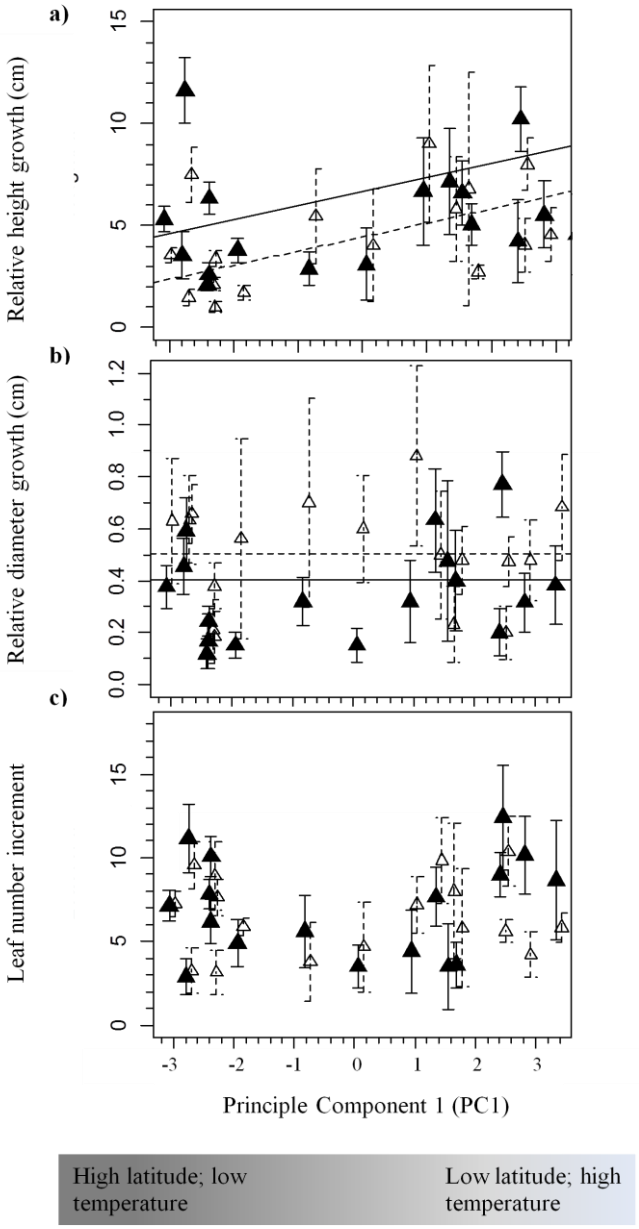

Figure 2.2 Relative height growth (a), relative diameter growth (b), and leaf number increment (c) for balsam poplar genotypes grown at 29/19 °C (HT) and 21/9 °C (LT) day/night temperatures. Points are plotted relative to population means for principal component 1 (PC1) along the abscissa, which correlates positively with temperature and inversely with latitude in the source environment. Error bars are standard error of the mean; HT population means are represented by closed triangles and solid lines, LT are open triangles and dotted lines. Points are slightly offset on the abscissa to reduce overlap and increase visibility.

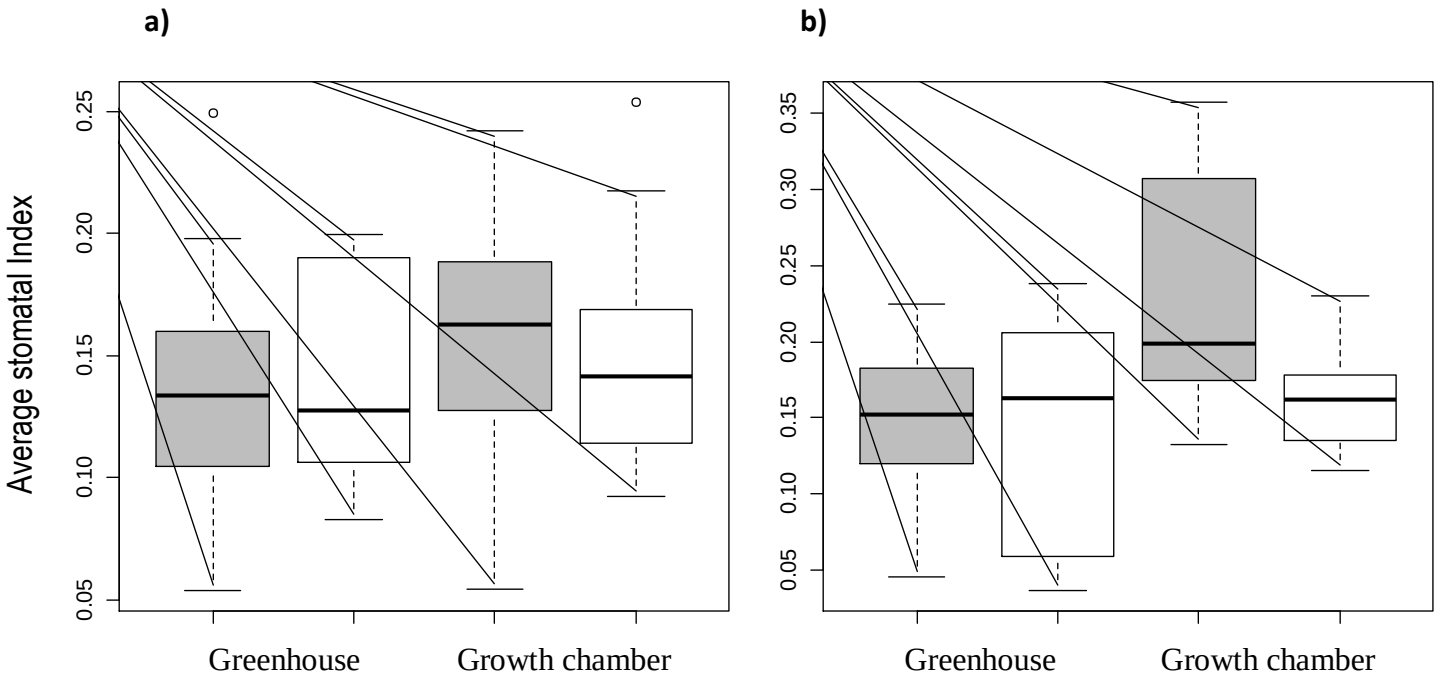

Figure 2.3 Box and whisker plots illustrating the average stomatal index for leaves that were measured in the greenhouse (before) and following the growth chamber experiment (after) for (a) the same leaves that developed in the greenhouse environment and were transferred to the growth chamber, and (b) leaves that developed in the greenhouse environment compared to new leaves that emerged while in growth chambers. Gray boxes indicate samples from the high temperature treatment (HT) and open boxes low temperature treatment (LT). Whiskers show the minimum and maximum values with exception of outliers.

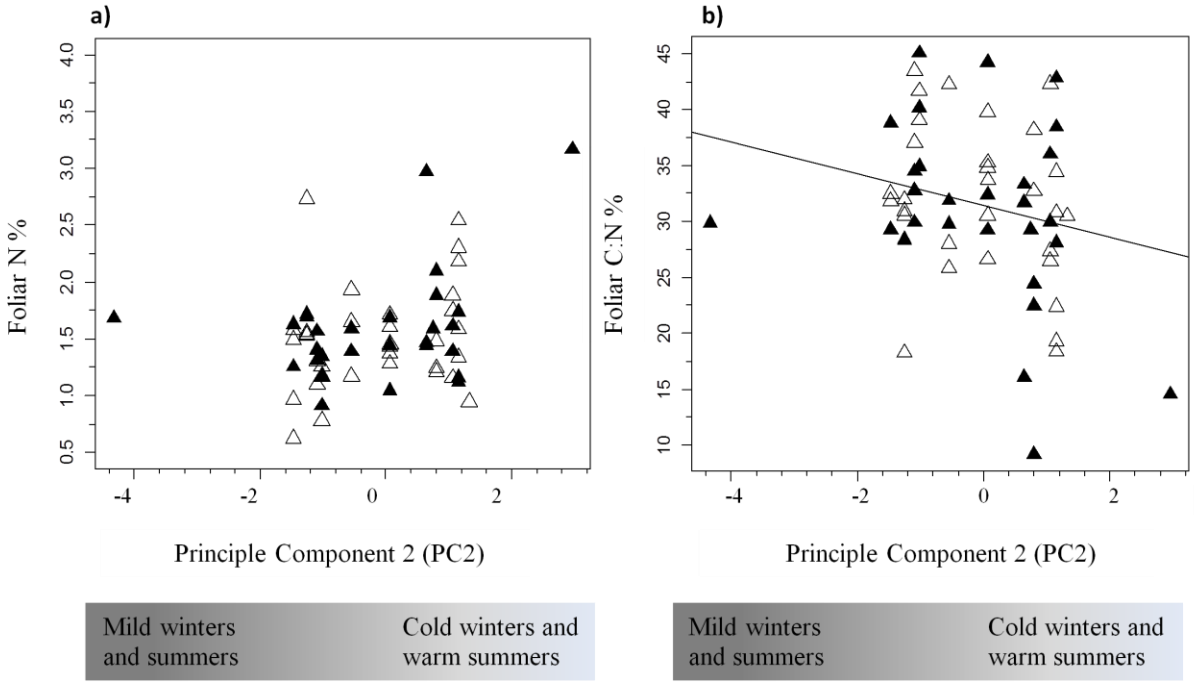

Figure 2.4 Foliar nitrogen concentration (a) and foliar carbon to nitrogen ratio (b) plotted against population averages for principal component 2 (PC2), which correlates positively with continentality. HT data points are represented by closed triangles and solid lines, LT are open triangles and dotted lines.

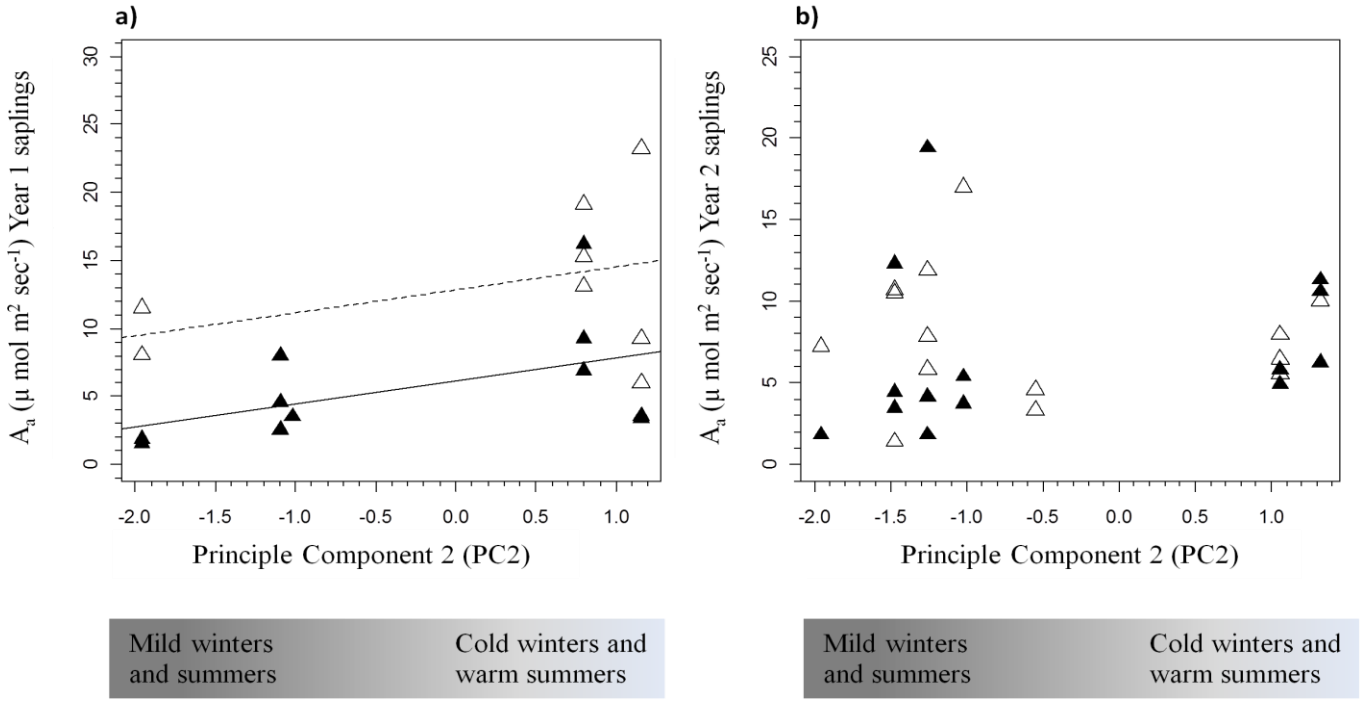

Figure 2.5 Instantaneous photosynthetic rate per unit leaf area plotted against principal component 2 (PC2) for balsam poplar cuttings rooted in (a) 2009 and (b) 2010. HT data points are represented by closed triangles and solid lines, LT are open triangles and dotted lines.

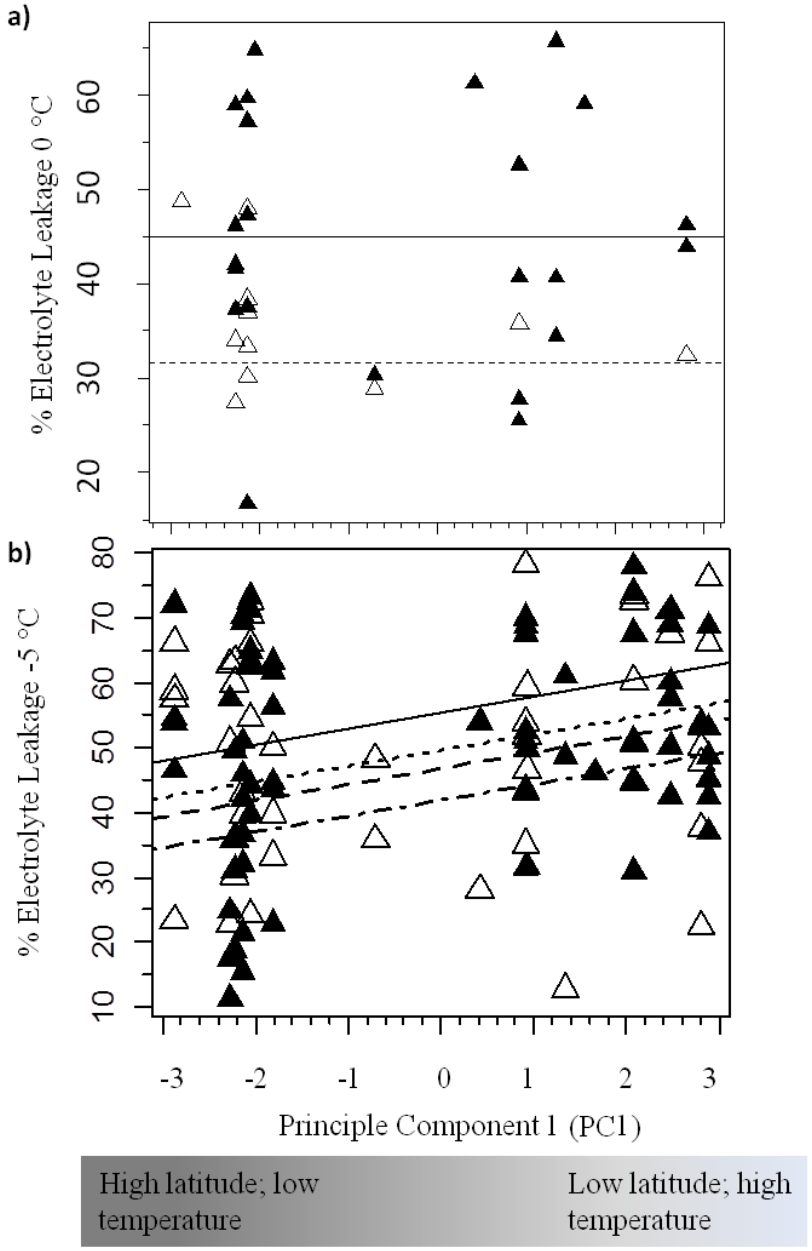

Figure 2.6 Percent electrolyte leakage after leaf tissues were exposed to 0 °C (a), and -5 °C (b), plotted against principal component 1 (PC1). HT data points are represented by closed triangles (solid line = HT, cuttings rooted in 2009; dotted line = HT, cuttings rooted in 2010), LT are open triangles (dashed line = LT, rooted in 2009; dot-dash line = LT, rooted in 2010).

Chapter 3

Acclimation and adaptation potential of balsam poplar, *Populus balsamifera* L., in a changing climate[1]

Abstract

Global climate change has, and is predicted to, increase both summer temperatures and growing season length in high-latitude forests. In response to these changes, boreal forests are projected to increase in productivity and experience biome-wide northward expansion of species' ranges. Within a species, adaptation to local photoperiod and climate regimes may either facilitate or limit the ability of genotypes to respond to changing environmental conditions. To determine how local adaptation influences the capacity for acclimation and migration to increased temperatures and longer growing seasons, we experimentally warmed balsam poplar (*Populus balsamifera* L.) trees collected from a latitudinal transect in a common garden located at 64.8 °N. The effects of increased temperature were measured on height growth, diameter growth, leaf number and lateral bud number, in addition to phenology, photosynthetic rate per unit leaf area, and cold injury. Warmed balsam poplar trees grew 69% taller, and were larger in diameter, had more leaves, and more lateral buds compared to those grown under ambient conditions. Warmed trees also delayed bud set by an average of 6.5 days, showing plasticity in growth cessation and bud set with respect to temperature cues. Source environment had the largest influence on growth and phenology. Genotypes from

[1] Robertson, A.L., N. Takebayashi, M.S. Olson. Prepared for submission to Ecology Letters.

southern populations grew taller and had longer growing season lengths (by up to two months) than those from northern populations in both warmed and control treatments. Trees with the longest growing seasons grew the tallest but also had higher occurrences of cold injury. Instantaneous photosynthetic rates were lower for warmed trees than for controls. Trends in both photosynthetic rates and height-growth increment with source environment shifted seasonally; northern genotypes exhibited higher growth and photosynthesis rates at the beginning of the growing seasons, which transferred to southern genotypes in the mid-latter part of the growing season. This study indicates that adaptation to local photoperiod/climate regimes does not limit the acclimation capacity of northern populations of balsam poplar in a warmer climate. It also suggests that local adaptation does not limit the northward migration of southern genotypes into northern latitudes, and that northward migration may increase the success of balsam poplar under scenarios of global climate change.

Introduction

Species range shifts due to increasing 21st century temperatures are inevitable, and range boundaries are widely expected to expand to higher latitudes and altitudes in order to track their temperature niches (Parmesan 2006, Thomas 2010, Chen et al. 2011). Boreal and temperate trees are often locally adapted and experience reduced fitness when planted in other areas of the species' range (Savolainen et al. 2007, Savolainen et al 2011). Thus, the extent and rate of migration is likely to be influenced by the degree and type of local adaptation, in addition to plasticity needed to cope with changing or novel

environments. Two important aspects of local adaptation in boreal and temperate forests are growth rate and freeze tolerance/avoidance (Saxe et al. 2001). Both photoperiod and temperature cues are central in timing phenological events, therefore this tradeoff results in strong latitudinal clines in phenology and growth (Aitken et al. 2008). Climate warming has, and will continue to, increase the available growing season at high latitudes (Juday et al. 2005, Linderholm 2006), but photoperiod does not change with climate. This may reduce the efficacy of local adaptation to historical temperature and photoperiod dynamics (Olson et al. in press). In other words, populations that are adapted to historical and current environments may not be the best performers in future climates.

As a result, it is widely expected that genotypes from southern populations, adapted to longer growing seasons and higher growth temperatures, will migrate northward, outcompeting northern genotypes with shorter growing seasons (Rehfeldt et al. 1999, Way and Oren 2010). In addition to increased average temperatures, extreme weather events, such as growing-season frosts, are also expected to increase in frequency in northern environments, particularly in the spring and fall (IPCC 2007). Given a potential phenological mismatch, it is unclear if trees that are locally adapted to photoperiod and temperature regimes in the south of a species' range will be able to successfully colonize northern latitudes, without adaptation to the new photoperiodic regimes (Saikkonen et al. 2012, Olson et al. in press).

In addition to increasing growing season lengths, boreal trees will be subject to direct effects of warming on physiological processes, which may differ for genotypes in different parts of the species' range (Ghannoum and Way 2011). Trees exhibit thermal

optima for physiological processes that are generally close to the growing temperatures of local climates (Lambers et al. 1998, Sage and Kubien 2008), although trees can readily acclimate to warming temperatures until a temperature threshold is met (Atkin and Tjoelker 2003). Acclimation responses and thermal tolerances often vary across a species' range (Silim et al. 2010). For example, high-latitude populations often grow below their thermal optima and are expected to respond positively to increased growing-season temperatures, whereas populations at the southern range limit may be stressed by future warming (Ghannoum and Way 2011, Wertin et al. 2011). Observations across temperature gradients, common gardens, and artificial warming/growth chamber experiments have shown that many boreal species, but not all, respond positively to warming (Goldblum and Rigg 2005, Lin et al. 2010, Way and Oren 2010). Deciduous boreal taxa, such as *Betula* (Hobbie and Chapin 1998, Kellomäki and Wang 2001) and *Populus* (Way et al. 2012), demonstrate positive growth trends in response to warming whereas there is evidence that high-latitude coniferous taxa, such as *Picea,* may currently be at or approaching their upper temperature thresholds as a result of temperature-induced drought stress (Barber et al. 2000, Goldblum and Rigg 2005, McGuire et al. 2010, Juday and Alix 2012). The differential responses of boreal tree species to increased growth temperature have biome-level implications in a changing climate.

Here we describe a warming experiment in which genotypes collected from a latitudinal transect in the western range of balsam poplar (*P. balsamifera* L.) were grown in a common garden environment at 64.8 °N, near the northern edge of the species distribution (Fig 3.1). A long history of common garden experiments show that balsam

poplar and its sister species, black cottonwood (*P. trichocarpa* Torr & A. Gray) is locally adapted to photoperiodic cues for phenological timing, particularly growth cessation and bud set (Pauley and Perry 1954, Howe et al. 1996, Soolanayakanahally et al. 2012, Olson et al. in press), resulting in a latitudinal cline of decreasing growing season length with increasing latitude. Genetic and niche modeling studies indicate that balsam poplar populations may have been located in the Rocky Mountain regions of the contiguous 48 states during the last glacial maximum and likely recolonized Canada and Alaska within the last 10-15k years (Breen et al. 2012, Keller et al. 2012, Levsen et al. 2012). Thus, the current cline in phenology is likely to have been lost and re-formed several times during the glacial periods of the Pleistocene.

Our goal was to identify the extent to which local adaptation benefits or limits the colonization success of genotypes of balsam poplar into northern environments under present and future temperature scenarios, by addressing the following research questions:

(i) Does temperature influence growth or components of growth (phenology, photosynthesis, cold injury) of balsam poplar trees when grown at 64.8 °N, near the northern limit of the species' distribution?

(ii) Does source latitude influence growth or components of growth of balsam poplar trees when planted at 64.8 °N?

(iii) Does a longer growing season length due to experimental warming or latitudinal differentiation among source populations have a positive or negative impact on overall growth?

Materials and methods

Plant material and common garden design

In March of 2009, 150 dormant trees of balsam poplar, representing 75 distinct genotypes (two trees of each genotype), were rooted in the Institute of Arctic Biology greenhouse in Fairbanks, Alaska. These trees originated from 15 source populations (five genotypes per population) from the western portion of balsam poplar's range spanning 50 – 70 °N (Fig. 3.1). A portion of these genotypes are part of the AgCanBaP collection of the Agriculture and Agri-Food Canada (AAFC), Agroforestry Division, Indian Head, Canada (Soolanayakanahally et al. 2009), and the remaining genotypes were collected separately for the experiment described by Olson et al. (in press). The rooted trees were planted in a fenced-in fallow field at the University of Alaska Fairbanks (64.8 °N, 147.7 °W) during the summer of 2009. Climate and environmental information are given for Fairbanks in Table 1. Genotypes were planted in an 8 x 20 grid with trees spaced at 2.5 m intervals. Paired genotypes were always planted adjacent to one another, with the locations of genotype pairs randomized within the garden. A shelter row of locally-collected balsam poplar trees was planted along the periphery to minimize edge effects. The land was tilled and applied with glycoside herbicide three weeks prior to planting, and the garden was weeded throughout the growing seasons mechanically and by hand. Herbivorous insects were removed by hand approximately once per week, but no insecticides were applied. Trees were watered during and shortly after planting but were otherwise not irrigated. Trees that experienced winter mortality were replaced with newly rooted trees of the same genotype the following summers.

Warming treatment

One individual of each genotype pair was randomly selected to be passively warmed and the other was grown under ambient conditions. Passive warming was achieved by surrounding tree transplants with 1.0 m diameter open-top chambers (OTCs) made from 0.7 mm clear plastic sheeting. Water containers (3.8 L) were painted black and placed inside the OTCs in order to store day-time solar heat and radiate it back into the chambers at night. This treatment increased both diurnal and nocturnal air temperatures. OTCs were placed around the trees on May 3 in 2010 and 2011, approximately 10 days before the average bud flush date for local balsam poplar. The chambers were removed on September 20 of each year, 2-3 weeks after the average first frost date.

iButton Thermochron dataloggers (Maxim Integrated Products, San Jose, California) placed near 50 randomly chosen trees (25 in OTCs, hereafter warmed, and 25 in ambient conditions, hereafter control) recorded air temperature at ground level and soil temperature at 10 cm soil depth hourly. Additionally, four randomly chosen control and genotype pairs were monitored for relative humidity, soil temperature at 10 cm and 20 cm depth, and soil volumetric water content (soil moisture averaged across 5 - 20 cm below ground) using a Campbell CR1000 datalogger, Campbell Scientific Inc., Logan Utah. OTCs increased air temperature surrounding the trees by an average of 3 ℃ compared to controls across the two growing seasons (Fig. 3.2; t-test; $t = 20.55$; $p < 0.0001$), and the amount of warming did not differ for the two experimental years.

Diurnal air temperature for warmed individuals was on average 3.42 °C ± 0.03 (SEM) higher than for control individuals and 2.51 ± 0.04 °C warmer nocturnally (Fig. 3.2). The OTCs also increased soil temperature at 10 cm depth by ~ 0.8 °C ($t = 9.84$; $p < 0.0001$; mean for warmed trees = 13.63 ± 0.06 °C; mean for control trees 12.84 ± 0.05 °C). There was no significant chamber effect on percent relative humidity 10 cm above ground ($t = -0.85$; $p = 0.40$; mean warmed = 66.82 ± 0.43; mean control 66.27 ± 0.45), soil temperature at 20 cm depth ($t = -0.55$, $p = 0.58$), or percent soil volumetric water content; $t = -0.78$; $p = 0.31$; mean warmed = 0.23 ± 0.01; mean control 0.23 ± 0.01).

Growth and growth components measurements

Height from soil level to apical meristem, stem diameter at 5 cm, number of leaves, and number of lateral buds were measured for each individual every 14 days. Genotypes from the Cottonwood population (69.1 °N) required diameter measurements at 2 cm above ground because of their small stature when transplanted. Height growth and diameter growth were calculated as the difference between final and initial measurements within a single growing season.

Measured growth components included vegetative bud phenology, photosynthesis, and cold injury. Bud flush was recorded as the date when leaf scales opened and leaves began to emerge from the bud, and was measured daily from the first week of May until all trees had flushed. Bud set, recorded when bud scales fully formed around the apical bud, was monitored every two to three days throughout the growing season and then measured daily from August 1 until all individuals had set bud. Bud flush

date and bud set date are reported as the calendar days of the year starting with January 1. Growing season length for each tree was calculated as the number of days between bud flush and set. Instantaneous photosynthetic rates per unit leaf area (A_a) were measured on cloudless days between 11:00 and 13:00 h once per month from June through September for a subset of 80 individuals (40 warmed and 40 control), using a LI-6400XT Portable Photosynthesis System (LI-COR Environmental, Lincoln, Nebraska) and 6400-40 Leaf Chamber Fluorometer with integrated LED light source. The block temperature was set to ambient air temperature, and for each measurement day, light levels inside the leaf chamber were matched to ambient conditions detected at the beginning of measurement; light levels were held constant for subsequent measurements. Overwinter mortality and cold injury were scored in the springs of 2010 and 2011. Growing-season mortality and damage from all sources were recorded biweekly. To determine mortality and injury due to cold rather than associated with planting, summer drought, herbivory or any other cause, we looked for evidence of frost damage in the form of forking and dead, but intact buds and stems, particularly in the upper third of the trees.

Geo-climatic data

Geo-climatic variables for each source population where tree cuttings were originally collected are listed in Table 1. Thirty-year climate normals (1971 - 2000) for weather stations located near source locations for sample populations were obtained from Environment Canada (http://climate.weatheroffice.gc.ca/climate_normals/index_e.html) and from the Alaska Climate Research Center

(http://akclimate.org/Climate/Normals/index.html). Geo-climate variables were chosen to be consistent with Soolanayakanahally *et al.* (2009) and include latitude (LAT; °N), longitude (LON; °W), elevation (ELV; m a.s.l.), frost free days, or the average number of consecutive days with low temperatures above freezing (FFD; days), mean annual air temperature (MAT; °C), mean annual summer (June, July, August) air temperature (MST; °C), mean temperature of the coldest month (MTCM; °C), mean temperature of the warmest month (MTWM; °C), mean annual precipitation (MAP; mm), and mean summer precipitation (MSP; mm). Continentality (CONT), which is defined as the difference between MTWM and MTCM, is a proxy of the effects of large land masses on temperature (Guy and Holowachuk 2001). Annual dryness index (ADI) and summer dryness index (SDI) were calculated following the equations in Guy and Holowachuk (2001), which related saturation of vapor pressure (as a proxy for potential evapotranspiration) to precipitation and temperature (annually or seasonally). We also included cumulative growing degree days (cGDD) with base temperature of 5 °C as described in Chapter 1.

Analyses

The latitudinal gradient chosen for this experiment is a proxy for gradients in mean annual temperature and photoperiod to which the genotypes chosen for this study may be locally adapted; however, the environments of the source populations differ in more than temperature and day length. To account for covariates in the source-provenance environment, we performed a principal components analysis (PCA) on the

geo-climatic variables for each population listed in Table 1. Principal components that combined to explain ≥ 90% of the variation in the geo-climatic data were included as independent variables to represent environmental gradients across source populations for subsequent analyses.

Environmental and warming treatment effects on trees were tested using MANCOVA on the four overall growth measurements: height growth, diameter growth, leaf number, and number of lateral buds. Independent variables included warming treatment and the two dominant environmental principal components, ePC1, and ePC2, experimental year (2010 and 2011 growing seasons), population, and genotype. Tree age (one or two years old, depending on needs for re-planting) and initial height, diameter, leaf count, and lateral bud count (as measured before the start of the experiment) were included as covariates. Trees with missing values for any response variable were discarded from the analysis. Dependent variables were transformed to conform to a multivariate normal distribution using a multivariate box-cox transformation (Weisberg 2005). Roy's greatest root was used to evaluate the significant effects of independent variables.

To further explore the effects of growing temperature and source environment on overall growth, seasonal relative height growth, phenology, and monthly photosynthetic rates over the two growing seasons, repeated-measures ANCOVA were implemented with linear mixed effects models following the procedure outlined in Chapter 1. In short, hierarchical models were constructed for each growth or growth component measurement that included warming treatment, the dominant environmental principal components

(ePC1 and ePC2), measurement year (2010, 2011), and all interaction terms as within-subject factors. Random effects included population, genotype, and individual tree number (subject ID for repeated measures). For biweekly growth comparisons, both growth increment during that bi-weekly time interval (hereafter referred to as time interval) and year, and their interactions were included as within-subject effects. Bi-weekly interval was handled as a discrete variable. Initial measurements for height, diameter, leaf number and bud number were included in all models of overall growth as covariates; the statistical significance of effects of independent variables on initial measurements was not calculated. Starting from the most parameter-rich model (full model), an interaction term (starting with the most complex) or a single independent variable was dropped and the likelihoods of the two models with and without the term were compared with -2 times the difference in the two log likelihoods ($-2 \Delta \, lnL$). All relevant model combinations were considered. Significance of the likelihood ratio test (a=0.05) was calculated using parametric bootstrapping with 5000 iterations (Faraway 2010). If the model fit was not significantly improved by the term, the term was dropped and another interaction or variable was tested. Cold injury and overwinter mortality were each analyzed as binomial data with general linear mixed effects models using the binomial family; warming treatment and ePC1 and ePC2 were included as independent variables.

As there is strong selective pressure for young trees to maximize height growth (Davis et al. 2005), we wanted to determine which growth variables and components contributed to height growth under warmed and control conditions. For example, were

trees that had the highest height-growth increment the same as those that grew the longest, had larger diameters, or more leaves or lateral buds (branches)? Significant relationships among growth variables could indicate successful aspects of the life history that allow for successful colonization at 64.8 °N. Linear mixed effects models were constructed with overall relative height growth as the response variable and season length, flush day, set day, relative diameter growth, leaf increment, and lateral bud increment as fixed effects, and genotype as a random effect.

All statistical analyses were conducted in R (v. 2.15; R Development Core Team, 2011), and the mixed effects models utilized the *lme4* R package (Bates and Maechler 2009). All dependent variables were transformed to fit normal distributions via box-cox transformations.

Results

Variation in source-provenance environment

Environmental variation across the latitudinal transect was almost completely accounted for in the first two principal components; ePC1 and ePC2 explained 76 % and 17% variation in the geo-climatic data (Table 3.1), respectively. ePC1 is largely representative of latitude and its correlates, temperature and precipitation (Table 3.2), whereas ePC2 largely represented variation in continentality, as influenced by warm summers and cold winters (mean temperature of the warmest and coldest months; Table 3.2).

The effects of increased temperature on growth and growth components

Over the course of the two growing seasons, experimentally warmed balsam poplar trees grew significantly larger than those grown under ambient conditions (MANCOVA, $F= 6.71$, $p < 0.001$; Table 3.3; Fig 3.3). By the end of the second growing season, warmed trees had grown 69% taller (mean relative height growth warmed = 31.0 ± 2.19 cm ; control = 18.32 ± 1.69 cm), were 34% greater in diameter (mean relative diameter growth warmed = 3.31 ± 0.23; control = 2.47 ± 0.17), had 34% more leaves (mean leaf number growth warmed = 75.0 ± 0.87; control = 65.7 ± 0.78) and 32% more buds (mean lateral bud number warmed = 38.63 ± 4.10; control = 29.33 ± 3.41) compared to controls. Growth responses were different in the two growing seasons, suggesting that developmental stage is an important factor in environmental influences on growth. In both the MANCOVA analysis (Table 3.3) and the univariate tests, there were significant interactions between independent variables and year for relative height growth, leaf number, and lateral bud number, thus the two growing seasons were analyzed separately for these growth variables (Table 3.4). In the first growing season (2010), the warming treatment did not result in taller trees or more leaves, but it did have a significant effect on relative diameter increment; diameter growth was higher in control trees than warmed trees (Table 3.4; mean warmed relative diameter growth = 1.32 ± 0.02 cm; control = 1.38 ± 0.03 cm).

Interestingly, the warming treatment resulted in a significant increase in growing season length (number of days between bud flush and bud set) for balsam poplar for both

growing seasons. Warmed trees had an average of 81.08 ± 2.28 growing days compared to 74.65 ± 1.98 for control trees, or a difference of about 6.5 days (Table 3.5, Fig. 3.4a). The increase in growing season length for warmed trees was primarily influenced by delayed bud set, rather than earlier bud flush. This is surprising because in poplar, timing of bud flush is generally thought to be cued in part by temperature whereas timing of bud set is considered to be determined primarily by photoperiodic cues (Howe et al. 2003, Way 2011). The mean date of bud set of warmed trees was on average five days later than for control trees (mean date of bud set for warmed seedlings = 216.12 ± 2.26; mean for control seedlings = 211.74 ± 2.12; Fig. 3.4b). Mean date of bud flush for warmed seedlings was 136.4 ± 0.80, and the mean for control trees was 137.09 ± 0.59 (Fig. 3.4c). There were no significant differences in phenology between 2010 and 2011.

Warming also influenced photosynthetic rates, but not as expected. Photosynthetic rates per unit leaf area were significantly lower for warmed seedlings compared to control seedlings in the months of June (mean warmed = 14.69 ± 0.74 µmol $m^{-2}sec^{-1}$; mean control = 17.05 ± 0.89 µmol $m^{-2}sec^{-1}$), July (mean warmed 23.68 ± 0.94 µmol $m^{-2}sec^{-1}$; mean control = 26.36 ± 0.78 µmol $m^{-2}sec^{-1}$) and August (mean warmed 20.67 ± 0.56 µmol $m^{-2}sec^{-1}$; mean control = 21.56 ± 0.45 µmol $m^{-2}sec^{-1}$) but the difference with warming treatment was not significant in September (mean warmed = 12.4 ± 0.72 µmol $m^{-2}sec^{-1}$; mean control = 13.02 ± 0.96 µmol $m^{-2}sec^{-1}$; Table 3.5; Fig 3.5). The increased growth observed in warmed trees was not a direct effect of increased photosynthesis.

Influence of source environment on growth and growth components

Growth differences among genotypes in the common environment were apparent. Source environment had a significant effect on growth as indicated by both the MANCOVA and for each independent growth variable and components of growth in both growing seasons (Tables 3.3 & 3.4). The influence of ePC1 (latitude and its correlates, temperature and precipitation) was associated with relative height growth, increased relative diameter growth, number of leaves and number of buds in both growing seasons (Table 3.4; Fig 3.3). In 2010, all four growth variables increased linearly with ePC1. In other words, the genotypes from southern source provenances had higher overall growth than those from mid latitudes or those from northern populations (Fig 3.3). This trend was similar in 2011; however, there was a significant curvilinear trend with relative height growth (Table 3.3). In this year, three mid- to high-latitude populations, Hay River (60.8 °N), Whitehorse (60.7 °N), and Fairbanks (64.8 °N) exhibited greater height increments than the other populations. In contrast, only one mid- to high-latitude population, Hay River, (60.8 °N) had greater diameter increment relative to other populations (Fig. 3.3 a,b). ePC2 (continentality) of source populations influenced the numbers of leaves and lateral buds in both experimental years (Table 3.4), showing that environmental variation other than that associated with latitude are important in influencing tree growth and morphology.

The relationship between growing season length and source environment shows strong genotypic differences in phenology across the distribution of balsam poplar (Fig.

3.4). Both date of bud flush and date of bud set occurred earlier for trees from northern environments (ePC1) (Fig. 3.4b,c; Table 3.5). Because of this strong pattern with season length and source environment, it is not surprising that incidence of cold injury showed a significant relationship with ePC1, with southern genotypes experienced higher rates of cold damage than northern genotypes (Table 3.5). Following the first winter31 trees, or about 21%, showed signs of cold injury in buds and stems. Incidence of cold injury following the second growing season, however, was only observed in eight individuals, all from source provenances below 55 °N latitude. The warming treatment did not influence occurrence of cold injury (Table 3.5) likely because the one-week extension of the growing season did not put trees at increased risk of cold damage. Despite the increasing occurrence of cold injury with decreasing latitude of origin, southern and mid-latitude trees experienced low rates of mortality and high growth increment the following summers. Cold injury may explain the curvilinear trend in height growth in the second growing season. Southern trees grew tallest in the first experimental year, but were also the most damaged by cold. Frost damage to the apical meristem results in forking and can lead to increased branching and lateral growth and decreased height growth. Winter mortality was more difficult to distinguish from mortality due to other causes. Total mortality was low (nine out of 150 trees died following the first winter and 2 following the second). There was no relationship of mortality with source environment (Table 3.5).

Seasonal effects of source latitude

The relationship of relative height growth with ePC1 varied across the growing season (Fig 3.6). This pattern highlights the importance of phenology in the final height growth patterns. In both years, genotypes from northern populations began growing earlier (Fig. 3.3c) and had higher growth rates early in the summer (Fig. 3.6a,b). By July, southern genotypes started to grow taller (per two-week interval) than those in the north, and this trend continued throughout the remainder of the growing season (Fig. 3.6a,b). Despite the higher growth rates early in the season, the shorter length of growing season limited overall growth increment in northern genotypes within a single summer. These seasonal trends were analyzed separately for 2010 and 2011 because the magnitude of the trends differed between years, as indicated by the significant interaction between growth period and year (Table 3.6). The overall pattern in seasonal growth was similar, however, in 2011, the trend was curvilinear, with mid- to high-latitude trees growing more before mid-July and southern genotypes growing more later in the season (Fig 3.6b).

Photosynthetic rates per unit leaf area also exhibited seasonal trends with ePC1 similar to those observed for growth (Table 3.5; Fig 3.5). Genotypes from more northern source populations (more negative values along the ePC1 axis) exhibited significantly higher instantaneous photosynthetic rates for the majority of the growing season (Fig. 3.5). The slope of the relationship of A_a with ePC1 is steeper earlier in the season and southern genotypes retain higher photosynthetic rates through August, past the time when the majority of trees from northern sources set bud and ceased growth (Table 3.5; Fig. 3.5).

Discussion

Recent climate warming has resulted in a 45% increase in the frost-free period and a 1.6 ℃ increase in the mean summer temperature since the beginning of the last century in Fairbanks, Alaska, the only location in central and northern Alaska with a continuous temperature record since the beginning of the 20th century (Wendler and Shulski 2009). These climate changes are likely to influence competitive outcomes among species influencing ecosystem level processes in the boreal forest. Our studies show that experimental warming increased the length of the growing season by 6-7 days and resulted in a 69% increase in height growth for rooted balsam poplar cuttings grown at 64.8 °N.

G*rowth response to warming*

Increased height growth in response to warming was a generalized response, with no differences among genotypes from the north and south. Both increased growth response due to warming and lack of interaction between warming and source latitude were also found in growth chamber experiments utilizing many of the same genotypes (Chapter 1). Adaptation to local climates and differing thermal optima for growth of trees originating from different regions, however, are expected to result in different effects of temperature on tree growth across a species' range (Ghannoum and Way 2011). Effects of source genotype on height growth in our *P. balsamifera* experiments were strong, with southern genotypes growing more than northern ones in both warming and control

treatments. Variation in growth response to warming across latitude of origin has also been identified in multi-site provenance trials spanning species' distributions. In Scots pine (*Pinus sylvestris* L), climate transfers of 1-4 °C resulted in positive growth from all latitudes, except at the southern (< 54 °N) and northern (> 62 °N) range extremes (Reich and Oleksyn 2008). In a reciprocal transplant experiment of European aspen (*Populus tremula*), southern genotypes consistently grew taller, larger in diameter, and had higher degree of branching in both northern and southern common gardens in Sweden (Luquez et al. 2008). Significant genotype by environment interactions were observed for both height growth and diameter growth, suggesting genetic variation for plasticity. Nonetheless, the latitudinal cline in growth indicated that southern genotypes have overall higher growth rates than northern genotypes in a variety of environments. Clearly there are population-level differences in growth potential that, if accounted for, may increase the accuracy of forecasts using species distribution models.

Recent efforts to include both genetic and ecological intraspecific variation in modeling species growth and distribution with respect to climate change produce drastically different outputs than those that predict a homogenous response. For example, O'Neill et al. (2008), integrated long-term provenance data from lodgepole pine (*Pinus contorta* Douglas) with climate data to predict species' future distributions and productivity. Models that assumed lodge pole growth responses were consistent across its range projected wholesale increases in productivity and northward range expansion. Models that included both genetic and ecological variation projected large growth declines in some parts of the species' range while increases in production in other

populations (O'Neill et al. 2008). Both environmental and genetic variation can influence which populations will be winners and which will be losers in a future climate.

Plasticity in bud set in response to warming

Poplar relies primarily on day-length cues for determining the date of bud set (Pauley and Perry 1954, Bradshaw and Stettler 1995, Howe et al. 1996, Olson et al. in press), but our experiment indicates that growth temperature also influences this trait. Importantly, the delay of bud set with warming suggests that balsam poplar can respond to projected lengthening of growing season *in situ*, without relying on northward migration by southern genotypes. This finding is consistent with recent experimental evidence that warming results in delayed growth cessation in hybrid poplars compared across a latitudinal range of field sites (Rohde et al. 2011). Surprisingly, warming did not influence the timing of bud flush, which is generally thought to be more sensitive to temperature cues than bud set (Howe et al. 2003), and is also unexpected given the observed trends of earlier greening across the boreal forest (Linderholm 2006). The lack of influence may have resulted partially from the initiation of warming at our sites beginning only 10 days before bud flush, whereas an earlier start for the warming treatment may have resulted in a more drastic effect.

Importance of photoperiod effects

Although warming did influence the timing of bud set, adaptation to local photoperiod explained 42% of the variance in growing season length. For instance, the

two-year average bud set date for the farthest south population (Portage, 49.9 °N) was August 30, compared to June 19 for the farthest north population (Cottonwood, 69.1 °N), a difference of over two months. Despite higher incidences of cold injury, longer growing seasons positively influenced tree growth.

Northern genotypes flushed bud earlier and exhibited higher growth increment early in the growing season, but the shortened growing season, due to early bud set, resulted in lower overall growth. In the second growing season, genotypes from mid to north latitudes (60 - 65 °N) had the highest relative height growth. These genotypes may have been able to capitalize on earlier flush dates and higher early-season growth, while still setting bud early enough to avoid frost damage. Photosynthetic rate also showed a similar seasonal pattern with source environment, suggesting that early-season growth and high photosynthetic rates are compensation for shorter growing seasons (Soolanayakanahally et al. 2009). Common garden studies in which photosynthesis is measured once during the growing season could report different trends of photosynthetic rates among populations depending on the time of measurement, although a correlation between mid-summer photosynthesis and ensuing fall hardiness was observed in Sitka alder (*Alnus sinuata* [Reg.] Rydb.) and paper birch (*Betula papyrifera* Marsh.), again demonstrating the influence of photoperiod on tree growth (Benowicz et al. 2000).

The duration of our experiment was too short to fully capture the variability in spring and fall temperatures and cold damage may be cumulative, as indicated by our data; therefore we cannot determine the true risk of extended growing seasons in northern environments. Over the course of the two experimental seasons, however, freezing

temperatures were recorded on 11 days in May, 6 days in August, and 7 days in September. Although spring and fall cold injury could impose a barrier to northward migration, there may be enough variability in shoulder-season frosts for colonizing seedlings to become established. Risk of cold injury and mortality for immigrating southern genotypes may decrease as trees age. First and second year seedlings have been observed to extend growth longer into the fall than adult trees, likely due to severe juvenile competition for light gaps (Howe et al. 2003).

Genetics of adaptive clines

Here we are interpreting the genetic differentiation among populations as evidence of local adaptation. Phenological clines along photoperiod/climate gradients in poplar have been recognized as having a genetic basis for nearly 70 years (Pauley & Perry 1954), and similar patterns have been demonstrated for multiple tree species (reviewed in Morgenstern 1996, Howe et al. 2000, Neale and Ingvarsson 2008). Population differences in traits such as phenology, growth, and cold tolerance are not random in that they are strongly associated with environmental or geographic gradients, which have been widely interpreted as evidence of adaptive variation (Howe et al. 2003, Savolainen et al. 2007, Rohde et al. 2011). The genetic basis of phenological clines, however, is poorly understood (Olson et al. in press).

Quantitative trail loci (QTL) studies indicate that traits involved in local adaptation to these environmental clines are generally the products of several genes of small effect, making it difficult to identify individual loci that control quantitative traits

(Howe et al. 2003), but these methods have been useful in determining the adaptive significance of phenotypic traits. In boreal and temperate trees, adaptive QTL have been identified for cold-tolerance traits (Chen et al. 2002, Neale and Savolainen 2004), bud phenology (Frewen et al. 2000, Chen et al. 2002, Scotti-Saintagne et al. 2004), height and diameter growth (Wu et al. 2003, Scotti-Saintagne et al. 2004) among others. Association mapping methods have also been useful in identifying individual loci underlying phenotypic variation. Single nucleotide polymorphisms (SNPs) in the gene *Phytochrome B1* (*PHYB1*) was found to associate with bud set (Ingvarsson et al. 2008), as did multiple SNPs in the *CO/FT* pathway genes *LEAFY* and GIGANTEA 5 (Olson et al. in press). SNPs within FRIGIDA were associated with bud flush (Olson et al. in press). Using F_{st} outlier analyses, the abscic acid gene *ABI1B* was found to significantly vary along temperature gradients and the circadian rhythm genes *ELF3* and *GI5* genes strongly varied with latitude and precipitation (Keller et al 2012). Association genetics has also identified loci related to height and diameter growth (Romšáková et al. 2012). Although these techniques provide strong evidence of local adaptation to environmental gradients, there are still many unanswered questions regarding the genomic architecture of adaptive clines, and if novel genotypes will be required in a directionally-changing climate.

Management Implications

Although boreal species have undergone repeated continental-scale migrations in response to Quaternary climate oscillations (Davis and Shaw 2001, Hewitt 2004), the rate of future projected climate warming may outpace expected migration rates (Davis et al.

2005). Moreover, habitat fragmentation due to land use can serve as a barrier to natural migration. Thus, assisted migration practices to help species track climate envelopes has been widely discussed in the conservation community, but not without controversy (McLachlan et al. 2007, Marris 2008, Vitt et al. 2010). If adaptation to novel photoperiod/temperature regimes is not required, managers may simply move species or populations to compensate for migration barriers (Olson et al. in press). Common gardens and artificial warming experiments, both in field and greenhouse settings (Aitken et al. 2008), should be implemented prior to any implementation of migration interventions (Gray et al. 2010). Our results suggest that if barriers to migration exist for balsam poplar, assisted migration would aid in introducing better growing genotypes than are currently located in interior Alaska. Since seed and pollen in *P. balsamifera* are windborne and gene flow is extensive across broad geographical ranges (Keller et al. 2010), assisted migration may not be necessary for this species.

Increasing energy costs and demand in northern communities are increasing the desire for renewable, local source fuel, such as biomass (Fresco and Chapin 2009). *Populus* has long been recognized as a viable genus for biomass fuel given its fast growth rates (Bradshaw et al. 2000, Dillen et al. 2011). Foresters are looking to the scientific community to assist in cultivation and management best practices for maximizing biomass yield, while maintaining ecological integrity, in a changing climate. Our common-garden experiment near the northern range limit of balsam poplar can begin to inform these types of management and conservation questions for both current and future, warmer climates.

Conclusion

Our data suggest that balsam poplar trees will positively respond to climate warming, *in situ*, in even the northernmost populations. This increased growth capacity may give balsam poplar and other deciduous boreal trees a competitive advantage over coniferous taxa (Way and Oren 2010). Despite the general positive response to warming in all populations, genotypes transferred from source populations 5 - 10° south of the common garden location performed the best under both ambient and warmed conditions. This may suggest that local genotypes are not the best adapted to local environments, perhaps as a result of 20th century warming. Regardless, evidence from this experiment suggests that adaptation to local photoperiod does not inhibit the successful migration of southern genotypes of balsam poplar into northern environments.

References

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications **1**:95-111.

Atkin, O. K. and M. G. Tjoelker. 2003. Thermal acclimation and the dynamic response of plant respiration to temperature. Trends in Plant Science **8**:343-351.

Barber, V. A., G. P. Juday, and B. P. Finney. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature **405**:668-673.

Bates, D. M. and M. Maechler. 2009. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-32.

Benowicz, A., R. Guy, M. R. Carlson, and Y. A. El-Kassaby. 2000. Genetic variation among paper birch (*Betula papyrifera* MARSH.) populations in germination, frost hardiness, gas exchange and growth. Silvae Genetica **50**:7-13.

Bradshaw, H. D., R. Ceulemans, J. Davis, and R. Stettler. 2000. Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. Journal of Plant Growth Regulation **19**:306-313.

Bradshaw, H. D. and R. F. Stettler. 1995. Molecular genetics of growth and development in *Populus*. IV. Mapping QTLs with large effects on growth, form, and phenology traits in a forest tree. Genetics **139**:963-973.

Breen, A. L., D. F. Murray, and M. S. Olson. 2012. Genetic consequences of glacial survival: the late Quaternary history of balsam poplar (*Populus balsamifera* L.) in North America. Journal of Biogeography **39**:918-928.

Chen, I.-C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid range shifts of species associated with high levels of climate warming. Science **333**:1024-1026.

Chen, T. H. H., G. T. Howe, and H. D. Bradshaw Jr. 2002. Molecular genetic analysis of dormancy-related traits in poplars. Weed Science **50**:232-240.

Davis, M. B. and R. G. Shaw. 2001. Range shifts and adaptive responses to Quaternary climate change. Science **292**:673-679.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology **86**:1704-1714.

Dillen, S., S. Vanbeveren, al. N. Afas, I. Laureysens, S. Croes, and R. Ceulemans. 2011. Biomass production in a 15-year old poplar short-rotation coppice culture in Belgium. Aspects of Applied Biology **112**:99-106.

Faraway, J. J. 2010. Changes to the mixed effects models chapters in ELM. URL: http://www.maths.bath.ac.uk/~jjf23/ELM/mixchange.pdf.

Fresco, N. and F. S. C. III. 2009. Assessing the Potential for Conversion to Biomass Fuels in Interior Alaska. Research Paper, United States Department of Agriculture, Pacific Northwest Research Station.

Frewen, B. E., T. H. H. Chen, G. T. Howe, J. Davis, A. Rohde, W. Boerjan, and H. D. Bradshaw. 2000. Quantitative trait loci and candidate gene mapping of bud set and bud flush in *Populus*. Genetics **154**:837-845.

Ghannoum, O. and D. A. Way. 2011. On the role of ecological adaptation and geographic distribution in the response of trees to climate change. Tree Physiology **31**:1273-1276.

Goldblum, D. and L. S. Rigg. 2005. Tree growth response to climate change at the deciduous-boreal forest ecotone, Ontario, Canada. Canadian Journal of Forest Research **35**:2709-2718.

Gray, L. K., T. Gylander, M. S. Mbogga, P.Y. Chen, and A. Hamann. 2010. Assisted migration to address climate change: recommendations for aspen reforestation in western Canada. Ecological Applications **21**:1591-1603.

Guy, R. D. and D. L. Holowachuk. 2001. Population differences in stable carbon isotope ratio of *Pinus contorta* Dougl. ex Loud.: relationship to environment, climate of origin, and growth potential. Canadian Journal of Botany **79**:274-283.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **359**:183-195.

Hobbie, S. E. and F. S. Chapin. 1998. The response of tundra plant biomass, aboveground production, nitrogen, and CO2 flux to experimental warming. Ecology **79**:1526-1544.

Howe, G. T., S. N. Aitken, D. B. Neale, K. D. Jermstad, N. C. Wheeler, and T. H. Chen. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany **81**:1247-1266.

Howe, G. T., G. Gardner, W. P. Hackett, and G. R. Furnier. 1996. Phytochrome control of short-day-induced bud set in black cottonwood. Physiologia Plantarum **97**:95-103.

Howe, G. T., P. Saruul, J. Davis, and T. H. H. Chen. 2000. Quantitative genetics of bud phenology, frost damage, and winter survival in an F2 family of hybrid poplars. TAG Theoretical and Applied Genetics **101**:632-642.

Ingvarsson, P. K., M. V. Garcia, V. Luquez, D. Hall, and S. Jansson. 2008. Nucleotide polymorphism and phenotypic associations within and around the phytochrome B2 locus in European aspen (*Populus tremula*, Salicaceae). Genetics **178**:2217-2226.

IPCC. 2007. Climate Change 2007: The Physical Science Basis: Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. *IN* S. Soloman, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. Cambridge University Press, Cambridge, UK.

Juday, G. P. and C. Alix. 2012. Consistent negative temperature sensitivity and positive influence of precipitation on growth of floodplain *Picea glauca* in Interior Alaska. Canadian Journal of Forest Research **42**:561-573.

Juday, G. P., V. Barber, P. Duffy, H. W. Linderholm, S. R. Rupp, S. Sparrow, E. Vaganov, and J. Yarie. 2005. Forest, Land Management, and Agriculture. Pages 782-854 *in* C. Symon, L. Arris, and B. Heal, editors. Arctic Climate Impact Assessment. Cambridge University Press, New York.

Keller, S. R., N. Levsen, M. S. Olson, and P. Tiffin. 2012. Local adaptation in the flowering time gene network of balsam poplar, *Populus balsamifera* L. Molecular Biology and Evolution.

Keller, S. R., M. S. Olson, S. Silim, W. Schroeder, and P. Tiffin. 2010. Genomic diversity, population structure, and migration following rapid range expansion in the Balsam Poplar, *Populus balsamifera*. Molecular Ecology **19**:1212-1226.

Kellomäki, S. and K.-Y. Wang. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. Annals of Botany **87**:669-682.

Lambers, H., F. S. C. III, and T. L. Pons. 1998. Plant Physiological Ecology. Springer, New York.

Levsen, N. D., P. Tiffin, and M. S. Olson. 2012. Pleistocene speciation in the genus *Populus* (Salicaceae). Systematic Biology.

Lin, D., J. Xia, and S. Wan. 2010. Climate warming and biomass accumulation of terrestrial plants: a meta-analysis. New Phytologist **188**:187-198.

Linderholm, H. W. 2006. Growing season changes in the last century. Agricultural and Forest Meteorology **137**:1-14.

Luquez, V., D. Hall, B. Albrectsen, J. Karlsson, P. Ingvarsson, and S. Jansson. 2008. Natural phenological variation in aspen (*Populus tremula*): the SwAsp collection. Tree Genetics & Genomes **4**:279-292.

Marris, E. 2008. Moving on assisted migration.112-113.

McGuire, A. D., R. W. Ruess, A. Lloyd, J. Yarie, J. S. Clein, and G. P. Juday. 2010. Vulnerability of white spruce tree growth in interior Alaska in response to climate variability: dendrochronological, demographic, and experimental perspectives. Canadian Journal of Forest Research **40**:1197-1209.

McLachlan, J. S., J. J. Hellmann, and M. W. Schwartz. 2007. A Framework for debate of assisted migration in an era of climate change. Conservation Biology **21**:297-302.

Morgenstern, E. K. 1996. Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Siviculture. UBC Press, Vancouver, British Columbia.

Neale, D. B. and P. K. Ingvarsson. 2008. Population, quantitative and comparative genomics of adaptation in forest trees. Current Opinion in Plant Biology **11**:149-155.

Neale, D. B. and O. Savolainen. 2004. Association genetics of complex traits in conifers. Trends in Plant Science **9**:325-330.

O'Neill, G. A., A. Hamann, and T. Wang. 2008. Accounting for population variation improves estimates of the impact of climate change on species' growth and distribution. Journal of Applied Ecology **45**:1040-1049.

Olson, M. S., N. Levsen, R. Y. Soolanayakanahally, R. D. Guy, W. R. Schroeder, S. R. Keller, and P. Tiffin. in press. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics **37**:637-669.

Pauley, S. S. and T. O. Perry. 1954. Ecotypic variation of the photoperiodic response in *Populus*. Journal of Arnold Arboretum **35**:167-188.

R Development Team, R. D. C. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rehfeldt, G. E., C. C. Ying, D. L. Spittlehouse, and D. A. Hamilton. 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation Ecological Monographs **69**:375-407.

Reich, P. B. and J. Oleksyn. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. Ecology Letters **11**:588-597.

Rohde, A., C. Bastien, and W. Boerjan. 2011. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. Tree Physiology **31**:472-482.

Romšáková, I., E. Foffová, J. Kmeť, R. Longauer, M. Pacalaj, and D. Gömöry. 2012. Nucleotide polymorphisms related to altitude and physiological traits in contrasting provenances of Norway spruce (*Picea abies*). Biologia **67**:909-916.

Sage, R. F. and D. S. Kubien. 2008. The temperature response of C3 and C4 photosynthesis. Plant, Cell & Environment **30**:1086-1106.

Saikkonen, K., K. Taulavuori, T. Hyvonen, P. E. Gundel, C. E. Hamilton, I. Vanninen, A. Nissinen, and M. Helander. 2012. Climate change-driven species' range shifts filtered by photoperiodism. Nature Clim. Change **2**:239-242.

Savolainen, O., T. Pyhäjärvi, and T. Knürr. 2007. Gene flow and local adaptation in trees. Annual Review of Ecology, Evolution, and Systematics **38**:595-619.

Savolainen, O., S. T. Kujala, C. Sokol, T. Pyhäjärvi, K. Avia, T. Knürr, K. Kärkkäinen, and S. Hicks. 2011. Adaptive potential of northernmost tree populations to climate change, with Emphasis on Scots Pine (Pinus sylvestris L.). Journal of Heredity **102**:526-536.

Saxe, H., M. G. R. Cannell, Ø. Johnsen, M. G. Ryan, and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. New Phytologist **149**:369-399.

Scotti-Saintagne, C., C. Bodénès, T. Barreneche, E. Bertocchi, C. Plomion, and A. Kremer. 2004. Detection of quantitative trait loci controlling bud burst and height growth in *Quercus robur* L. TAG Theoretical and Applied Genetics **109**:1648-1659.

Silim, S., N. Ryan, and D. Kubien. 2010. Temperature responses of photosynthesis and respiration in *Populus balsamifera* L.: acclimation versus adaptation. Photosynthesis Research **104**:19-30.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, E. C. Drewes, and W. R. Schroeder. 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment **32**:1821-1832.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, and M. Song. 2012. Timing of photoperiodic competency causes phenological mismatch in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment:no-no.

Thomas, C. D. 2010. Climate, climate change and range boundaries. Diversity and Distributions **16**:488-495.

Vitt, P., K. Havens, A. T. Kramer, D. Sollenberger, and E. Yates. 2010. Assisted migration of plants: Changes in latitudes, changes in attitudes. Biological Conservation **143**:18-27.

Way, D. A. 2011. Tree phenology responses to warming: spring forward, fall back? Tree Physiology **31**:469-471.

Way, D. A., J.-C. Domec, and R. B. Jackson. 2012. Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. Plant, Cell & Environment:no-no.

Way, D. A. and R. Oren. 2010. Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. Tree Physiology **30**:669-688.

Weisberg, S. 2005. Applied Linear Regression. John Wiley & Sons, Hoboken, New Jersey.

Wendler, G. and M. Shulski. 2009. A century of climate change for Fairbanks, Alaska. Arctic **62**:295-300.

Wertin, T. M., M. A. McGuire, and R. O. Teskey. 2011. Higher growth temperatures decreased net carbon assimilation and biomass accumulation of northern red oak seedlings near the southern limit of the species range. Tree Physiology **31**:1277-1288.

Wu, R., C.-X. MA, M. C. K. Yang, M. Chang, R. C. Littell, U. Santra, S. S. Wu, T. Yin, M. Huang, M. Wang, and G. Casella. 2003. Quantitative trait loci for growth trajectories in *Populus*. Genetics Research **81**:51-64.

Tables

Table 3.1 Geo-climatic data of source populations included in this study: latitude (LAT), longitude (LON), elevation (ELV), frost free days (FFD), mean annual air temperature (MAT), mean summer (June, July, August) air temperature (MST), mean air temperature warmest month (MTWM), mean air temperature coldest month (MTCM), mean annual precipitation (MAP), mean summer precipitation (MSP), cumulative growing degree days (cGDD), continentality (CONT), annual dryness index (ADI), summer dryness index (SDI).

Source populations	LAT (°N)	LON (°W)	ELEV (m)	FFD	MAT (°C)	MST (°C)	MTCM (°C)	MTWM (°C)	MAP (mm)	MSP (mm)	cGDD (°5 C)	CONT	ADI	SDI
Cottonwood*	69.1	147.89	353	33	-11.3	5.8	-26.8	7.7	105.9	56.4	214	34.5	2.46	18.82
Norman Wells	65.28	126.80	73	133	-5.5	15.2	-26.5	17.0	291.0	123	1123	43.5	1.40	14.18
Fairbanks	64.8	147.87	248	142	-2.4	15.4	-22.2	16.9	274.6	137.4	1286	39.1	1.89	14.15
Galena	64.71	156.73	74	124	-3.7	12.7	-21.3	14.2	532.0	171	902	35.5	0.88	9.57
Nome	64.56	165.34	75	129	-2.5	10.1	-14.9	11.2	427.0	160	570	26.1	1.20	8.40
Denali National Park	63.87	149.02	594	122	-3.2	11.8	-22.3	16.1	235.0	159	1245	38.4	2.08	11.62
Hay River	60.8	115.78	168	144	-2.9	14.3	-23.1	15.9	320.4	125.2	1093	39	1.56	14.57
Whitehorse	60.7	135.33	770	138	-0.7	12.8	-18.4	14.8	267.4	111.1	895	33.2	2.19	15.30
La Ronge	55.15	105.26	379	237	-0.1	15.8	-20.4	17.2	483.8	215.8	1323	37.6	1.27	9.18
Grande Prairie	54.75	118.63	769	167	1.9	15.0	-15.0	15.9	446.6	208.7	3023	30.9	1.59	8.74
Boyle*	54.6	112.89	649	165	2.1	15.2	-14.9	16.2	503.7	258.8	1370	31.1	1.43	7.19
Edmonton	53.31	113.58	723	273	2.4	15.0	-13.5	15.9	482.7	252.9	1360	29.4	1.52	7.21
Grand Rapids	53.16	99.28	223	232	0.8	17.1	-19.7	18.6	473.7	214.4	1508	38.3	1.38	10.10
Sioux Lookout	50.08	91.9	384	168	1.6	17.2	-18.6	18.6	716.1	271.1	1578	37.2	0.97	7.98
Portage	49.9	98.26	259	174	3.1	18.5	-16.3	18.5	514.6	224.8	1848	34.8	1.50	9.57

*Climate stations within 300 km of source populations

Table 3.2 Loadings for each geo-climatic variable onto the two primary principal components, ePC1 and ePC2, and correlations of each original geo-climate variable on ePC1 and ePC2.

	PCA Loadings		Correlations	
Geo-climatic variables	ePC1	ePC2	ePC1	ePC2
LAT	-0.94	-0.01	-0.32601	-0.00835
LON	-0.75	-0.36	-0.26167	-0.23208
ELEV	0.28	-0.53	0.096375	-0.33917
FFD	0.83	0.05	0.289467	0.031503
MAT	0.95	-0.13	0.33181	-0.08643
MST	0.85	0.46	0.294677	0.299952
MTCM	0.73	-0.65	0.254375	-0.41699
MTWM	0.77	0.55	0.266578	0.354726
MAP	0.86	-0.01	0.300557	-0.0034
MSP	0.95	-0.11	0.330669	-0.06879
cGDD	0.71	0.05	0.246938	0.031186
CONT	-0.19	0.95	-0.06456	0.615261
ADI	-0.62	-0.17	-0.21525	-0.11213
SDI	-0.85	0.29	-0.29717	0.189881

Table 3.3 MANCOVA results for four growth response variables of balsam poplar transplants: relative tree height growth, relative diameter growth, leaf number, and number of lateral buds. Significance of independent variables was determined using Roy's greatest root. ePC1 and ePC2 are the dominant environmental principal components.

Independent Variable	DF	Value	F	num DF	den DF	P
Warming treatment	1	0.15335	6.709	4	177	**<0.001**
Year	1	2.69301	117.819	4	177	**<0.0001**
ePC1	1	2.52722	110.566	4	177	**<0.0001**
ePC2	1	0.4404	19.268	4	177	**<0.0001**
Population	12	0.77189	11.45	12	180	**<0.0001**
Genotype	58	2.3097	7.088	58	180	**<0.0001**
Warming treatment x Year	1	0.07557	3.306	4	177	**0.01**
Warming treatment x ePC1	1	0.03043	1.331	4	177	0.26
Warming treatment x ePC2	1	0.02837	1.241	4	177	0.3
Year x ePC1	1	0.12063	5.277	4	177	**<0.001**
Year x ePC2	1	0.03149	1.377	4	177	0.24
Warming treatment x Year x ePC1	1	0.03072	1.344	4	177	0.26
Warming treatment x Year x ePC2	1	0.01361	0.595	4	177	0.67
Warming treatment x ePC1 x ePC2	1	0.00575	0.251	4	177	0.91
Year x ePC1 x ePC2	1	0.02522	1.104	4	177	0.36
Warming treatment x Year x ePC1 x ePC2	1	0.03297	1.442	4	177	0.22

Table 3.4. Significant fixed and random effects for growth response variables, representing the best-fit linear mixed effects model. ePC1 and ePC2 are the first two principle components representing relative source environmental factors from each population. Year refers to the two experimental seasons (2010, 2011).

Response variable	Best-fit model	Significant fixed effects	Log likelihood ratio[a]/t-value*	p-value	Significant random effects	Log likelihood ratio[a]	p-value
Height growth 2010	ePC1 + Genotype	ePC1	26.52	< 0.0001	Genotype	14.44	< 0.0001
Leaf number 2010	ePC1 + ePC2+ Genotype	ePC1	21.89	< 0.0001	Genotype	91.5	< 0.0001
		ePC2	7.05	0.01			
Lateral bud number 2011	ePC1 + ePC2	ePC1	48.98*	< 0.0001	none	n/a	n/a
		ePC2	8.15*	< 0.01			
Height growth 2011	Warming Treatment + ePC1 + Genotype + $ePC1^2$	Warming Treatment	29.62	< 0.0001	Genotype	14.44	< 0.0001
		ePC1	7.11	<0.01			
		$ePC1^2$	21.43	< 0.0001			
Leaf number 2011	Warming Treatment + ePC1 + ePC2 + Genotype	Warming Treatment	7.04	0.01	Genotype	91.5	< 0.0001
		ePC1	30.16	< 0.0001			
		ePC2	7.66	< 0.01			
Lateral bud number 2011	Warming Treatment + ePC1 + ePC2	Warming Treatment	22.40*	< 0.0001	none	n/a	n/a
		ePC1	48.72*	< 0.0001			
		ePC2	11.26*	< 0.001			
Diameter growth	Warming Treatment + ePC1 + Year + Genotype	Warming Treatment	4.65	0.03	Genotype	6.72	< 0.01
		ePC1	16.67	< 0.0001			
		Year	85.79	< 0.0001			

Table 3.5. Significant fixed and random effects for growth components, representing the best-fit linear mixed effects model. ePC1 and ePC2 are the first two principle components representing relative source environmental factors from each population. Year refers to the two experimental seasons (2010, 2011).

Response variable	Best-fit model	Significant fixed effects	Log likelihood ratio[a]/t-value*	p-value	Significant random effects	Log likelihood ratio[a]	p-value
Growing Season Length	Warming Treatment + ePC1 + Genotype	Warming Treatment	14.40	< 0.001	Genotype	14.9	< 0.0001
		ePC1	79.69	< 0.0001			
Flush Day	ePC1 + Genotype + Population	ePC1	21.66	< 0.0001	Genotype	5.82	0.01
					Population	5.82	< 0.001
Set Day	Warming Treatment + ePC1 + Genotype	Warming Treatment	12.43	< 0.001	Genotype	24.09	< 0.0001
		ePC1	92.85	< 0.0001			
A_a June	Warming Treatment + ePC1 + ePC2 + Year + ePC1 x ePC2	Warming Treatment	3.92	0.05	none	n/a	n/a
		ePC1	5.87	0.02			
		ePC1 x ePC2	8.37	< 0.01			
A_a July	Warming Treatment + ePC1	Warming Treatment	2.81*	< 0.01	none	n/a	n/a
		ePC1	-3.31*	< 0.01			
A_a Aug	Warming Treatment + ePC1 + ePC2 + Year + ePC1 x Year	Warming Treatment	4.89	0.02	none	n/a	n/a
		ePC1	23.26	< 0.0001			
		ePC1 x Year	9.83	< 0.01			
A_a September	ePC1	ePC1	1.95	0.05	none	n/a	n/a
Cold Injury	ePC1 + Year	ePC1	4.42*	< 0.0001	none	n/a	n/a
		Year	-4.09*	<0.001			
Mortality	Year	Year	-4.13*	<0.0001			

Table 3.6 Significant fixed and random effects of seasonal patterns in relative height growth as measured from bi-weekly growth intervals (Interval) and best-fit linear mixed effects models. ePC1 and ePC2 are the first two principal components representing relative source environmental factors from each population.

Response variable	Best-fit model	Significant fixed effects	Log likelihood ratio[a]	p-value	Significant random effects	Log likelihood ratio[a]	p-value
Biweekly height growth (2010 &	Warming Treatment + ePC1 + Interval +	Warming Treatment	34.88	< 0.0001	Genotype	14.9	< 0.0001
2011 combined)	Interval x ePC1 +	ePC1	9.98	< 0.01			
	Interval x Warming Treatment	Warming Treatment x Interval	23.93	0.04			
		Interval x ePC1	238.59	< 0.0001			
		Interval x Year	212.87	< 0.0001			
Biweekly height growth 2010	Warming Treatment + ePC1 + ePC2 +	Warming Treatment	8.88	< 0.01	Genotype	21.93	< 0.0001
	Interval + Interval x	Interval	293.40	< 0.0001			
	ePC1	Interval x Warming Treatment	16.04	<0.001			
		Interval x ePC1	34.96	< 0.0001			
Biweekly height growth 2011	Warming Treatment + ePC1 + ePC2 +	Warming Treatment	8.35	0.02	Genotype	4.44	0.02
	Interval + Interval x	ePC1	7.67	< 0.0001			
	ePC1 + Warming	Interval	19.03	< 0.0001			
	Treatment x ePC1	Interval x ePC1	16.87	< 0.0001			
		Warming Treatment x ePC1	4.97	0.02			

Figures

Figure 3.1 Locations of the 15 source populations chosen for this study are shown with the western range of balsam poplar (*Populus balsamifera* L.) shaded in gray.

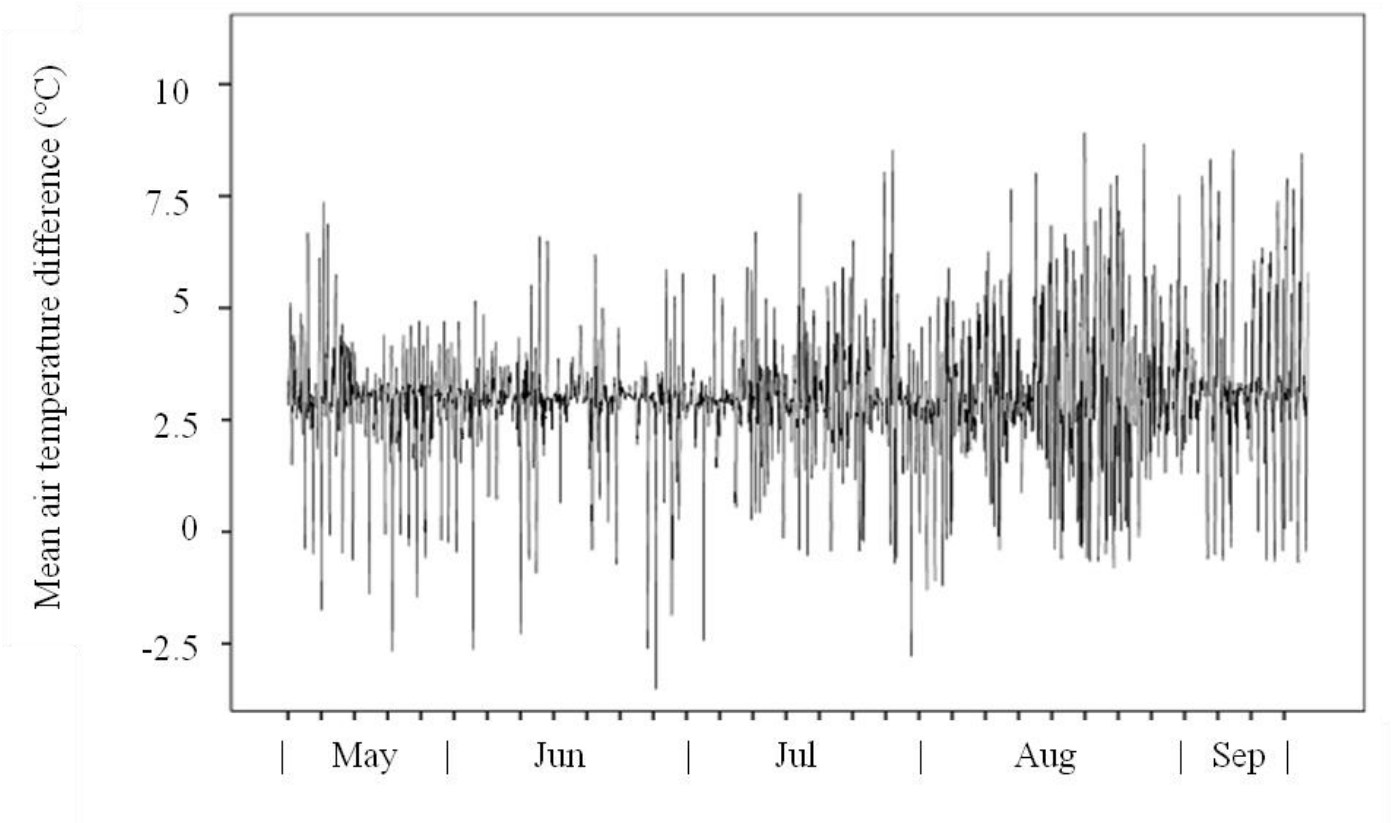

Figure 3.2 The average hourly air temperature difference between warmed and control trees (3.06 °C ± 0.02 mean ± SEM) for two growing seasons of an artificial warming experiment. Both diurnal and seasonal variation contributed to variation in solar radiation.

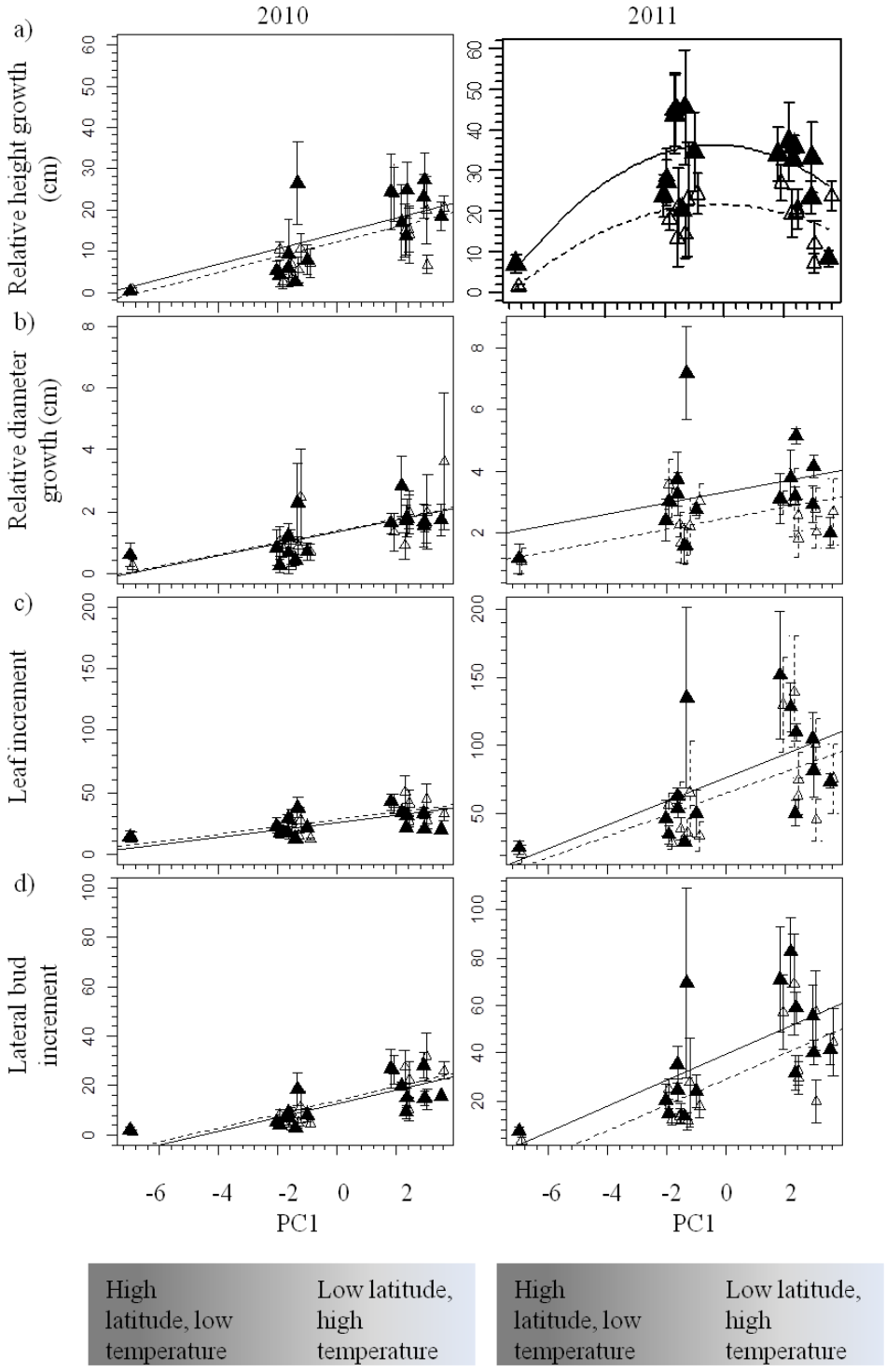

Figure 3.3 Relative height growth (a), relative diameter growth (b), leaf number increment (c), and lateral bud increment (d) for balsam poplar genotypes grown under warmed (closed triangles and solid lines) and control (open triangles and dashed lines) in a common garden environment over two growing seasons (2010, left; 2011, right), plotted against principal component 1 (PC1).

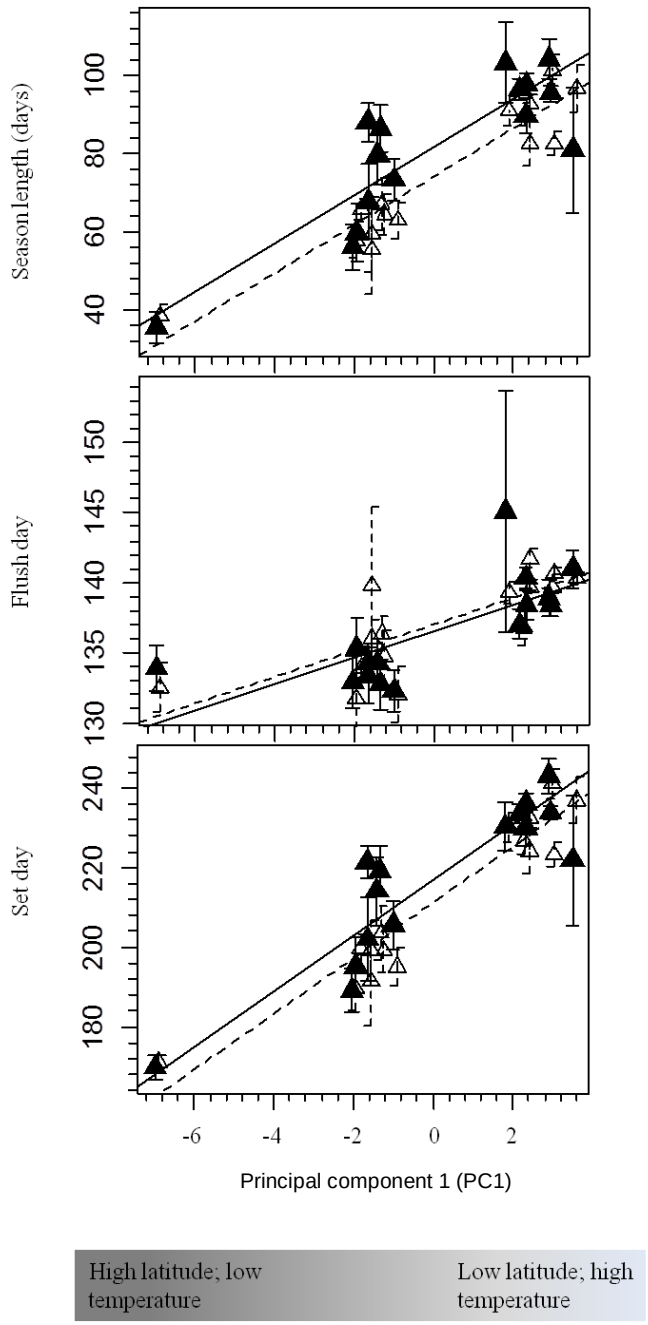

Figure 3.4 Growing season length as measured as the number of days between bud flush and bud set (a), calendar date of bud flush; b), and calendar date of bud set; c) for artificially warmed balsam poplar trees (closed triangles, solid lines) and trees grown under ambient conditions (open triangles, dashed lines).

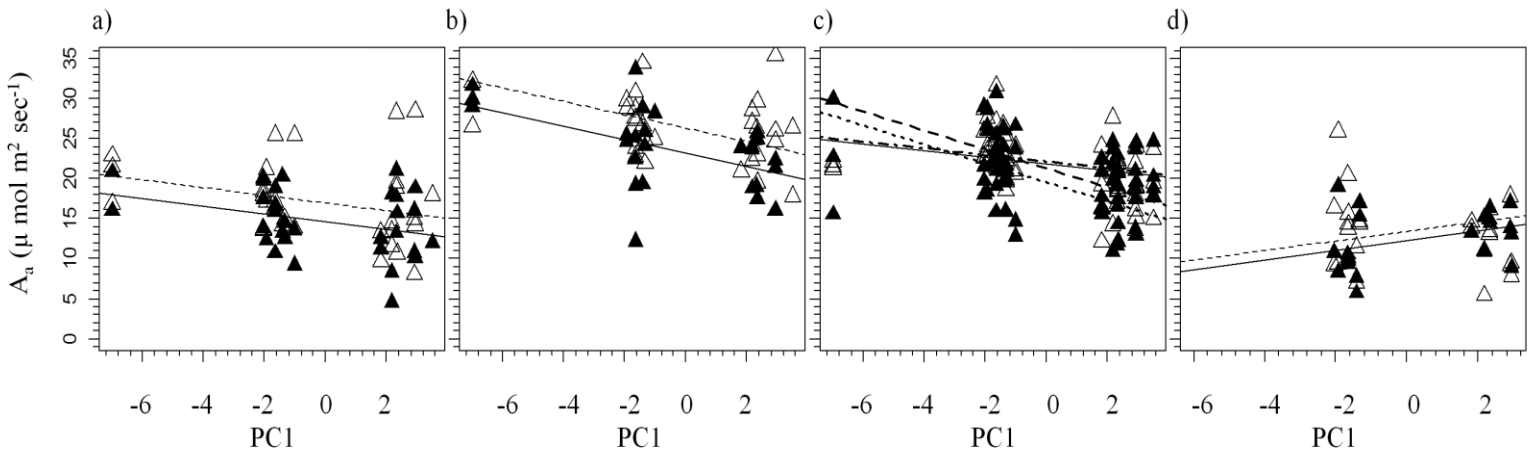

Figure 3.5 Photosynthetic rate per unit leaf area (A_a) plotted against ePC1. ePC1 correlates positively with temperature, mean summer precipitation and inversely with latitude of tree source provenance environment. A_a measurements were taken once monthly in June (a), July (b), August (c), and September (d). Closed triangles and solid lines correspond to warmed trees; open triangles and dashed lines to control trees. Data were pooled for 2010 and 2011; A_a was significantly different between years in August (2010 warmed = dotted line, 2011 warmed = solid line; 2010 control = dashed line, 2011 control = dot-dash line).

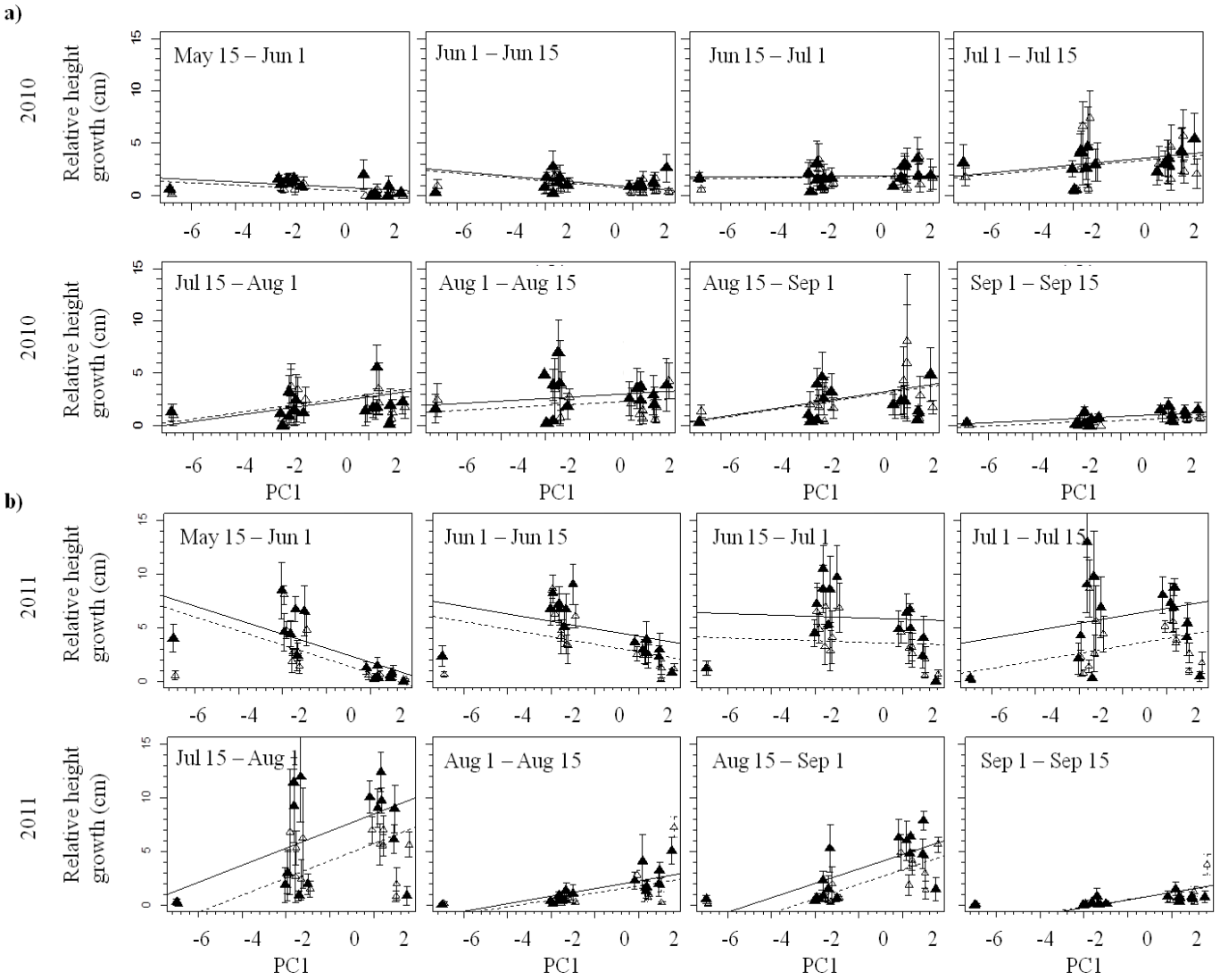

Figure 3.6 Relative height growth increment for biweekly intervals throughout the 2010 (a) and 2011 (b) growing seasons for balsam poplar trees grown under warmed (closed triangles, solid lines) and ambient conditions (open triangles, dashed lines) in a common garden environment at 64.8°N, plotted against principal component 1 (PC1).

Chapter 4

The role of epigenetics in plant adaptation[1]

Abstract

Recent work in the field of plant epigenetics is adding to a growing understanding of how epigenetic variation can be an important source of phenotypic variation in natural populations. Therefore, it has the potential to play a major role in adaptation to environmental change. Most epigenetic variation is reset between generations, however, in some instances environmentally-induced epigenetic variation can result in heritable phenotypic plasticity that invokes Lamarkian-like inheritance. Epigenetic variation can also be the result of random *epimutations* that can have both higher mutation and reversal rates than DNA sequence mutations. We discuss several examples documenting epigenetic variation in wild populations. We also discuss laboratory studies that investigate the rate of epimutations and reversals, and how that has been incorporated into evolutionary theory. We suggest that modern evolutionary theory will benefit from the incorporation of epigenetics, but it is not in need of a complete revision, as has been suggested.

Epigenetics in ecology and evolution

There has been long-standing evidence of transgenerational epigenetic inheritance, such as paramutation in maize (1) and imprinting in mammals,(2) but the

[1] Robertson, A.L, D.E. Wolf. 2012. The role of epigenetics in plant adaptation. Trends in Evolutionary Biology. 4;e4. 19-25.

general biological community did not take notice until it became abundantly clear that it was a widespread phenomenon in plants and animals, and not limited to a few very specific examples. The fact that environmental cues in one generation can cause epigenetic changes that are inherited for multiple generations, which has been referred to as Lamarkian inheritance or inheritance of acquired characteristics,(3, 4) has particularly intrigued evolutionary biologists.

Epigenetics involves meiotically and mitotically stable alterations in gene expression that are not based on DNA sequence changes, but involve processes that impact the packaging of DNA (chromatin structure).(5) These processes include the addition of methyl groups to the fifth carbon in cytosine molecules (DNA methylation), histone modifications that may be influenced by transposable elements, which are often methylated, and small RNAs which can direct DNA methylation and chromatin remodeling at their target loci. (6-9) Chromatin structure then alters the availability of DNA to transcription factors, and influences whether genes can be expressed.(10) Although believed to have evolved in part to protect against genome perturbations, such as transposable elements and retroviruses,(11) epigenetic processes play a crucial role in cell differentiation and development, and are probably responsible for many aspects of behavior and phenotypic plasticity.(12)

Epimutations can create heritable epialleles, the epigenetic equivalent of genetic alleles. They may be caused by errors in methylation maintenance,(13, 14) *de novo* methylation,(15) or other chromatin remodeling factors,(10) or they may be triggered by a particular environmental stimulus, creating a type of transgenerational plasticity.(16)

Although epigenetic variation can occur in the absence of genetic variation, genetic variation can influence epigenetic variation and the epimutation rate in a number of ways. For instance, variation in the presence of cytosines that can be methylated,(17) transposable elements, (18-21) small RNA production,(22) and genes controlling histone modifications and chromatin structure (10) can all influence whether a gene is subject to epigenetic silencing. Thus, selection on the epigenotype may act directly on transgenerationally heritable epialleles, or it may proceed by selection on DNA polymorphisms that influence epigenetic state.

Epigenetic variation can be a significant source of natural phenotypic variation; therefore, it has the potential to play a major role in adaptation to environmental change. A simple hypothetical scenario may illustrate the possibility that adaptive phenotypic evolution may occur via epigenetic modification even though the population is genetically homogeneous (Figure 1).

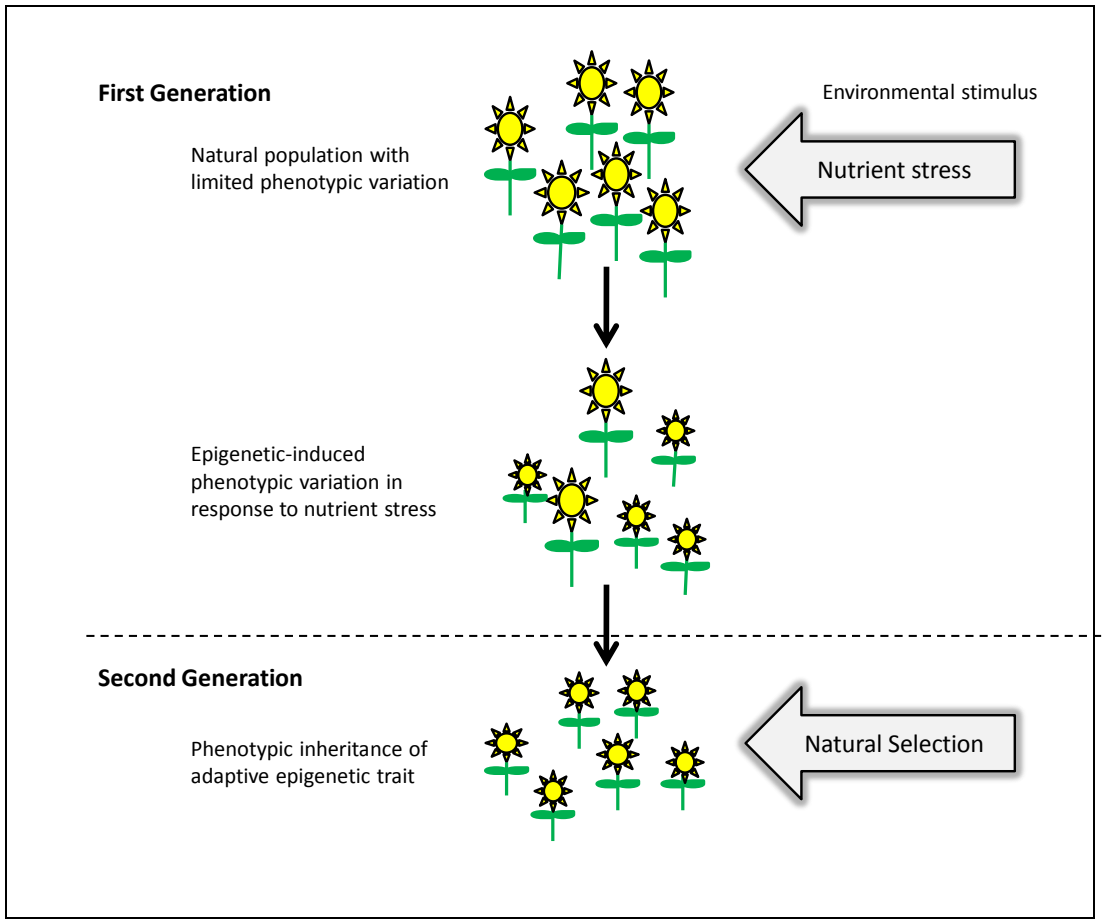

Figure 1. A hypothetical example of epigenetic-induced phenotypic variation in response to an environmental cue. In the parental generation, a natural plant population is exposed to nutrient stress which induces phenotypic plasticity that is of epigenetic origin. The new phenotypic mean is skewed towards smaller phenotypes which require lower nutrient levels to successfully reproduce. The adaptive phenotypic trait is inherited by the offspring, as are the associated epigenetic markers.

This example illustrates how environmentally-induced phenotypic change may be mediated via epigenetic mechanisms. In this case, nutrient stress could cause phenotypic

variation within an otherwise genetically homogeneous population. There are various reasons why some plants may change phenotypes while others do not. For instance, there may be some stochasticity such that the methylation probability of a particular region given the environmental cue may be less than 1, there may be micro-heterogeneity in the environmental cue, or there may be genetic differences among individuals that affect the availability of sites that can be methylated within a particular region. Although the phenotypic change is not necessarily adaptive in the environment that cues it, the induced phenotypic variation (*e.g.* a smaller flower size in some plants) may offer the opportunity for the selection to act. In this example, small flowers may require fewer resources and result in higher seed maturation and therefore higher fitness. If the change in methylation is transgenerationally inherited (transgenerational plasticity), the new epimutation can spread into the population, and adaptive evolution can occur even in the absence of genetic change.(23) Alternatively, even if the methylation change is not inherited, but there is genetic variation in the ability to be cued by the environment (plasticity), selection can act on this genetic variation.

Modern evolutionary theory is primarily based on the inheritance of random genetic variation, so there has been ample discussion whether evolutionary theory requires revision in light of epigenetics.(4, 24-27) In order to assess the importance of epigenetics in evolutionary processes, it is first necessary to show that epigenetic variation exists in wild populations, and second that this variation correlates with phenotypic variance that is subject to selection. Next, it is necessary to determine what epigenetic variation is transgenerationally inherited. In this review, we first describe

examples of naturally-occurring epigenetic variation that influences plant reproduction, followed by recent evidence for transgenerational plasticity in response to stress. Next, we discuss studies of DNA methylation variation in wild populations, followed by an overview of recent laboratory experiments in which heritability of methylation variation is directly analyzed. We conclude with a discussion on how epigenetics fits into post-Modern Synthesis evolutionary theory from both a mechanistic and theoretical viewpoint.

Natural epigenetic variation and reproduction

Research on epiallelic variation traces its roots to a seminal paper showing that the first natural morphological mutant described by Linnaeus is actually a transgenerationally heritable epimutation, caused by hypermethylation, and not by a DNA mutation.(28) *Linaria vulgaris* flowers are typically bilaterally symmetrical and bee pollinated.(29) The epimutation suppresses transcription of the *Linaria-like-CYCLOIDEA* (*Lcyc*) gene in developing flowers, causing them to become radially-symmetrical,(28) and not likely to be effectively pollinated by bees. Shifts from bilateral to radial symmetry are often associated with a change in pollination syndrome.(30) Another spontaneous, heritable epimutation, caused by hypermethylation in the promoter-region of the *COLORLESS NON_RIPENING* (*CNR*) locus of tomatoes, is thought to cause non-ripening fruits,(31) and is perhaps regulated by small, non-coding RNAs.(32) Although this study was in cultivated tomato, it demonstrates the impact of natural epigenetic variation on fruit color and ripening characteristics, which play a central role in seed dispersal.

In *Arabidopsis thaliana*, two highly studied genes influencing the timing of flowering are regulated, at least in part, by epigenetic mechanisms. In *Arabidopsis, FLOWEIRING LOCUS (C)* (*FLC*) is an important gene for synchronizing floral timing with seasonal cues. Specifically, *FLC* suppresses flowering until a sufficiently long cold period has been experienced (vernalization), so that plants know when to time flowering in spring.(33) Vernalization causes epigenetic changes in the chromatin structure of *FLC*, suppressing FLC expression, and permitting flowering.(34) There is variation among ecotypes in the genes that control *FLC* chromatin structure, and therefore epigenetic variation at the *FLC* locus among ecotypes, which results in variation in flowering time.(22, 35, 36) Further, there are associations between variation in these genes and latitude, winter temperatures, and precipitation,(35, 36) suggesting that their influence on the epigenetic control of *FLC* could be important for adaptation to seasonal environments associated with local climates.(35)

Epigenetic variation in the *FLOWERING WAGENINGEN* (*FWA*) gene can also influence flowering time. *FWA* is expressed only in the endosperm of wild-type *A. thaliana*, but heritable (37) lab-induced epialleles cause *FWA* to be expressed in vegetative tissue, producing a late-flowering phenotype.(38) These epialleles are independent of DNA variation. There is natural variation within and among other *Arabidopsis* species in both the level of *FWA* promoter methylation and level of vegetative expression, which may be caused by DNA variation in the *FWA* promoter.(39) This natural variation outside of *A. thaliana* does not appear to influence flowering time,(40) however there may be an effect on other phenotypes, such as endosperm

development. Further, the fact that *FWA* epialleles occur in the lab shows they are possible and may occur in nature.

These examples give weight to the argument that epigenetics could result in ecologically-important phenotypic variation. While the heritable *Lcyc* epiallele in *Linaria vulgaris* and *CNR* epiallele in tomatoes appear to be entirely epigenetic, and do not appear to be linked to DNA variation, the natural epigenetic variation in *Arabidopsis* flowering time appears to be controlled, at least in part, by DNA sequence variation. Thus, selection on epigenetic variation may act either directly on the epigenotype or on DNA variation that influences the epigenotype.

Transgenerational plasticity in response to stress

Epigenetic mechanisms can play an important role in plastic responses to the environment (34) and have been particularly studied in relation to plant stress responses. As sessile organisms, plants often display high levels of phenotypic plasticity to cope with stress. Priming, an effect in which stress exposure causes a plant to either exhibit higher resistance or faster response to that stress in the future, can, in certain examples, be linked to epigenetic marks that activate transcription of stress-related gene pathways.(41) In some cases, *stress memory* has been shown to pass from parental generations to unstressed offspring,(41) presumably to prepare offspring for an environment containing the same stressors.(6)

Two ground-breaking studies have linked epigenetic variation to the transmission of stressed phenotypes from the parental generation to unstressed offspring. In *Mimulus*

guttatus, simulated herbivory (leaf damage) induced trichome production on the underside of leaves, a well-known response to deter future herbivory. The response was linked to the epigenetic down-regulation of a specific candidate gene (*MgMYBML8*). This epimutation was inherited by unstressed offspring that also displayed increased trichome production when compared to control plants.(42) In a different approach, genome-wide DNA methylation profiles were compared between control individuals of apomictic dandelions (*Taraxacum officinale*) and those exposed to chemical simulation of herbivore or pathogen attack. Significant genome-wide methylation changes were observed in stressed plants which displayed stunted phenotypes; the stressed phenotype was inherited for three generations as were most of the methylation changes.(43) The genetic uniformity of asexual plants makes this an ideal system for demonstrating the impact of environmental cues on epigenetic inheritance.(43)

These two studies are among the first to document transgenerational plasticity in plants that is directly correlated to epigenetic modifications, although it has long been speculated. Other noteworthy studies have linked ecologically-important epigenetic responses to stress factors, although transgenerational inheritance was either unexplored or has been unapparent. These include global hypomethylation in hemp (*Cannabis sativa*) that is exposed to heavy metals,(44) drought-induced methylation changes in rice, *Oryza sativa*, that may increase drought tolerance,(45) and transcription activation of repetitive elements due to chromatin modification in *Arabidopsis thaliana* that is exposed to prolonged heat stress.(46) Activation of repetitive elements in response to stress is extremely interesting, since this is likely to increase the mutation rate and increase

phenotypic variation, potentially increasing the chance that a stress-adapted mutant will arise.(47)

In an extreme example of environmentally-induced plasticity, exposure to acute salt stress in the salt-tolerant plant, *Mesembryanthemum crystallinum*, resulted in the methylation-directed down-regulation of loci responsible for switching from the C3 photosynthetic pathway to crassulacean acid metabolism (CAM) pathway.(48, 49) Even if not transgenerationally inherited, these transient stress responses can increase fitness while avoiding the cost of constitutive expression of stress-related genes.(41) A great deal of additional research is needed to determine how frequently stress-response traits, as well as other phenotypic traits, can be transgenerationally inherited.

Methylation variation in natural populations

In order to understand the role of epigenetic effects on plant adaptation, it is necessary to understand the occurrence and structure of epigenetic variation in nature.(26, 50) To date, there have been only a few studies on natural populations, however, tools borrowed from early DNA sequence variation analysis, such as amplified fragment length polymorphisms (AFLPs) modified to detect differences in cytosine methylation (methylation-sensitive AFLPs; MSAP), have allowed the quantification of epigenetic variation to reach beyond the laboratory and model organisms.

In one study using this technique, Herrera and Bazaga (51) showed that both MSAP epigenotypes and AFLP genotypes of individuals are correlated with long-term herbivory levels in natural populations of the wild violet (*Viola cazorlensis*). They

identified six AFLP loci related to 44% of variation in herbivory, and showed that the epigenotype was significantly correlated with genotype at these six herbivory-related AFLP loci.(51) It is difficult to make strong conclusions about causal relationships, however, as the differences among epigenotypes could be caused by variation in herbivory, or the differences in herbivory (herbivore resistance) could be caused by variation among epigenotypes. Methods such as common-garden experiments that control the environment, or studies of genetically uniform plants are necessary to distinguish among environmental, epigenetic, and genetic sources of variance.(6) Nonetheless, this study clearly shows the importance of the interplay of epigenetics and genetics in herbivory dynamics in a natural population.

Several recent studies have applied the MSAP technique to compare global methylation patterns among individuals collected from contrasting environments. A surprising consistency in findings has emerged from these early population-level studies. First, levels of genome-wide epigenetic variation are higher than genetic variation, even when the epigenotype was scored in a single tissue and single developmental stage.(51-55) Second, among-population epigenetic variation is higher than within-population variation, even when there is no overall genetic differentiation among populations.(54, 55) Further, epigenetic variation is highly correlated with environment, both within and among populations.(9, 51, 54-57) This may be due to environmental influences on the epigenetic state (plasticity), but could also be due to selection on the epigenotype, or on genes influencing the epigenotype. These findings are being interpreted as evidence that

epigenetic mechanisms are important for responding to the environment, and that they may contribute to adaptive divergence among populations.(7, 58)

Another source of natural epigenetic variation arises from the processes of polyploidization and hybridization, a common phenomenon believed to be in part responsible for the extreme levels of species diversity in plants.(59) Genome-wide epigenetic changes are induced by genome duplication events and are believed to be a coping mechanism for the genome shock caused by these processes.(19) Moreover, the novel epigenetic variants produced by genome duplication provide the potential for phenotypic and ecological divergence between polyploids and their parental taxa,(60) or among sister polyploid taxa that have arisen from the same parental taxa.(59, 61, 62) MSAP comparisons among three sister allopolyploid species of the orchid, *Dactylorhiza*, growing in three different environments, showed a striking divergence in methylation profiles that were highly correlated to growing environment.(61)

The examples included in the section highlight an emerging and rapidly growing field of *population epigenetics* but they also reflect some of the challenges. Studies on natural populations to date have only speculated about transgenerational inheritance of the observed epigenetic variation, and are complicated by the correlation between genetic and epigenetic variance. Further, it is typically not determined whether the observed variation in DNA methylation has any functional consequences.(9) Despite these obstacles, these studies are leading the way forward to a better understanding of how epigenetic processes contribute to adaptation to local environments and their role in adaptive divergence.

Methylation variation in Recombinant Inbred Lines

Unlike the studies on natural populations discussed above, laboratory populations have been used to directly study the inheritance of methylation polymorphisms, and their link to phenotypic variation. By creating highly inbred lines that are virtually genetically identical but have introduced epi-mutations, epigenetic Recombinant Inbred Lines (epiRILS) have been used to decouple the effects of the genotype and epigenotype as sources of trait inheritance.(63) Two groups developed isogenic lines of *Arabidopsis thaliana*, both bred from a wild type parent and a parent with a single loss-of-function mutation in a gene associated with methylation control, *MET1* (64) and *DDM1;*(37) thus both of these studies eliminated genetic variation and exaggerated epigenetic variation.

The first major finding from both studies is that extensive epigenetic variation not only differed greatly from the parents, but it persisted over at least 8 generations in the absence of selection.(37) Second, this epigenetic variation resulted in increased phenotypic variation in ecologically-important traits such as flowering time,(37, 64) and traits that can influence plant fitness, such as plant height (37) and biomass.(64)

Within the epiRIL populations, the vast majority (70%) of methylation changes reverted to the wild-type state within eight generations.(37, 64) This has been interpreted as evidence of the instability of epialleles and of a genomic *rescue system* to maintain genomic integrity.(65) Interestingly, broad-sense heritability estimates derived from these epiRIL populations is similar to heritability for many quantitative traits presumed to have a genetic basis.(37, 66) These exaggerated *MET1*/*DDM1* loss of function mutants are not likely reflective of natural populations, however, and there is clearly a need for

this type of study on natural ecotypes. Nonetheless, these epiRILs show that methylation variation is a trangenerational source of phenotypic variation, and offer some insight into how epialleles contribute to the heritability of complex quantitative traits. Additionally, these papers suggest how it may be possible to map variation in cytosine methylation to disentangle the genetic and epigenetic contributions to natural variation in quantitative traits, and identify the functional consequences of variation in DNA methylation.

Epimutations and evolutionary theory

Modern evolutionary theory is generally based on a strict definition of inheritance of random genetic variation. Because epigenetic variation can play a role in inheritance, it is necessary to consider how it should be incorporated into evolutionary theory and population genetics. Although some researchers have even suggested that a complete revision of evolutionary and population genetics theory is needed,(4, 25, 27) we believe that epigenetics can be incorporated into existing theory with some simple modifications. First, random epimutations, which are not induced by the environment, can be treated very much like random genetic mutations, with minor modifications to theory. Second, some epimutations are very different from traditional genetic mutations because they are influenced by the environment. These environmentally-cued epimutations, which are also referred to as transgenerational plasticity, can be modeled much like adaptive plasticity or adaptive maternal effects, which have been relatively well studied.(67-69) In this section, we discuss data on several features of random epimutations, relevant to evolutionary

theory, and some approaches that have been used to model random and environmentally-cued epimutations.

The rate of natural random epimutation and the stability of epialleles is not yet well understood, however this is a critical factor for incorporating epigenetics into evolutionary theory. The epimutation rate is likely to influence epigenetic diversity, equilibrium frequencies of epialleles, and therefore how random epigenetic variation will contribute to adaptation. One detailed study in *Arabidopsis thaliana* makes great strides towards understanding the rate of natural, spontaneous, random epimutations in a single growth environment. Becker *et al*. (70) compared genome-wide variation in DNA methylation among 10 *Arabidopsis thaliana* lines that were derived from a common ancestor 30 generations ago. The epimutation rate for single cytosines was far higher than the genetic mutation rate. However, the epimutation rate of larger, contiguous regions of methylation, which are more likely to have functional consequences, was similar to the genetic mutation rate. Further, the methylation status of certain sites was highly mutable while other sites were stable. Thus, epimutation has the potential to occur at rate much higher than the mutation rate, at least at some sites. Although this study investigated the natural epimutation rate in plants that were not subject to demethylating agents such as 5-azacytidine, the study was conducted in the lab. Epimutation rates in natural populations could be influenced by the environment, and could be quite different. Thus similar studies in more natural environments will be valuable. Research is also needed to understand how these changes in cytosine methylation correspond to phenotypic changes, and to measure the epimutation rate for phenotypic traits.

Another important empirical observation is that reverse epimutations are much more common than reverse nucleotide mutations.(70) This is due to the high epimutation rate at some sites and the fact that an individual cytosine has only two possible states (methylated or un-methylated), whereas nucleotide sites can have four different states. The rate of reversals is important for the incorporation of epigenetics into population genetics models. Further, frequent reversals facilitate switching back and forth between two phenotypes, which may be beneficial if the environment fluctuates between two different states.

Similar to nucleotide mutations, epimutations have the potential to be beneficial, neutral or deleterious. In a stable environment, where most individuals are well-adapted, mutations or epimutations are likely to reduce an individual's fitness, creating genetic or epigenetic load. Stenøien and Pederson (71) modeled the negative effects of epigenetic load. They show that the fitness consequences of epimutations are analogous to the effects of deleterious genetic mutations, and load is primarily determined by the epimutation rate and degree of reversibility. Since the heritable epimutation rate may be quite high relative to the mutation rate, epimutation has the potential to increase load considerably. Even epimutations that cannot be transgenerationally inherited have the potential to considerably decrease fitness. They suggest that these epimutations are similar to somatic mutations, and because the epimuation rate can be orders of magnitude higher than the somatic mutation rate, especially as individuals age, epigenetic load will be much more severe than somatic genetic load. However, to understand the impact of

epigenetic load relative to genetic load, we need better estimates of the fitness consequences of both heritable and non-heritable epimutations.

In contrast to a stable environment, mutations or random epimutations may be beneficial in a temporally or spatially variable environment. If there are two environments, and two heritable epialleles, where one has higher fitness in each environment, a high rate of environmental change favors a high rate of epimutation.(72-74) Epimutations in some fraction of the progeny allow an individual to produce offspring with a mix of phenotypes in the face of unpredictable environmental fluctuations from one generation to the next. The probability of each phenotype should be determined by the probability of being subject to selection in each environment.(73) This is basically a bet-hedging strategy.(75) Since epigenetic mechanisms are more likely to permit frequent switching between two allelic states than genetic mechanisms,(70) epigenetic mechanisms may be favored for traits that influence survival in a variable environment.(73)

Models have also investigated the adaptive significance of heritable, environmentally-cued epimutations (transgerational plasticity) vs. a purely genetic strategy of phenotype determination or a purely plastic strategy (environmentally-cued, but not transgenerational).(74) Jablonka *et al.* (1995) suggests that transgenerational plasticity is an intermediate strategy between plastic and genetic strategies. On the other hand, Shea (2011) views transgenerational plasticity as being identical to adaptive maternal effects. Like the models of random epimutation, these models also focus on environmental variation, and one advantage of transgenerational plasticity could be the

production of offspring with a mix of phenotypes in the face of an unpredictable environment. The frequency of each phenotype should be determined by the probability of being exposed to selection in each environment,(74) The pattern and frequency of environmental change is likely to determine when transgenerational plasticity is beneficial.(67, 73, 74) If the environment changes frequently within a generation, there would seem to be no benefit to transgenerational plasticity. Likewise, if it remains stable for hundreds of generations, selection would likely fix a single genetically-determined phenotype before the environment changed. Yet if it remains stable for a few generations so that the parent's environment predicts the offspring's environment with some accuracy, it may be beneficial to inherit the parent's phenotype rather than relying on an environmental cue to direct development.(73) This inheritance of cues from the parental environment may be especially beneficial if there is some time lag between detection of the environmental cue, and assumption of the appropriate phenotype.(73) Similarly, transgenerational plasticity could be beneficial because the parent can detect the environmental cue more reliably than the offspring.(67) For instance, if the parent experiences herbivory, and herbivore abundance cycles with a period of several years, it is likely that her offspring will experience the same herbivory. Offspring may then benefit by producing defenses such as trichomes (42, 76-78) or glucosinolates (77) in anticipation of herbivory. Similarly, if the parent does not experience herbivory, there it is likely that offspring will not either, and they can avoid the costs of producing defenses.

Considerable progress has been made in incorporating epigenetics into evolutionary theory, however many avenues of research remain yet to be explored. For

example, further research is needed to understand why some sites are highly epi-mutable, but others are more stable. Is the explanation purely mechanistic, reflecting different mechanisms of methylation maintenance, or has selection shaped the epimutation rate just as it has shaped the mutation rate? Are the more stable epimutations more likely to have functional consequences? Perhaps, like non-synonymous DNA sites, the stable sites are subject to purifying selection, while methylation at unstable sites have no phenotypic consequence and are therefore neutral with respect to selection, similar to synonymous DNA sites. Additionally, at a small number of sites, a high epimutation rate could be beneficial, and therefore positively selected.

We still know very little about natural epimutation rates at the phenotypic level and the transgenerational stability of epimutations that influence phenotype. These epimutations are far more likely to be subject to selection, and have more potential to contribute to adaptive evolution. Other unanswered questions include: Are random epimutations more stable than environmentally-cued epimutations? How many generations does an environmentally-cued epimutation persist in a non-matching environment? What conditions would selectively favor the maintenance of an environmentally-cued phenotype for multiple generations in a non-matching environment?

Concluding thoughts

Much remains to be explored in the field of epigenetics, both mechanistically and ecologically before the true impact of epigenetics on plant adaptation is understood. It is

clear however, that both heritable and non-heritable epigenetic variation is an important source of variance in ecologically important traits such as reproduction and stress tolerance. Epigenetic differences between contrasting habitats are further evidence that epigenetic mechanisms are important in plant responses to the environment in natural populations. This variation can result in environmentally-induced phenotypic plasticity, which may be transgenerationally inherited, although there are currently only a few good examples of epigenetically-induced transgenerational plasticity. Nonetheless, studies in natural environments demonstrate that epigenetics are important for adaptation to environmental change.

Epigenetic variation may be controlled by environmental variation and/or genetic variation, or it may be independent of both. Thus selection may influence epigenetic traits either through selection on the genes that control epigenetic variation or on heritable epialleles. Future research efforts to untangle the sources of epigenetic variation within specific pathways or systems will be necessary to better understand genotype by epigenotype by environment interactions and how they relate to selection. Epigenetic variation can contribute to a large fraction of phenotypic variance, and may be especially important in populations with little genetic variance, or in habitats exposed to rapid environmental change. Research addressing the level of heritable and non-heritable phenotypic variation caused by epigenetic variation in populations with low genetic diversity will be especially useful.

The rapid pace of advancement, coupled with increased affordability, of next generation sequencing technology will allow for more comprehensive studies on genome-

wide epigenetic variation in non-model organisms and natural populations. For example, whole-genome bisulfite treatment of DNA, or chromatin immunoprecipitation, followed by next generation sequencing provides genome-wide information on site-specific methylated sites or histone modifications, respectively.(79) The next step in epigenetics research is to link gene expression levels to observed epigenetic variation. Entire transcriptomes (including those for small RNAs) can now be obtained in a few days, allowing for direct comparisons in expression levels between contrasting environments. Most notably, these methods do not require an annotated genome. At this level, it will be easier to connect variation in DNA methylation or other epigenetic marks to phenotypic and environmental variation. Linking epigenetic variation to differential gene expression is the next step in epigenetics research. Quantitative trait loci mapping and association studies are needed to solidify the relationship between the epigenotype, genotype, and phenotype.

To understand the role of epigenetics in plant adaptation, it will take the collaboration of molecular biologists and evolutionary ecologists to combine mechanistic information into population genetics models and ecological theory. The rapid pace of advancement in the field of epigenetics will continue to shape our understanding of the mechanisms controlling and creating phenotypic variation, and its implications for evolution.

References

1. Arteaga-Vazquez MA, Chandler VL. Paramutation in maize: RNA mediated trans-generational gene silencing. Curr Opin Genet Dev 2010;20:156-63.

2. Ferguson-Smith AC. Genomic imprinting: the emergence of an epigenetic paradigm. Nat Rev Genet 2011;12:565-75.

3. Richards EJ. Inherited epigenetic variation - revisiting soft inheritance. Nat Rev Genet 2006;7:395-401.

4. Jablonka E, Lamb MJ. Soft inheritance: challenging the modern synthesis. Genet Mol Biol 2008;31:389-95.

5. Boyko A, Kovalchuk I. Epigenetic control of plant stress repsonse. Environ Mol Mutagen 2008;49:61-72.

6. Bossdorf O, Richards CL, Pigliucci M. Epigenetics for ecologists. Ecol Lett 2008;11:106-15.

7. Eric J R. Natural epigenetic variation in plant species: a view from the field. Curr Opin Plant Biol 2011;14:204-9.

8. Matzke MA, Birchler JA. RNAi-mediated pathways in the nucleus. Nat Rev Genet 2005;6:24-35.

9. Vaughn MW, Tanurdžić M, Lippman Z, et al. Epigenetic Natural Variation in Arabidopsis thaliana. PLoS Biol 2007;5:e174.

10. Gilbert DM, Wallrath LL. Chromatin and chromosomes. Mol Biol Cell 2011;22:717.

11. Johnson L. The genome strikes back: the evolutionary importance of defence against mobile elements. Evolution Biol 2007;34:121-9.

12. Scott FG. Ecological developmental biology: developmental biology meets the real world. Dev Biol 2001;233:1-12.

13. Peter M. DNA methylation systems and targets in plants. FEBS Letters 2011;585:2008-15.

14. Vanyushin BF, Ashapkin VV. DNA methylation in higher plants: past, present and future. Biochim Biophys Acta 2011;1809:360-8.

15. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet 2010;11:204-20.

16. Angers B, Castonguay E, Massicotte R. Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Mol Ecol 2010;19:1283-95.

17. Feinberg AP, Irizarry RA. Stochastic epigenetic variation as a driving force of development, evolutionary adaptation, and disease. Proc Natl Acad Sci USA 2010;107Suppl1:1757-64.

18. Gazzani S, Gendall AR, Lister C, Dean C. Analysis of the molecular basis of flowering time variaion in Aribidopsis accessions. Plant Physiol 2003;132:1107-14.

19. Parisod C, Alix K, Just J, et al. Impact of transposable elements on the organization and function of allopolyploid genomes. New Phytologist 2010;186:37-45.

20. Mirouze M, Paszkowski J. Epigenetic contribution to stress adaptation in plants. Curr Opin Plant Biol 2011;14:267-74.

21. Furner IJ, Matzke M. Methylation and demethylation of the Arabidopsis genome. Curr Opin Plant Biol 2011;14:137-41.

22. Zhai J, Liu J, Liu B, Li P, et al. Small RNA-directed epigenetic natural variation in Arabidopsis thaliana. PLoS Genet 2008;4:e1000056.

23. Beldade P, Mateus ARA, Keller RA. Evolution and molecular mechanisms of adaptive developmental plasticity. Mol Ecol 2011;20:1347-63.

24. Kalisz S, Purugganan MD. Epialleles via DNA methylation: consequences for plant evolution. Trends Ecol Evol 2004;19:309-14.

25. Eva Jablonka Raz G. Transgeneration epigenetic inheritance: Prevalence, mechanisms, and implications for the study of heridity and evolution. Q Rev Biol 2009;84:131-76.

26. Richards CL, Bossdorf O, Pigliucci M. What role does heritable epigenetic variation play in phenotypic evolution? BioSci 2010;60:232-7.

27. Pigliucci M. Do we need an extended evolutionay synthesis? Evolution 2007;61:2743-9.

28. Cubas P, Vincent C, Coen E. An epigenetic mutation responsible for natural variation in floral symmetry. Nature 1999;401:157-61.

29. Burkle LA, Irwin RE, Newman DA. Predicting the effects of nectar robbing on plant reproduction: implications of pollen limitation and plant mating system. Am J Bot 2007;94:1935-43.

30. Kalisz S, Ree RH, Sargent RD. Linking floral symmetry genes to breeding system evolution. Trends Plant Sci 2006;11:568-73.

31. Manning K, Tor M, Poole M, et al. A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. Nat Genet 2006;38:948-52.

32. Moxon S, Jing R, Szittya G, et al. Deep sequencing of tomato short RNAs identifies microRNAs targeting genes involved in fruit ripening. Genome Res 2008;18:1602-9.

33. Sung S, Amasino RM. Vernalization and epigenetics: how plants remember winter. Curr Opin Plant Biol 2004;7:4-10.

34. Bastow R, Mylne JS, Lister C, et al. Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 2004;427:164-7.

35. Stinchcombe JR, Caicedo AL, Hopkins R, et al. Vernalization sensitivity in Arabidopsis thaliana (Brassicaceae): the effects of latitude and FLC variation. Am J Bot 2005;92:1701-7.

36. Mendez-Vigo B, Pico XF, Ramiro M, et al. Altitudinal and climatic adaptation is mediated by flowering traits and FRI, FLC, and PhyC genes in Arabidopsis thaliana. Plant Physiol 2011;157:1942-55.

37. Johannes F, Porcher E, Teixeira FK, et al. Assessing the impact of transgenerational epigenetic variation on complex traits. PLoS Genet 2009;5:e1000530.

38. Soppe WJJ, Jacobsen SE, Alonso-Blanco C, et al. The late flowering phenotype of FWA mutants is caused by gain-of-function epigenetic alleles of a homeodomain gene. Mol Cell 2000;6:791-802.

39. Fujimoto R, Kinoshita Y, Kawabe A, et al. Evolution and Control of Imprinted FWA Genes in the Genus Arabidopsis. PLoS Genet 2008;4:e1000048.

40. Fujimoto R, Sasaki T, Kudoh H, et al. Epigenetic variation in the FWA gene within the genus Arabidopsis. Plant J 2011;66:831-43.

41. Bruce TJA, Matthes MC, Napier JA, Pickett JA. Stressful memories of plants: evidence and possible mechanisms. Plant Sci 2007;173:603-8.

42. Scoville AG, Barnett LL, Bodbyl-Roels S, et al. Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in Mimulus guttatus. New Phytologist 2011;191:251-63.

43. Verhoeven KJF, Jansen JJ, van Dijk PJ, Biere A. Stress-induced DNA methylation changes and their heritability in asexual dandelions. New Phytologist 2010;185:1108-18.

44. Aina R, Sgorbati S, Santagostino A, et al. Specific hypomethylation of DNA is induced by heavy metals in white clover and industrial hemp. Physiol Plantarum 2004;121:472-80.

45. Wang W-S, Pan Y-J, Zhao X-Q, et al. Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (Oryza sativa L.). J Experiment Bot 2011;62:1951-60.

46. Pecinka A, Dinh HQ, Buaubec T, et al. Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in Arabidoposis. Plant Cell 2010;22:3118-29.

47. Fonville NC, Ward RM, Mittelman D. Stress-induced modulators of repeat instability and genome evolution. J Mol Microbiol Biotechnol 2011;21:36-44.

48. Dyachenko O, Zakharchenko N, Shevchuk T, et al. Effect of hypermethylation of CCWGG sequences in DNA of Mesembryanthemum crystallinum plants on their adaptation to salt stress. Biochemistry (Moscow) 2006;71:461-5.

49. Huang N-C, Li C-H, Lee J-Y, Yen HE. Cytosine methylation changes in the ice plant Ppc1 promoter during transition from C3 to Crassulacean acid metabolism. Plant Sci 2010;178:41-6.

50. Richards CL, Wendel JF. The hairy problem of epigenetics in evolution. New Phytol 2011;191:7-9.

51. Herrera CM, Bazaga P. Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. Mol Ecol 2011;20:1675-88.

52. Salmon A, Ainouche ML, Wendel JF. Genetic and epigenetic consequences of recent hybridization and polyploidy in Spartina (Poaceae). Mol Ecol 2005;14:1163-75.

53. Keyte AL, Percifield R, Liu B, Wendel JF. Infraspecific DNA methylation polymorphism in cotton (Gossypium hirsutum L.). J Hered 2006;97:444-50.

54. Lira-Medeiros CF, Parisod C, Fernandes RA, et al. Epigenetic variation in mangrove plants occurring in contrasting natural environment. PLoS ONE 2010;5:e10326.

55. Herrera CM, Bazaga P. Epigenetic differentiation and relationship to adaptive genetic divergence in discrete populations of the violet Viola cazorlensis. New Phytologist 2010;187:867-76.

56. Marfil C, Camadro E, Masuelli R. Phenotypic instability and epigenetic variability in a diploid potato of hybrid origin, Solanum ruiz-lealii. BMC Plant Biol 2009;9:21.

57. Herrera CM, Pozo MI, Bazaga P. Jack of all nectars, master of most: DNA methylation and the epigenetic basis of niche width in a flower-living yeast. Mol Ecol 2011 Dec 15. [Epub ahead of print]

58. Richards CL, Bossdorf O, Verhoeven KJF. Understanding natural epigenetic variation. New Phytologist 2010;187:562-4.

59. Salmon A, Ainouche ML. Polyploidy and DNA methylation: new tools available. Mol Ecol 2010;19:213-5.

60. Doyle JJ, Flagel LE, Paterson AH, et al. Evolutionary genetics of genome merger and doubling in plants. Ann Rev Genet 2008;42:443-61.

61. Paun O, Bateman RM, Fay MF, et al. Stable epigenetic effects impact adaptation in allopolyploid orchids (Dactylorhiza: Orchidaceae). Mol Biol Evolut 2010;27:2465-73.

62. Hegarty MJ, Batstone TOM, Barker GL, et al. Nonadditive changes to cytosine methylation as a consequence of hybridization and genome duplication in Senecio (Asteraceae). Mol Ecol 2011;20:105-13.

63. Johannes F, Colot V, Jansen RC. Epigenome dynamics: a quantitative genetics perspective. Nat Rev Genet 2008;9:883-90.

64. Reinders J, Wulff BBH, Mirouze M, et al. Compromised stability of DNA methylation and transposon immobilization in mosaic Arabidopsis epigenomes. Gene Dev 2009;23:939-50.

65. Johannes F, Colomé-Tatché M. Quantitative epigenetics through epigenomic perturbation of isogenic lines. Genetics 2011;188:215-27.

66. Roux F, Colomé-Tatché M, Edelist C, et al. Genome-wide epigenetic perturbation jump-starts patterns of heritable variation found in nature. Genetics 2011;188:1015-7.

67. Shea N, Pen I, Uller T. Three epigenetic information channels and their different roles in evolution. J Evolution Biol 2011;24:1178-87.

68. Galloway LF. Maternal effects provide phenotypic adaptation to local environmental conditions. New Phytologist 2005;166:93-100.

69. Bradshaw AD. Evolutionary significance of phenotypic plasticity in plants. In: Caspari EW. Advances in Genetics. New York, USA: Academic Press; 1965.

70. Becker C, Hagmann J, Muller J, et al. Spontaneous epigenetic variation in the Arabidopsis thaliana methylome Nature 2011;480:245-9.

71. Stenøien HK, Pedersen B. Mutation and epimutation load in haploid and diploid life forms. J Theoret Biol 2005;233:119-26.

72. Liberman U, Van Cleve J, Feldman MW. On the evolution of mutation in changing environments: recombination and phenotypic switching. Genetics 2011;187:837-51.

73. Lachmann M, Jablonka E. The inheritance of phenotypes: an adaptation to fluctuating environments. J Theoret Biol 1996;181:1-9.

74. Jablonka E, Oborny B, Molnar I, et al. The adaptive advantage of phenotypic memory in changing environments. Philosophical transactions of the Royal Society of London Series B: biological sciences. 1995;350:133-41.

75. Childs DZ, Metcalf CJE, Rees M. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. Proceedings of the Royal Society B: Biological Sciences. 2010;277:3055-64.

76. Holeski LM. Within and between generation phenotypic plasticity in trichome density of Mimulus guttatus. J Evolution Biol 2007;20:2092-100.

77. Agrawal AA, Strauss SY, Stout MJ. Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. Evolution 1999;53:1093-104.

78. Agrawal AA. Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? Am Natural 2001;157:555-69.

79. Cullum R, Alder O, Hoodless PA. The next generation: using new sequencing technologies to analyse gene regulation. Respirology 2011;16:210-22.

Chapter 5

General Conclusions

Synthesis of warming experiments

Through artificial warming experiments, both in the greenhouse and in the field, we show that growth of balsam poplar trees collected from across a latitudinal gradient responded positively to increased growing temperature, with increases in height growth ranging from 27-69 % in response to 3-8 °C average warming. Genotypes from southern populations grew consistently taller in both field and greenhouse experiments, and there was no significant interaction between warming and source environment. The warmed trees in the growth chamber experiment grew taller but were smaller in diameter compared to control trees, indicating a change in allometry in response to increased temperatures. In the field experiment, we were able to also investigate the effects of warming and source latitude on balsam poplar phenology. Both experimental warming and source latitude influenced bud flush and bud set; both warmed trees and individuals originating from more southern latitudes grew larger and exhibited longer growing seasons (more days of active growth). Warmed trees grew taller, were larger in diameter, and had more leaves and lateral buds than control trees. The increased growth of northern genotypes early in the season may be an adaptation to a short growing season. Using height growth as a surrogate for fitness, we can infer that balsam poplar trees will have higher fitness in a future, warmer environment. At the community level, differences

among taxa in responses to warming can lead to changes in competitive interactions and thus have implications for community assemblage and forest productivity.

Photosynthesis was also consistently lower for warmed trees compared to controls in both experiments, and thus photosynthesis alone did not directly prompt higher growth. In balsam poplar, photosynthetic capacity was found to be relatively insensitive to temperature within a certain range. Silim et al. (2010) found that photosynthesis in balsam poplar was limited by RuBisCo capacity from 17 - 37 °C, regardless of growth temperature. They also found that autotrophic respiration decreased in warmed trees, showing an acclimation response to temperature. This acclimation capacity did not differ among northern or southern genotypes grown under increased temperatures (Silim et al. 2010), however, and this positive shift in leaf carbon balance may explain the similarity in growth responses to temperature among genotypes from all latitudes.

An interesting finding from the field experiment was evidence that the timing of bud set was influenced by increased ambient temperatures. This is in contrast to widely held views that bud set in *Populus* is primarily determined by photoperiod (Pauley and Perry 1954, Howe et al. 1996, Olson et al. in press); although other environmental cues, such as drought and nutrient stress, have been shown to induce bud set in poplar (Howe et al. 2003). Also interesting is that timing of bud flush was not influenced by the warming treatment. Empirical evidence suggests that spring greening (bud flush) is happening earlier in boreal forest ecosystems in response to recent warming (Euskirchen et al. 2006, Linderholm 2006, Robin et al. 2008). Our experimental warming started only 10 days before average bud flush in local genotypes and perhaps we would have seen a

warming effect on bud flush had we started the warming experiment sooner. Because we used passive warming techniques, the open-top chambers were not effective in April, however, and earlier warming would have required an active heating method. Tree age may also influence the ability to flush earlier in the spring. For example, adult trees may be more sensitive to temperature cues than juvenile trees.

Longer growing seasons as a result of both warming and genotype resulted in increased height growth. This is important because it suggests that northern genotypes in a warmer climate can delay bud set in order to maximize height growth. This will increase productivity of forests; however, growing season was only lengthened by approximately one week. The success of southern genotypes (which had up to 2 months longer growing seasons) when planted in Fairbanks, Alaska shows that 6-7 days of longer growth still may be too conservative to utilize the entire possible growing season. In this case, northward migration of alleles or seeds from southern populations may increase the capacity for balsam poplar to capitalize on longer summer seasons.

By comparing phenotypic variation of different genotypes growing in the same environment, we were able to demonstrate that there are genetic differences among populations of balsam poplar that affect growth in northern environments. By growing the same genotypes in different environments (different photoperiod and/or temperature regimes) we demonstrated a plastic response in balsam poplar to warming and increased growing season length. Populations from the north differ in growth traits from those in the south, but genotypes from all populations displayed plasticity in growth and phenology phenotypes. The lack of genotype by plasticity interaction shows that

genotypes from northern and southern populations respond similarly to increased temperatures.

Local genotypes of balsam poplar (trees originally collected from Fairbanks source populations) were not the best performers at either ambient or warmed conditions, when planted in Fairbanks, Alaska. This may be evidence of an adaptational lag in response to the recent 1.4 °C warming and ≥ 45 % increase in the growing season length (Wendler and Shulski 2009) documented for the region. Aitken et al. (2008) suggest a framework for modeling adaptational lags as shown in Fig. 5.1. Genetic clines (here bud set and height growth; Fig. 1a,b) are plotted against mean annual temperature of source environments. The horizontal arrow illustrates the average warming effect from our experimental treatment (3 °C). The difference between the two horizontal lines when transferred to the y-axis shows the severity of the expected adaptational lag given that degree of warming. This type of analysis could be used to select the best-fit genotypes in Interior Alaska in a future, warmer climate.

Overall, our experimental evidence suggests that balsam poplar, and likely other deciduous boreal trees, will respond positively to global climate change. This is a generalization as regional differences based on slope, aspect, and available soil moisture will likely result in heterogeneous responses to warming. Increased evapotranspiration as a result of higher temperatures will likely lower water availability in a warmer climate, even given the slight projected increase in precipitation for this region (www.snap.uaf.edu). Recent field observations in south-facing Alaskan birch (*Betula neoalaskana* Sarg.) stands have shown decline in growth and regeneration and may be

due to drought stress (Bob Ott and F. Stuart Chapin III, pers. comm.). Evidence of declining growth and survival in response to warming temperatures in the dominant coniferous taxa in the northwestern boreal forest, such as black spruce (*Picea mariana* [Mill.] B.S.P.) and white spruce (*Picea glauca* [Moench] Voss), indicates that increases in temperature may lead to different species compositions in the northwestern boreal forest of North America (Barber et al. 2000, Wilmking et al. 2004, Way and Sage 2008a). This trend in declining growth in spruce is primarily attributed to temperature-induced drought stress, but experimental evidence also suggests that autotrophic respiration in black spruce has limited acclimation capacity to increased temperatures (Way and Sage 2008b), resulting in decreased growth regardless of moisture stress. Treeline spruce growing at the extremes of the species' range are generally considered to be more temperature limited than precipitation limited thus may continue to show increased growth trends with warmer temperatures (Grace et al. 2002, Danby and Hik 2007). The interaction between warming and drought stress is not explored in our experiments. Quaking aspen (*Populus tremuloides*) seedlings grown under increased temperature had different hydraulic properties than those grown under ambient temperatures, leading to increased leaf (but not stem) cavitation (Way et al. 2012). Therefore, our warming experiments demonstrate an over-simplified temperature response. Species migrating from southern latitudes in response to increasing temperature, such as lodgepole pine, *Pinus contorta* Douglas, (Johnstone and Chapin 2003, O'Neill et al. 2008), may contribute to novel species assemblages in the boreal forest. In conclusion, boreal tree species will respond independently to changes in climate. The complex interactions

between species composition, fire dynamics, nutrient and hydrological cycles are difficult to model; however, experimental evidence of the effects of warming on boreal species can help inform scientists and land managers of species' responses to a changing climate.

Is balsam poplar locally adapted to temperature, photoperiod, or both?

Growth differences among latitudinally-sampled populations in common garden settings are often attributed to differences in growing season length, or in other words, due to adaptation to photoperiod (Soolanayakanahally et al. 2009). But growth also varies along temperature clines in which there are no or little differences in photoperiod, as in altitudinal gradients. In the two warming experiments we described growth clines that vary with both latitude and mean annual temperature gradients, as latitude and temperature are correlated. Is the higher growth observed in southern populations due to local adaptation to photoperiod or local adaptation to temperature? This can be addressed by comparing the growth patterns found in each warming experiment. In the common-garden field experiment (Chapter 3), we observed a strong relationship of increasing growth with decreasing latitude (or photoperiod, as photoperiod and latitude are directly related). This trend could be due to adaptation to photoperiod or temperature. In the growth chamber experiment photoperiod was held constant, yet we also observed a significant relationship of increasing growth with decreasing latitude. This relationship, described in Chapter 2, had more variability in mean population growth and the regression was not as strong, however, there were significant differences in mean growth among populations that were interpreted as a result of local adaptation to growing

temperatures. When photoperiod and temperature were part of the experimental treatment (common garden), the regression fit was stronger and there was less variability in population growth means. By comparing the two experiments, we can infer that balsam poplar is locally adapted to both temperature and photoperiod regimes (but see next section).

Genotypic variation in growth and phenology: evidence of local adaptation?

In both warming experiments, genotypic differences in observed growth and phenology are being interpreted as evidence of adaptive variation in response to local photoperiod/climate regimes. Latitudinal clines with timing of bud flush, bud set, and growth traits have been recognized as having a genetic basis since the mid 20th century (Pauley and Perry 1954). Although the same patterns could be the result of genetic drift or isolation by distance (Savolainen et al. 2007), common gardens and provenance trials have documented clear clines in phenology, growth, and cold tolerance with latitude for multiple tree species (reviewed in Morgenstern 1996, Howe et al. 2000, Neale and Ingvarsson 2008). This ubiquitous trend has been widely attributed to local adaptation to photoperiod/climate regimes in the scientific literature (Rohde et al. 2011). But there are numerous reasons why plants grow differently across latitudinal or altitudinal gradients that are the result of environmental plasticity rather than adaptation (Körner 1989). Constraints to growth at the northern extremes of species' ranges can include low soil temperatures, which hinder soil microbial activity and nutrient cycling (Jarvis and Linder

2000), the presence of permafrost (Chapin 1983), short growing seasons (Loehle 1998), and costs of maintaining metabolic rates at low temperatures (Reich 1996). Therefore, it is important to look for other lines of evidence when interpreting genetic differences among populations as local adaptation.

Local adaptation is defined as a pattern within a species in which genotypes within each population have higher relative fitness at their home site (habitat) than genotypes originating from other habitats (Kawecki and Ebert 2004). The extent of local adaptation is determined by a balance of geneflow and natural selection (Savolainen et al. 2007). Can common-garden experiments demonstrate local adaptation? Savolainen et al. (2007) provide three criteria by which common gardens can be used to demonstrate this phenomenon. First, experimental sites must include home sites of the populations. In other words, reciprocal transplants are necessary for the second criterion, the fitness of the local genotypes must be compared to the fitness of transplanted genotypes. Lastly, the phenotypic traits compared must be reasonable surrogates for fitness. The experiments described in Chapters 2 and 3 did not address all three criteria, however, many forest provenance trials do substantially test for local adaptation across the environmental gradients used in this study, which provide strong evidence of local adaptation to photoperiod/climate regimes (Howe et al. 2003, Aitken et al. 2008).

Genetic analyses, such as quantitative trail loci (QTL) and association mapping can be used to show that latitudinal clines are adaptive (Hall et al. 2007, Savolainen et al. 2011, Olson et al. in press). One way to measure local adaptation is to compare estimates of F_{st} (estimate of the total genetic variation that is attributed to differences among

populations) with Q_{st} (estimate of the proportion of total genetic variation for quantitative traits among populations). If Q_{st} is greater than F_{st} then there is evidence of divergent selection among populations (Howe et al. 2003). Comparing among population variation using nuclear single nucleotide polymorphism loci (SNPs) in genotypes from across the species' range of balsam poplar, Keller et al. (2011) found evidence of local adaptation in 13 ecophysiology and phenology traits, including bud phenology, petiole length, and foliar nitrogen content. They also found evidence of local adaptation in northern populations to shorter, drier growing seasons (Keller et al. 2011). Likewise, QTL mapping studies identified several adaptive traits along latitudinal clines including bud phenology (Frewen et al. 2000, Chen et al. 2002, Scotti-Saintagne et al. 2004), cold tolerance (Chen et al. 2002, Neale and Savolainen 2004), and growth (Wu et al. 2003, Scotti-Saintagne et al. 2004). Association of phenotypic variation with candidate loci allows for the detection of functionally important SNPs. In balsam poplar, several SNPs in genes in the flowering-time network *CONSTANS/FLOWERING TERMINAL* (*CO/FT*) regulon which includes photoreceptors, circadian rhythm and vernalization genes have been associated with adaptive variation in bud phenology (Olson et al. in press).

Given the extreme range shifts in balsam poplar during the Last Glacial Maximum (18-21 k years ago), these latitudinal clines must have formed relatively recently (Breen et al. 2012, Levsen et al. 2012). The strength of this adaptive cline is exemplified by northern genotypes, which may cease growth if days are shorter than 19 - 20 hours (Olson et al. in press), suggesting that there must of been sufficient genetic diversity for new combinations of genotypes to respond to changing photoperiodic

conditions. Indeed, association studies in balsam poplar show that SNPs associated with bud phenology are widespread throughout the species' range, except in the far north, suggesting that there is sufficient underlying genetic variation for genotypes to adapt *in situ* to novel photoperiod regimes (Olson et al. in press).

Height growth as a surrogate for fitness

In the two warming experiments I used height growth as a surrogate for fitness when interpreting the relative success of balsam poplar genotypes in warmed and control environments. For forest trees, traits that provide suitable measures of fitness are debatable (Ying and Yanchuk 2006), but height growth and survival are the two most commonly used traits in the literature (Rehfeldt et al. 1999, Wu and Ying 2004, Ying and Yanchuk 2006, Savolainen et al. 2007, Reich and Oleksyn 2008, Savolainen et al. 2011). Mortality as a fitness trait is less controversial, but trees have been shown to survive in environments far from their home sites even though this generally comes at a height growth reduction (Rehfeldt et al. 2002). In the warming experiments described here, mortality was too low to assess relative fitness influences of either warming or source environment. Height growth as a fitness surrogate has been widely accepted as it is heritable (Wu and Ying 2004, Ying and Yanchuk 2006) and within the same species, taller trees have higher probabilities of flowering and producing seed due to competitive advantage for light and nutrients (Ying et al. 1985). Height growth is particularly advantageous at the juvenile stage when competition for light gaps is severe. In an experimental forest in Michigan from 1991 - 1998, sugar maple (*Acer saccharum*) seed

dispersal was measured to be 3,000 - 10,000,000 seeds ha^{-1} yr^{-1}. The number of seedlings that germinated was 725,000 seedlings ha^{-1}. Out of those seedlings 500 individuals ha^{-1} survived to the sapling/understory tree stage and only 145 trees ha^{-1} became adult trees. Of the adult trees, one tree ha^{-1} yr^{-1} reached the dominant canopy stage (Davis et al. 2005). Although boreal forests have lower turnover rates than the one used in this example, the degree of competition and stand thinning for juvenile trees should be underscored. Juvenile trees are also observed to have riskier behavior in terms of bet hedging with environmental stressors as a result of this severe competition (Howe et al 2003).

It should be noted, however, that there are selective advantages in northern environments to investing in cold tolerance traits at the expense of growth (Loehle 1998), thus height growth may not be a true surrogate across a species range. Moreover, taller trees in northern environments may be more susceptible to breaking under snow or ice loads (King et al. 2011) or may be more vulnerable to wind damage and uprooting in general. Particularly in the juvenile stage, however, if there are genotypes in northern environments that can grow taller, while still coping with environmental stress, such as cold, those genotypes would have competitive advantage and thus inferred higher fitness. Therefore, if southern genotypes transplanted into northern environments can survive and grow, they will likely have higher fitness due to greater height growth than local genotypes that have more conservative growth strategies. Moreover, northern genotypes experience strong stabilizing selection for cold-tolerance traits, but that is balanced with directional selection for maximizing height growth - this is the basis for the height

growth/cold tolerance tradeoff that is common in boreal tree species (Loehle 1998, Saxe et al. 2001, Aitken et al. 2008).

Anecdotal observations with potential relevance to local adaptation

During the course of the two warming experiments, I made observations on traits that were not part of the experimental design and thus were not systematically measured and analyzed, but that could potentially be interesting explanations of some of the reported phenomena in Chapters 2 and 3. For example, in the common-garden field experiment, I noticed a growth habit primarily in southern genotypes but also from those from mid-latitudes that may explain why southern genotypes experienced the highest incidences of cold injury but retained some of the highest relative growth increment. Starting in August and continuing through September, southern genotypes were recorded as having set bud (bud scales fully encapsulating the terminal bud) and ceased height growth. Each week the trees were re-measured for height increment, diameter increment, number of leaves and number of lateral buds. From one week to the next these southern genotypes would be recorded as having set bud, yet I would measure increases in height growth as well as increases in the number of lateral buds; all buds except the apical were considered lateral. What I observed was a particular growth pattern in which these genotypes from southern latitudes were still growing, but doing so in a way that buds on the primary stem were forming with very little new stem growth between them as if the buds were stacking on top of each other. Although the mechanism for this growth

behavior is unknown, it appeared to be a bet-hedging strategy in that if the tree experienced cold damage, the apical bud may be damaged or killed, but there would be a fully protected, set bud close underneath the apical bud, limiting total stem damage to a few centimeters compared to the tens of centimeters that generally span the distance between lateral buds or branches. This 'conservative' growth pattern allowed southern and mid-latitude genotypes to continue to grow longer into the growing season at a lesser risk than if they exhibited full active growth behavior as was typical of earlier in the growing season.

Additionally, I wanted to know if northern genotypes, adapted to long days during the growing season, were able to photosynthesize for more hours per day than their southern counterparts, which are adapted to shorter day lengths. To test this, I measured diurnal photosynthetic rates on a subset of genotypes hourly from mid-day to midnight on the longest day of the year, June 21. I found no significant differences in diurnal photosynthetic rates among genotypes and by midnight, there was no evidence of active photosynthesis despite the 400 μmol $m^{-2}sec^{-1}$ of available photosynthetically active radiation.

Epigenetics: the missing link?

As described above, the strength and repeatability of phenotypic clines in traits such as phenology and growth are strong indicators of genetic differences among populations and adaptation to local environments. Even with new genomic tools,

however, the genetics behind local adaptation remains elusive (Aitken et al. 2008, Gienapp et al. 2008, Olson et al. in press). Epigenetic mechanisms, such as DNA methylation, may help in explaining the gap between strong phenotypic clines and genetic evidence of adaptation. In order to completely address this issue, studies will need to compare differences in transcription levels and gene expression among and within populations, rather than just quantify epigenetic differences as in the experiment described in Appendix 1. But epigenetics may influence the amplitude of plasticity, or norm of reaction, in response to changing environments which may provide long-lived, sedentary organisms, such as trees, extra acclimation capacity to cope with changing environmental conditions *in situ*, or when migrating into novel environments.

References

Aitken, S. N., S. Yeaman, J. A. Holliday, T. Wang, and S. Curtis-McLane. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications **1**:95-111.

Barber, V. A., G. P. Juday, and B. P. Finney. 2000. Reduced growth of Alaskan white spruce in the twentieth century from temperature-induced drought stress. Nature **405**:668-673.

Breen, A. L., D. F. Murray, and M. S. Olson. 2012. Genetic consequences of glacial survival: the late Quaternary history of balsam poplar (*Populus balsamifera* L.) in North America. Journal of Biogeography **39**:918-928.

Chapin III, F. S. 1983. Direct and indirect effects of temperature on arctic plants. Polar Biology **2**:47-52.

Chen, T. H. H., G. T. Howe, and H. D. Bradshaw Jr. 2002. Molecular genetic analysis of dormancy-related traits in poplars. Weed Science **50**:232-240.

Danby, R. K. and D. S. Hik. 2007. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. Global Change Biology **13**:437-451.

Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. Ecology **86**:1704-1714.

Euskirchen, E. S., A. D. McGuire, D. W. Kicklighter, Q. Zhuang, J. S. Clein, R. J. Dargaville, D. G. Dye, J. S. Kimball, K. C. McDonald, J. M. Melillo, V. E. Romanovsky, and N. V. Smith. 2006. Importance of recent shifts in soil thermal dynamics on growing season length, productivity, and carbon sequestration in terrestrial high-latitude ecosystems. Global Change Biology **12**:731-750.

Frewen, B. E., T. H. H. Chen, G. T. Howe, J. Davis, A. Rohde, W. Boerjan, and H. D. Bradshaw. 2000. Quantitative Trait Loci and Candidate Gene Mapping of Bud Set and Bud Flush in *Populus*. Genetics **154**:837-845.

Gienapp, P., C. Teplitsky, J. S. Alho, J. A. Mills, and J. MerilÄ. 2008. Climate change and evolution: disentangling environmental and genetic responses. Molecular Ecology **17**:167-178.

Grace, J., F. Berninger, and L. Nagy. 2002. Impacts of Climate Change on the Tree Line. Annals of Botany **90**:537-544.

Hall, D., V. Luquez, V. M. Garcia, K. R. S. Onge, S. Jansson, and P. K. Ingvarsson. 2007. Adaptive population differentiation in phenology across a latitudinal gradient in European aspen (*Populus tremula* L.): a comparison of neutral markers, candidate genes and phenotypic traits Evolution **61**:2849-2860.

Howe, G. T., S. N. Aitken, D. B. Neale, K. D. Jermstad, N. C. Wheeler, and T. H. Chen. 2003. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Canadian Journal of Botany **81**:1247-1266.

Howe, G. T., G. Gardner, W. P. Hackett, and G. R. Furnier. 1996. Phytochrome control of short-day-induced bud set in black cottonwood. Physiologia Plantarum **97**:95-103.

Howe, G. T., P. Saruul, J. Davis, and T. H. H. Chen. 2000. Quantitative genetics of bud phenology, frost damage, and winter survival in an F2 family of hybrid poplars. TAG Theoretical and Applied Genetics **101**:632-642.

Jarvis, P. and S. Linder. 2000. Botany: Constraints to growth of boreal forests. Nature **405**:904-905.

Johnstone, J. F. and F. S. Chapin. 2003. Non-equilibrium succession dynamics indicate continued northern migration of lodgepole pine. Global Change Biology **9**:1401-1409.

Kawecki, T. J. and D. Ebert. 2004. Conceptual issues in local adaptation. Ecology Letters **7**:1225-1241.

Keller, S. R., R. Y. Soolanayakanahally, R. D. Guy, S. N. Silim, M. S. Olson, and P. Tiffin. 2011. Climate-driven local adaptation of ecophysiology and phenology in

balsam poplar, *Populus balsamifera* L. (Salicaceae). American Journal of Botany **98**:99-108.

King, D. A. 2011. Size-Related Changes in Tree Proportions and Their Potential Influence on the Course of Height Growth Size- and Age-Related Changes in Tree Structure and Function. Pages 165-191 *in* F. C. C. Meinzer, B. Lachenbruch, and T. E. E. Dawson, editors. Springer Netherlands.

Körner, C. 1989. The Nutritional Status of Plants from High Altitudes. A Worldwide Comparison. Oecologia **81**:379-391.

Levsen, N. D., P. Tiffin, and M. S. Olson. 2012. Pleistocene Speciation in the Genus *Populus* (Salicaceae). Systematic Biology.

Linderholm, H. W. 2006. Growing season changes in the last century. Agricultural and Forest Meteorology **137**:1-14.

Loehle, C. 1998. Height growth rate tradeoffs determine northern and southern range limits for trees. Journal of Biogeography **25**:735-742.

Morgenstern, E. K. 1996. Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Siviculture. UBC Press, Vancouver, British Columbia.

Neale, D. B. and P. K. Ingvarsson. 2008. Population, quantitative and comparative genomics of adaptation in forest trees. Current Opinion in Plant Biology **11**:149-155.

Neale, D. B. and O. Savolainen. 2004. Association genetics of complex traits in conifers. Trends in Plant Science **9**:325-330.

O'Neill, G. A., A. Hamann, and T. Wang. 2008. Accounting for population variation improves estimates of the impact of climate change on species' growth and distribution. Journal of Applied Ecology **45**:1040-1049.

Olson, M. S., N. Levsen, R. Y. Soolanayakanahally, R. D. Guy, W. R. Schroeder, S. R. Keller, and P. Tiffin. in press. The adaptive potential of *Populus balsamifera* L. to phenology requirements in a warmer global climate. Molecular Ecology.

Pauley, S. S. and T. O. Perry. 1954. Ecotypic variation of the photoperiodic response in *Populus*. Journal of Arnold Arboretum **35**:167-188.

Rehfeldt, G. E., N. M. Tchebakova, Y. I. Parfenova, W. R. Wykoff, N. A. Kuzmina, and L. I. Milyutin. 2002. Intraspecific responses to climate in Pinus sylvestris. Global Change Biology **8**:912-929.

Rehfeldt, G. E., C. C. Ying, D. L. Spittlehouse, and D. A. Hamilton. 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation Ecological Monographs **69**:375-407.

Reich, P. B. and J. Oleksyn. 2008. Climate warming will reduce growth and survival of Scots pine except in the far north. Ecology Letters **11**:588-597.

Reich, P. B. O. 1996. Needle respiration and nitrogen concentration in Scots Pine populations from a broad latitudinal range: A common garden test with field-grown trees. Functional Ecology **10**:768-776.

Robin, J., R. Dubayah, E. Sparrow, and E. Levine. 2008. Monitoring start of season in Alaska with GLOBE, AVHRR, and MODIS data. J. Geophys. Res. **113**:G01017.

Rohde, A., C. Bastien, and W. Boerjan. 2011. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. Tree Physiology **31**:472-482.

Savolainen, O., T. Pyhäjärvi, and T. Knürr. 2007. Gene Flow and Local Adaptation in Trees. Annual Review of Ecology, Evolution, and Systematics **38**:595-619.

Savolainen, O., S. T. Kujala, C. Sokol, T. Pyhäjärvi, K. Avia, T. Knürr, K. Kärkkäinen, and S. Hicks. 2011. Adaptive Potential of Northernmost Tree Populations to Climate Change, with Emphasis on Scots Pine (Pinus sylvestris L.). Journal of Heredity **102**:526-536.

Saxe, H., M. G. R. Cannell, Ø. Johnsen, M. G. Ryan, and G. Vourlitis. 2001. Tree and forest functioning in response to global warming. New Phytologist **149**:369-399.

Scotti-Saintagne, C., C. Bodénès, T. Barreneche, E. Bertocchi, C. Plomion, and A. Kremer. 2004. Detection of quantitative trait loci controlling bud burst and height growth in *Quercus robur* L. TAG Theoretical and Applied Genetics **109**:1648-1659.

Silim, S., N. Ryan, and D. Kubien. 2010. Temperature responses of photosynthesis and respiration in *Populus balsamifera* L.: acclimation versus adaptation. Photosynthesis Research **104**:19-30.

Soolanayakanahally, R. Y., R. D. Guy, S. N. Silim, E. C. Drewes, and W. R. Schroeder. 2009. Enhanced assimilation rate and water use efficiency with latitude through increased photosynthetic capacity and internal conductance in balsam poplar (*Populus balsamifera* L.). Plant, Cell & Environment **32**:1821-1832.

Way, D. A. and R. F. Sage. 2008a. Elevated growth temperatures reduce the carbon gain of black spruce (*Picea mariana* (Mill.) B.S.P.). Global Change Biology **14**:624-636.

Way, D. A. and R. F. Sage. 2008b. Thermal acclimation of photosynthesis in black spruce (*Picea mariana* (Mill.) B.S.P.). Plant, Cell & Environment **31**:1250-1262.

Wendler, G. and M. Shulski. 2009. A century of climate change for Fairbanks, Alaska. Arctic **62**:295-300.

Way, D. A., J.-C. Domec, and R. B. Jackson. 2012. Elevated growth temperatures alter hydraulic characteristics in trembling aspen (*Populus tremuloides*) seedlings: implications for tree drought tolerance. Plant, Cell & Environment:no-no.

Wilmking, M., G. P. Juday, V. A. Barber, and H. S. J. Zald. 2004. Recent climate warming forces contrasting growth responses of white spruce at treeline in Alaska through temperature thresholds. Global Change Biology **10**:1724-1736.

Wu, H. X. and C. C. Ying. 2004. Geographic pattern of local optimality in natural populations of lodgepole pine. Forest Ecology and Management **194**:177-198.

Wu, R., C.-X. MA, M. C. K. Yang, M. Chang, R. C. Littell, U. Santra, S. S. Wu, T. Yin, M. Huang, M. Wang, and G. Casella. 2003. Quantitative trait loci for growth trajectories in *Populus*. Genetics Research **81**:51-64.

Ying, C., K. Illingworth, and M. Carlson. 1985. Geographic variation in lodgepole pine and its implications for tree improvement in British Columbia. Washington State University, Cooperative Extension, Pullman Washingtington (USA):8-16.

Ying, C. C. and A. D. Yanchuk. 2006. The development of British Columbia's tree seed transfer guidelines: Purpose, concept, methodology, and implementation. Forest Ecology and Management **227**:1-13.

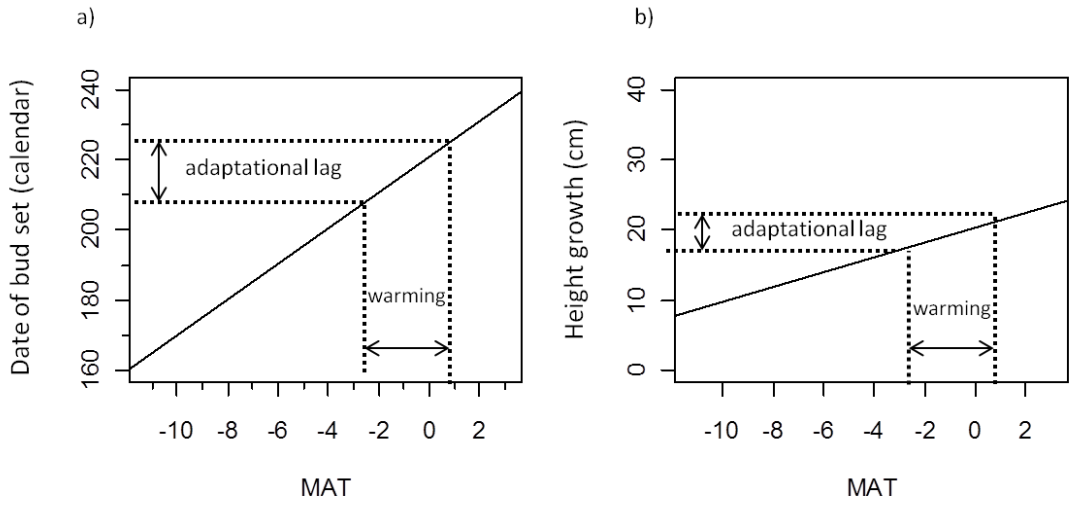

Figure 5.1 Genetic clines along gradients in mean annual temperature for mean calendar date of bud set (a), and for total height growth (b). The horizontal arrow illustrates the degree of experimental warming (3 °) and the vertical arrow represents the predicted adaptational lag for that amount of warming. Data are from Chapter 4; figure is adapted from Aitken et al. (2008).

Appendix 1Methylation-sensitive Amplified Polymorphism Protocol

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Appendix 1 describes a protocol for quantification of global levels of DNA methylation that has been optimized for balsam poplar. Methylation-sensitive amplified polymorphism (MSAP) is a modified version of Amplified Fragment Length Polymorphism (AFLP) techniques developed by Vos et al (1995). Xiong et al (1999) replaced the standard rare and frequent cutter restriction enzymes in traditional AFLP with two isoschizomer restriction enzymes *Hpa*II and *Msp*I. Both enzymes recognize the same restriction sites (5'-CCGG-3') but have different cytosine methylation sensitivities. The MSAP technique allows for quantification of cytosine methylation variation without knowledge of genome sequences which allows it to be used outside of model organisms, but also means that this method does not identify which loci are methylated.

I extracted genomic DNA from the balsam poplar trees used in the warming experiments described in Chapters 2 and 3 for quantification of differences in DNA methylation. Specifically, I am asking:

1) Is there greater DNA methylation variation among- than within-populations of balsam poplar sampled from across the species' latitudinal range?

2) Are levels of DNA methylation correlated with phenotypes induced by experimental warming?

This project is designed to be one of the first landscape-scale epigenetic surveys in natural populations and will attempt to address questions about the role of epigenetic mechanisms in tree adaptation and acclimation to climate.

In the spring of 2012, the laboratory protocol described below was optimized for use in balsam poplar. Prior to optimization, development and testing of the protocol was carried out over a period of six months. MSAP data collection has been completed using six primer pairs for the trees used in the common-garden field experiment (Chapter 3). Data analysis is expected to be completed in the spring of 2013. When integrated with the warming experiments in Chapters 2 and 3, and with body of literature on the genetics of adaptive clines in forest trees, this project has the potential to further our understanding of the mechanisms by which trees acclimate and adapt to novel environments.

References

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. v. d. Lee, M. Hornes, A. Friters, J. Pot, J. Paleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research **23**:4407-4414.

Xiong, L. Z., C. G. Xu, M. A. Saghai Maroof, and Q. Zhang. 1999. Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. Molecular and General Genetics MGG **261**:439-446.

Methylation-sensitive Amplified Polymorphism Protocol

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Prepare reactions on ice

1. Restriction Digest (2 separate rxns)

Restriction Digest Hpa11
37.5 ng DNA

0.0375 µl EcoRI NEB (20,000 U/ml) = 0.75 U
0.15 µl HpaII NEB (10,000 U/ml) = 1.5 U*
1.0 µl 10X NEB Buffer #4

Q.S. to 10 µl with ddH20

Restriction Digest MspI
37.5 ng DNA

0.0375 µl EcoRI NEB (20,000U/ml) =
0.75 U
0.0375 µl MspI NEB (20,000 U/ml) =
0.75 U
1.0 µl 10X NEB Buffer #4

Q.S. to 10 µl with ddH20

Incubate at 37º C for 3 hours, heated lid
*HpaII volume is doubled due to 50% optimality in NEB buffer #4

2. Ligation of adaptors (2 separate rxns)
after incubation, add to each 10 µl digest (for a total 12.5 µl):

0.125 µl EcoRI DS (double stranded) adaptor (50 pm/µl)
1.0 µl H/M DS adaptor (50 pm/µl)
0.25 µl 10X T4 Ligase buffer
0.125 µl T4 DNA ligase
1.0 µl ddH20
Incubate at 16 ℃ for 3 hours, heat inactivation 65 ℃ for 10 mintues

Store ligation reactions at either 4 or -20 ℃

3. Pre-amplification (2 rxns)
25 µl volume
Program:
95º C 02:00*
95º C 00:30
60º C 00:30
72º C 1:00
repeat 30 cycles
hold 10º C

*Change program to optimize amplification for specific taq

2 µl template DNA (from ligation rxn, undiluted)
5.0 µl 5x Taq buffer
0.5 µl dNTPs
2.5 µl EcoRIpre-primer (@ 10 µM)
2.5 µl H/Mpre-primer (@ 10 µM)
0.125 µl Taq
12.375 µl ddH20

dilute 1:19 with ddH2O, store at 4 or -20º C
Run gel (smear)

4. Selective amplification
10 µl volume

Program:
95º C 2:00
95º C 0:30
-1º C / 68º C 0:30 67,66,65,64,63,62,61,60,59
72º C 1:00
repeat 9x
95º C 00:30s
58º C 00:30s
72º C 1:00
repeat 25x
hold 10º C

2.5 µl template DNA (from diluted pre-amp)
2 µl 5X taq buffer
0.2 µl dNTPs
0.5 µl EcoRI+N primer (10 µM, fluorescently labeled)
0.5 µl H/M+N primer (10 µM)
0.1 µl taq
4.2 µl ddH20

5. Submit fragments for analysis
Formamide and denature
Can multiplex several reactions using fluorescently labeled primers (EcoRI +N)

9.5 µl Formamide
0.5 µl Size Standard (LIZ500 or LIZ600)
1 µl total volume PCR product and water

Optimize loading concentrations for each marker; If multiplexing markers, total volume of PCR products should equal 1.0 µl total product; may need to dilute with water

Adaptor preparation:
Centrifuge dehydrated primers
Vortex after hydrating, do not re-centrifuge once hydrated

Dilute adaptors to 100 µM in TE and NaCl:
Mix:
60 µl 5 M NaCl
Q.S. to equal 100 µM in 1xTE (total solution 10 x nmoles of dehydrated oligo)

(use sterile TE and NaCl)

Anneal adaptors:
10 µl forward adaptor (100 µM)
10 µl reverse adaptor (100 µM)
Do for both frequent and rare cutters
Results in final concentration 50 pm/µl

Heat to 95°C and allow to cool to room temperature slowly

EcoRI adaptorF	5'-CTCGTAGACTGCGTACC	5'-G/AATTC
EcoRI adaptorR	CATCTGACGCATGGTTAA-5'	CTTAA/G-5'
H/M adaptorF	5'-GATCATGAGTCCTGCT	5'-C/CGG
H/MadaptorR	AGTACTCAGGACGAGC-5'	GGC/C-5'
MseIadaptorF	5'-GACGATGAGTCCTGAG	5'-T/TAA
MseIadaptorR	ATGAGTCCTGAGTA-5'	AAT/T-5'

ADAPTOR:

95 °C	3 min
90 °C	90 sec
5º C / 90º C (to 20°C)	90 sec
10°	hold
end	