American Journal of Botany

American Journal of Botany 98(1): 99–108. 2011.

Climate-driven local adaptation of ecophysiology and phenology in balsam poplar, *Populus balsamifera* L. (Salicaceae)[1]

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- *Premise of the study*: During past episodes of climate change, many plant species experienced large-scale range expansions. Expanding populations likely encountered strong selection as they colonized new environments. In this study we examine the extent to which populations of the widespread forest tree *Populus balsamifera* L. have become locally adapted as the species expanded into its current range since the last glaciation.
- *Methods*: We tested for adaptive variation in 13 ecophysiology and phenology traits on clonally propagated genotypes originating from a range-wide sample of 20 subpopulations. The hypothesis of local adaption was tested by comparing among-population variation at ecologically important traits (Q_{ST}) to expected variation based on demographic history (F_{ST}) estimated from a large set of nuclear single nucleotide polymorphism loci.
- *Key results*: Evidence for divergence in excess of neutral expectations was present for eight of 13 traits. Bud phenology, petiole length, and leaf nitrogen showed the greatest divergence (all $Q_{ST} > 0.6$), whereas traits related to leaf water usage showed the least (all $Q_{ST} \leq 0.30$) and were not different from neutrality. Strong correlations were present between traits, geography, and climate, and they revealed a general pattern of northern subpopulations adapted to shorter, drier growing seasons compared with populations in the center or eastern regions of the range.
- *Conclusions*: Our study demonstrates pronounced adaptive variation in ecophysiology and phenology among balsam poplar populations. These results suggest that as this widespread forest tree species expanded its range since the end of the last glacial maximum, it evolved rapidly in response to geographically variable selection.

Key words: adaptation; balsam poplar; drift; ecophysiology; F_{ST}; phenology; *Populus balsamifera*; Q_{ST}; selection; SNP.

Studies on the evolution of plant populations during historical fluctuations in climate can reveal the capacity for and constraints on adaptive evolution and may help inform predictions about evolutionary responses to future environments (Davis and Shaw, 2001; Aitken et al., 2008; Petit et al., 2008). When demography is relatively stable and environmental change occurs gradually, the distribution of diversity within and among populations and the extent of local adaptation across a species' range will reflect the long-term balance between migration, genetic drift, and selection (Wright, 1951; Kirkpatrick and Barton, 1997). However, rapid changes in the environment may trigger changes in population demography, differences in the direction or magnitude of selection, or both. Such changes may drive shifts in geographical distributions, population genetic diversity, and locally adapted trait variation (Davis and Shaw, 2001; Excoffier et al., 2009a).

The warming of climate conditions that marked the end of the last glacial maximum (~18 thousand years ago) was a rapid environmental change that increased population sizes and led to range expansion in many plant species. For species of forest trees, populations generally increased in distribution and migrated out of refugia to recolonize vast continental land masses over a relatively brief span of a few hundred generations (Taberlet et al., 1998; Petit et al., 2003). This wave of migrations affected genomic diversity within populations, as colonists subsampled alleles from the ancestral gene pool during the process of expansion (Lascoux et al., 2004; Petit et al., 2004; Savolainen and Pyhäjärvi, 2007). Thus, much of the diversity within and the divergence between populations would have initially arisen as a consequence of stochastic sampling and founder effects. However, colonists undoubtedly encountered novel selective environments as well. Changes in temperature, precipitation, photoperiod, and biotic diversity all occur with latitude, leading to potentially strong selective gradients coincident with the direction of expansion. The resolution of these two processes during expansion, stochastic sampling of genetic diversity on the one hand, and selection favoring different allelic variants across diverse environments on the other, is often not apparent, and approaches are needed that distinguish the effects of selection from drift during expansion (e.g., Keller and Taylor, 2008; Coop et al., 2009).

[1] Manuscript received 22 August 2010; revision accepted 10 November 2010.

The authors thank K. Ritland for advice on the heritability analysis and the University of Minnesota Supercomputing Institute (MSI) for computational support. The manuscript was improved by the constructive comments of J. Ross-Ibarra and three anonymous reviewers. Financial support came from NSF Plant Genome (award DBI-0701911 to M.S.O. and P.T.), Agri-Environment Services Branch, Agriculture and Agri-Food Canada, under the Student Research Affiliate Program (to R.Y.S.), and a Natural Sciences and Engineering Research Council (Canada) Discovery Grant (to R.D.G.).

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doi:10.3732/ajb.1000317

Balsam poplar (*Populus balsamifera* L., Salicaceae) is a widespread, deciduous hardwood tree in North America that underwent a massive range expansion at the end of the Pleistocene (Keller et al., 2010). This expansion led to the development of three regional clusters of genetic diversity—a northern region cluster distributed primarily in Alaska and northern Canada, a widespread central region cluster distributed from the Great Lakes to the Canadian prairie provenances, and an eastern region cluster primarily distributed in Quebec and maritime Canada. Evidence from marker diversity and coalescent models of migration and population size suggest a large, diverse population in the center and highly asymmetrical migration away from the center toward the north and east, with genetic diversity declining toward the range peripheries. Thus, the history of balsam poplar's postglacial expansion is reasonably well characterized and includes a large-scale northward migration out of southern refugia that subsampled ancestral diversity into different parts of the current range (Keller et al., 2010).

A greenhouse study was recently conducted in balsam poplar by Soolanayakanahally et al. (2009) in which dormant stem whips from 10 genotypes from each of 21 populations were grown without resource limitation. Multiple ecophysiological (photosynthesis, stomatal conductance, water- and nitrogen [N]-use efficiencies, growth rate) and morphological traits (leaf mass area, petiole length, stomatal density) displayed significant population differences. In addition to the greenhouse study, Soolanayakanahally et al. (unpublished manuscript) monitored spring and autumn phenology in balsam poplar planted in a common garden and found strong clines with latitude of origin. Parallel latitudinal clines for growth and photosynthesis have been observed in black cottonwood (Gornall and Guy, 2007), paper birch, and Sitka alder (Benowicz et al., 2000), which suggests a general adaptive response among woody angiosperms to selective pressures encountered at higher latitudes. Population differences may be consistent with local adaptation; however, it is unclear whether divergence in ecophysiological and phenological traits exceeds that expected from neutral founder effects and genetic drift during range expansion.

In this study, we examined the evolutionary causes of population divergence during the postglacial expansion of balsam poplar by combining genetic diversity estimated at a set of reference nuclear single nucleotide polymorphisms (SNPs) loci with greenhouse and common garden measurements of 13 ecophysiological and phenological traits in a range-wide sample of 20 populations studied previously for both phenotypic and population genetic variation (Soolanayakanahally et al., 2009; Keller et al., 2010). We had three primary goals: (1) to test whether current variation in plant ecophysiological and phenological traits reflects a history of selection or demography as poplar migrated in response to a changing climate, (2) to compare the adaptive nature of trait divergence at regional vs. local spatial scales, and (3) to identify the traits most strongly affected by geographically varying climate and environmental factors. Our analysis applied a widely used method of comparing the distribution of variance for quantitative traits and neutral markers (Q_{ST} vs. F_{ST}) to identify traits putatively under local selection ($Q_{ST} > F_{ST}$) or those under stabilizing selection ($Q_{ST} < F_{ST}$) (Lewontin and Krakauer, 1975; Spitze, 1993; Whitlock, 1999; McKay and Latta, 2002; Whitlock, 2008). We also explored how among-population trait variation was associated with gradients in latitude, summer temperature and precipitation, and background genetic ancestry.

MATERIALS AND METHODS

Study species—Balsam poplar has a natural range that covers the boreal and northeastern temperate forests of North America (Fig. 1). For this study we collected ecophysiological data for 10 genotypes from each of 20 sampling localities (hereafter referred to as *subpopulations*) ($N = 200$) and phenology data on 12–15 genotypes from each of the same populations. Ecophysiological traits were measured in greenhouse-grown plants, and phenology traits were scored on plants grown in an outdoor common garden ($N = 255$) in Indian Head, Saskatchewan. All the genotypes we included are part of the Agriculture Canada Balsam Poplar (AgCanBaP) collection (described in Soolanayakanahally et al., 2009). The data on ecophysiological traits from these same genotypes were previously reported in Soolanayakanahally et al. (2009).

Greenhouse ecophysiology data collection—All leaf-based observations were recorded on fully expanded mature leaves. Light saturated photosynthetic rates (A) and stomatal conductance (g_s) were recorded with a portable gas exchange system (Analytical Development Co., Ltd., Hoddesdon, UK) during the summer of 2006 in a greenhouse on all genotypes maintained under free growth over 90 d without light, water, or nutrient limitation. The natural photoperiod was extended to 21 h with artificial lighting providing a minimum photosynthetic photon flux density (PPFD) of 400 μmol · m^{-2} · s^{-1}. Water-use efficiency (WUE) was estimated as the ratio of A/g_s. Immediately after the gas exchange measurements, the chlorophyll content index of the same leaves was determined with a CCM-200 meter (Opti-Sciences, Hudson, New Hampshire, USA). Leaf punches then were collected to determine leaf mass area (g · cm^{-2}), leaf $\delta^{13}C$ (‰), and leaf N (μmol N · cm^{-2}). Nail polish impressions of adaxial and abaxial leaf surfaces were taken for counts of stomatal density. Stem tissue was sampled at the end of the experiment for wood $\delta^{13}C$ (‰). Height increment (cm) was recorded on all genotypes over a 15-d interval. For additional details, refer to Soolanayakanahally et al. (2009).

Common garden phenology observations—The common garden was established in May 2007 near the southern edge of the species range at Indian Head (50.33°N, 103.39°W), Saskatchewan, Canada. Fifteen distinct genotypes from each subpopulation were planted in a group at 2 × 2 m spacing, and groups (subpopulations) then were randomly assigned within five blocks. Spring bud flush and, later, bud set were monitored in one block in the year 2008. At the common garden site, mean annual precipitation is 435 mm, and mean maximum and minimum temperatures during the growing season (May–August) are 22°C and 8°C, respectively.

SNP survey—The marker data consist of 410 of the 412 SNPs originally reported in Keller et al. (2010) (we removed SNPs 642336 and 227771_5 because they were monomorphic in the present set of populations). Single nucleotide polymorphisms were developed by haphazardly selecting one polymorphic site per region sampled from across the genome in a previous resequencing survey and genotyped with the Sequenom iPlex platform (Sequenom, San Diego, California, USA) (see also Keller et al., 2010; Olson et al., 2010). All SNP data are available for download at the Poplar Population Genomics website (http://www.popgen.uaf.edu/).

Analysis—We tested for mean differences in ecophysiological traits using mixed-model nested ANOVA (SAS: PROC MIXED). For this, we defined a hierarchical population structure that consisted of three genetically based regions (North, Center, and East) and local subpopulations nested within each region (Fig. 1). Regions were defined on the basis of the analysis by Keller et al. (2010), which grouped SNP genotypes by using the Bayesian clustering algorithm Instruct (Gao et al., 2007); all genotypes in this study were included in that analysis. In ANOVA, region was treated as a fixed effect with three levels, and subpopulation was nested within region as a random effect. Significance tests for the random subpopulation effect were performed by running the model under restricted maximum likelihood estimation (REML), with and without the subpopulation effect included, and then calculating likelihood ratio tests with 1 degree of freedom.

Hierarchical estimates of quantitative genetic divergence among all subpopulations (Q_{ST}), among regions (Q_{CT}), and among local subpopulations within regions (Q_{SC}) were estimated from the phenotypic data by using the following equations:

$$Q_{ST} = \frac{\sigma^2_{pop}}{\sigma^2_{pop} + 2\left(h^2\right)\sigma^2_{resid}} \quad (1)$$

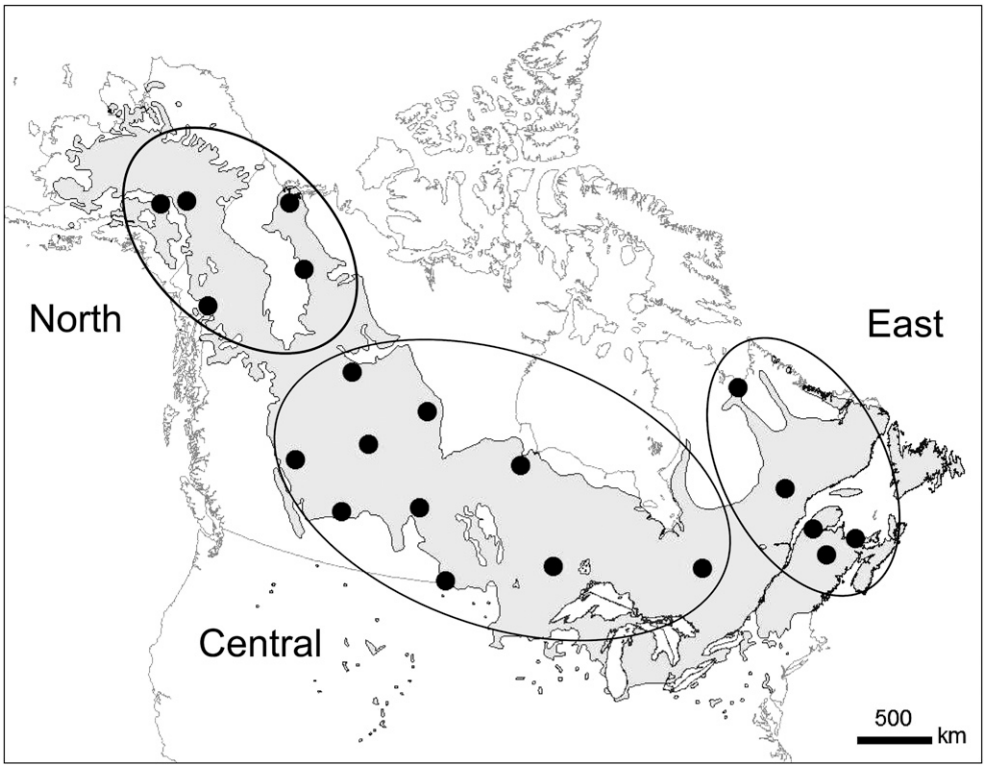


Fig. 1. Map of sampling localities and geographic range of balsam poplar (shaded area). Circles around groups of subpopulations denote membership in the three genetically defined regions identified by Bayesian clustering analysis of single nucleotide polymorphism genotypes (see Keller et al., 2010).

$$Q_{CT} = \frac{\sigma^2_{region}}{\sigma^2_{region} + \sigma^2_{pop(region)} + 2\left(h^2\right)\sigma^2_{resid}} \quad (2)$$

$$Q_{SC} = \frac{\sigma^2_{pop(region)}}{\sigma^2_{pop(region)} + 2\left(h^2\right)\sigma^2_{resid}} \quad (3)$$

where σ^2_{pop} is the variance among all 20 subpopulations considered independently, σ^2_{region} is the variance among regions, $\sigma^2_{pop(region)}$ is the variance among populations nested within regions, σ^2_{resid} is the residual variance among individual genotypes, and h^2 is the narrow-sense heritability (the proportion of total phenotypic variance attributable to additive genetic effects). Variance components for σ^2_{pop}, σ^2_{region}, $\sigma^2_{pop(region)}$, and σ^2_{resid} were obtained by random effects ANOVA by using REML estimation (SAS: PROC VARCOMP). Because our genotypic data were collected on only a single individual of each genotype, we lacked estimates of either heritability or within-population additive genetic variance for each trait. Whereas all genotypes were grown under relatively uniform conditions (either greenhouse or common garden), microenvironmental differences and measurement error undoubtedly contributed to some of the observed phenotypic variance. Therefore, we used the method of Ritland (1996, 2000) to obtain indirect estimates of h^2 (hereafter referred to as $\hat{h}^2$) based on the covariance between phenotypes and marker-inferred relatedness among individuals. To keep our estimates of h^2 independent from distributions of F_{ST}, we randomly selected 100 of the SNP loci for estimating $\hat{h}^2$ and used the remaining 310 loci for determining the distribution of F_{ST}. We estimated $\hat{h}^2$ by using maximum likelihood with K. Ritland's MARK program (available at http://genetics.forestry.ubc.ca/ritland/programs.html). We used a distance class of 0.1 degrees (roughly ~10 km), which restricted the pairwise calculations of relatedness and phenotypic similarity to between individuals from the same local subpopulation. Calculation of 95% confidence intervals on $\hat{h}^2$ was done by randomly resampling the data with 1000 bootstrap replicates. Second, we explored the degree to which uncertainty in heritability may affect our inference by recalculating estimates of quantitative divergence across a range of h^2 values from 0.1 to 1.0 (Saether et al., 2007).

We used AMOVA in Arlequin version 3.5 (Excoffier and Lischer, 2010) to partition genetic variance among regions and subpopulations (within regions) using the 310 SNPs that we did not use for estimating h^2. Significance of variance components was assessed following 1000 permutations of the data. To evaluate which of our traits showed excessive divergence at each hierarchical level relative to neutral demographic expectations, we compared our values of Q_{ST}, Q_{CT}, and Q_{SC} with the corresponding F statistics for the 310 SNP loci. We looked for evidence of local adaptation by assessing whether Q_{ST} values from traits are outliers from the empirical distribution of F_{ST}—i.e., the expected distribution of traits evolving under neutrality. Empirical distributions of F statistics were estimated from locus-by-locus AMOVA in Arlequin, and the significance threshold for outlier Q_{ST} values was set to $\alpha = 0.01$ to be conservative in the face of multiple comparisons.

Lastly, we performed an exploratory analysis to assess the correlations between phenotypic traits, genetic ancestry, and environmental gradients. Ancestry was summarized by the first two eigenvectors from a principal components analysis (PCA) on subpopulation SNP allele frequencies, which collectively explain 25% of the variance (Keller et al., 2010). The environmental variables were average summer temperature (AST), number of frost-free days (FFD), latitude (LAT), and an index of summer dryness (SDI). Climate data were based on 30-yr averages (1971–2000) obtained from Environment Canada and NOAA's National Climatic Data Center. Additional details on climate data are presented in Soolanayakanahally et al. (2009). We examined pairwise correlations among traits, ancestry, and environment using Pearson correlation coefficients. Because many of these variables were highly correlated (see Results), we performed canonical correlation analysis (CCA) using the *cca* package in R (R Development Core Team. 2009) to explore the joint associations between phenotypic traits and ancestry/environment. Canonical correlation analysis identifies linear combinations of one matrix (here, a 13 × 20 matrix of trait means across subpopulations) that are highly correlated with linear combinations of a second matrix (here, a 6 × 20 matrix of genetic ancestry/environment across subpopulations). This allowed us to assess which combinations of climate and ancestry were most correlated with subpopulation variation in quantitative traits.

RESULTS

We used range-wide data for genotypes from 20 balsam poplar subpopulations, grown under greenhouse and common garden conditions, and assayed for 410 nuclear SNP loci and for 13 phenotypic traits that gauge plant growth, resource use, and seasonal phenology. On the basis of relatedness calculated from 100 SNPs, nine of 13 traits showed marker-based estimates of heritability ($\hat{h}^2$) with 95% confidence intervals greater than zero. Bud set, height increment, and photosynthetic rate showed the greatest proportion of genetically based phenotypic variation within populations, whereas the remaining 10 traits all had $\hat{h}^2$ values < 0.5 (Fig. 2). Four traits related to water use (wood $\delta^{13}C$, leaf $\delta^{13}C$, stomatal density, and stomatal conductance) had 95% confidence intervals that overlapped zero.

Variation in phenotypic traits was structured hierarchically among regions and among local subpopulations. After Bonferroni correction, four traits showed significant differences in ANOVA among the three regions, and nine traits were differentiated among local subpopulations within regions (Table 1). Three traits (bud set, height increment, and leaf mass area) showed divergence at both regional and local subpopulation scales (all $P < 0.001$, Table 1). Averaged across all 13 traits, 20% of total phenotypic variance was explained by differences among regions (range 0–74%) and 18% by differences among subpopulations within regions (range 1%–52%; Fig. 3). The percentage of variance explained by differences among all 20 subpopulations in ANOVA was positively related to the magnitude of trait heritability (Spearman's $\rho = 0.68$, $P = 0.01$). With the exception of leaf $\delta^{13}C$, traits with weak heritability showed little evidence of divergence among regions or subpopulations (Table 1).

In contrast to quantitative traits, variance at 310 additional independent SNP loci showed much lower but still significant genetic structure, with most of the divergence in AMOVA occurring among regions (4.7%) and less among subpopulations within regions (2.0%; both $P < 0.0001$). When estimates of quantitative genetic divergence (Q_{ST}) were compared with the empirical distribution of divergence at SNPs (F_{ST}), eight of the 13 traits showed excessive divergence relative to neutral expectations, indicating pervasive adaptation of poplar ecophysiology and phenology in response to spatially varying selection (Fig. 4A). The traits with the greatest among-subpopulation divergence were bud set, petiole length, bud flush, and leaf N (all $Q_{ST} > 0.60$), whereas stomatal density, chlorophyll index, leaf $\delta^{13}C$, wood $\delta^{13}C$, and stomatal conductance all showed levels of divergence consistent with neutral demography ($Q_{ST} \leq 0.3$; Fig. 4A). Estimates of quantitative divergence for each trait are provided in Appendix S1 (see Supplemental Data online at http://www.amjbot.org/cgi/content/full/ajb.1000317/DC1).

When trait divergence was analyzed hierarchically (Fig. 4B), evidence existed for adaptive divergence among regions for four traits, including bud set ($Q_{CT} = 0.70$), petiole length ($Q_{CT} = 0.45$), leaf mass area ($Q_{CT} = 0.44$), and height increment ($Q_{CT} = 0.38$). Divergence among regions for bud set was especially large relative to the distribution of SNPs and other phenotypic traits. On average, genotypes from the North set bud 50 d earlier than those from the Center and >70 d earlier than those from the East (Fig. 5). Height increment and leaf mass area were both highest in the North and diminished to the Center and East, whereas petiole length was shortest in the North and longer in the Center and East. Several traits related to water use (leaf and wood $\delta^{13}C$ and WUE) showed significant phenotypic differences among regions in ANOVA but fell within the empirical distribution of SNP F_{CT} values, consistent with neutral divergence due to genetic drift (Table 1, Fig. 4B).

At the scale of local subpopulations within regions, outlier Q_{SC} values were observed for most traits, with the magnitude of Q_{SC} greatest for bud phenology (bud flush and bud set) and leaf N (Fig. 4C). Interestingly, while height increment showed significant divergence among regions ($Q_{CT} > F_{CT}$), it was not a significant outlier in the distribution of divergence among local subpopulations ($Q_{SC} = 0.11$). In fact, across traits there was generally poor correspondence between divergence among regions vs. among local subpopulations (Spearman's $\rho = -0.02$, $P = 0.96$), indicating differences in the strength and/or direction of selection operating at different spatial scales.

Inference about which Q statistics were significantly above neutral expectations was generally robust to the exact magnitude of heritability for traits (Appendix S2). Exceptions mostly involved Q_{SC} values for stomatal density, chlorophyll index, and leaf mass area, which became nonsignificant as $\hat{h}^2$ increased.

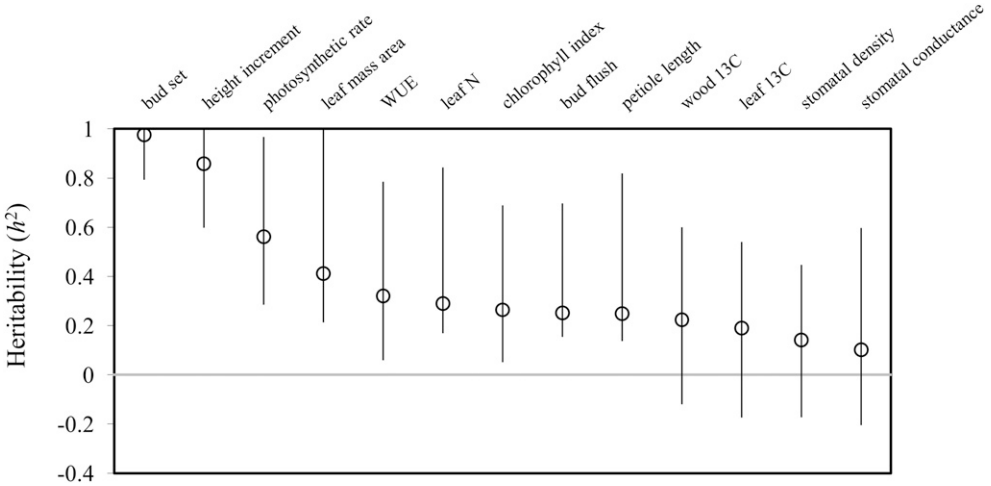


Fig. 2. Marker-based heritability estimates for phenological and ecophysiological traits. Error bars are 95% confidence intervals from 1000 bootstrap replicates. WUE, water-use efficiency.

TABLE 1. Results from mixed-model ANOVA for hierarchical divergence for phenological and ecophysiological traits among 20 subpopulations belonging to three regions defined by Bayesian clustering analysis of single nucleotide polymorphism genotypes. Variables significant at $\alpha = 0.05$ are followed by an asterisk (before multiple testing correction) or are in boldface type (after Bonferroni correction).

Trait	*N*	Region (*F* value)	Population (Δ-2LL)[a]
Phenology			
Bud flush	255	3.61	**127.5***
Bud set	255	**27.27***	**294.3***
Growth			
Chlorophyll index	200	2.55	**11.0***
Height increment	200	**20.28***	**13.6***
Leaf mass area	200	**14.06***	**17.2***
Leaf N	200	1.43	**86.1***
Petiole length	200	7.68*	**47.9***
Photosynthetic rate	200	3.11	**36.0***
Water use			
Leaf $\delta^{13}C$	200	**8.39***	0.1
Wood $\delta^{13}C$	200	3.60*	2.8
Stomatal conductance	200	0.70	0.7
Stomatal density	200	1.02	6.2*
Water-use efficiency	200	4.48*	**14.1***

[a] Likelihood ratio test statistic from models with and without the random effect of population.

Given this uncertainty, our analyses do not provide strong evidence that subpopulation variation in these traits reflects local adaptation. By contrast, other traits showing significant *Q* statistics represent robust evidence of adaptive divergence.

Extensive covariation was evident between latitude, climate, and genetic ancestry (Table 2). Canonical correlation analysis showed the structure of this covariation could be summarized by a single, highly significant multivariate dimension that explained 99% of the variance among the 20 populations (CCA1: $F_{78,12} = 7.24$, $P < 0.001$), with additional dimensions possessing little explanatory power (CCA2: $F_{60,13} = 1.80$, $P = 0.12$; CCA3: $F_{44,13} = 1.41$, $P = 0.25$). The environmental gradient described by CCA1 was characterized at one end by North region subpopulations that experience short growing seasons at high latitudes, with relatively cool, dry summers (Fig. 6). This environment was associated with a suite of ecophysiological traits consisting of early phenology (bud set and bud flush), high capacity for growth and carbon fixation (height increment, leaf mass area, and photosynthesis), increases in WUE (leaf and wood $\delta^{13}C$), and short leaf petioles. In contrast, Center and East region subpopulations experience longer growing seasons at lower latitudes, characterized by warmer summers and lower evaporative demand. Ecophysiological traits in these populations consisted of more extended phenology, slower growth rates, less efficient water use, and longer leaf petioles. Traits that showed evidence of local adaptation ($Q_{ST} > F_{ST}$) loaded strongly along this environmental gradient, whereas traits showing variation consistent with neutral demography loaded more weakly (e.g., stomatal conductance and density, leaf and wood $\delta^{13}C$). This generated a significant correlation between Q_{ST} and CCA1 (Spearman's $\rho = 0.77$, $P = 0.003$; Fig. 6), indicating that adaptive divergence in ecophysiology was likely driven by differences in climate and growing season length across a latitudinal gradient.

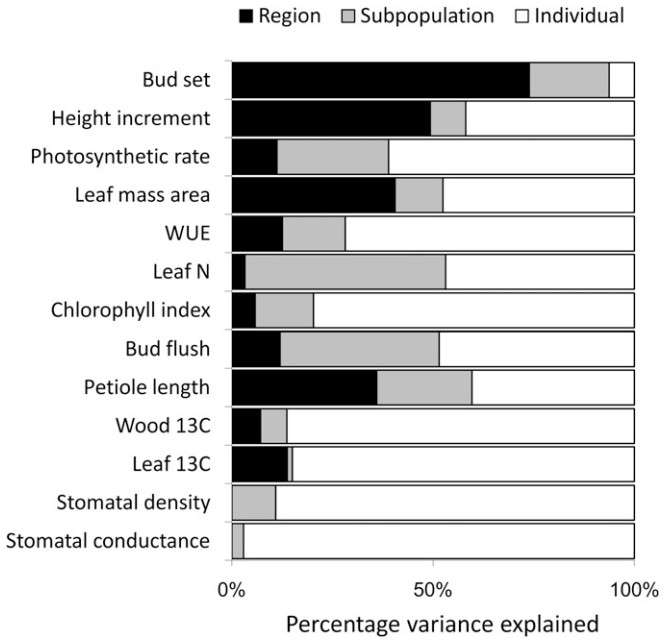
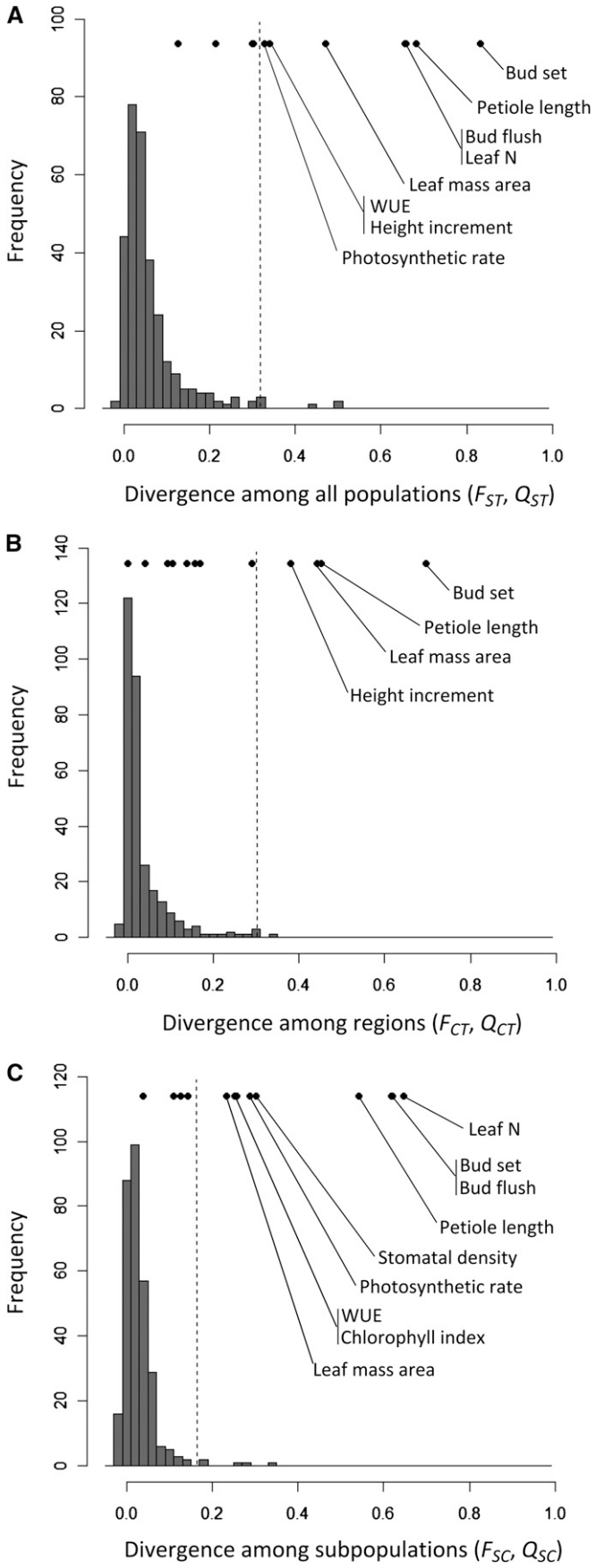


Fig. 3. Partitioning of phenotypic variance among regions, subpopulations, and individuals. Traits are ranked from top to bottom in descending order according to their estimated level of heritability. (*Figure abbreviation*: WUE, water-use efficiency.)

DISCUSSION

Forest tree species often exhibit large population sizes, high gene flow, and low population genetic structure (Savolainen et al., 2007; Neale and Ingvarsson, 2008), which should limit the effects of neutral genetic drift on population differentiation. However, the evolutionary history of balsam poplar resulted in small N_e, significant genetic structure at regional and local scales, and gradients of decreasing genetic diversity along the pathways of postglacial expansion (Keller et al., 2010; Olson et al., 2010). Similar effects of past demographic events during colonization have affected genetic structure in other forest trees (e.g., Taberlet et al., 1998; Petit et al., 2004; Eckert et al., 2010; Holliday et al., 2010a). In this study, we investigated whether genetic drift as a result of this demographic history is responsible for population differentiation of quantitative traits, or whether instead trait variation reflects local adaptation by natural selection. Our comparison of molecular and quantitative trait divergence across a range-wide sample of 20 balsam poplar populations revealed selection as the primary process structuring variability in ecophysiological and phenological traits at both regional and local spatial scales, suggesting strong adaptive evolutionary responses to historical changes in climate.

When populations were analyzed collectively, eight of 13 traits showed evidence of divergence above neutral demographic expectations (Fig. 4). Two of the largest divergence estimates were for the phenology traits bud flush and bud set ($Q_{ST} = 0.659$ and 0.832, respectively). These traits determine the start and end of the period of active stem elongation during the growing season and hence are important targets of selection for cold hardiness and the coordination of seasonal dormancy with permissive growing conditions (Howe et al., 2003, St. Clair, 2006; Hall et al., 2007; Jansson and Douglas, 2007). Divergence for bud set exceeded neutral expectations at both the regional scale and at the local scale among subpopulations



(within regions), whereas bud flush showed evidence of adaptation only at the local scale (Fig. 4). Both traits also showed very strong correlations with latitude and climate, as summarized by CCA1 (Fig. 6). The relation between latitude and bud set among balsam poplar populations is similar to that observed in the European aspen, *P. tremula* (Hall et al., 2007; Luquez et al., 2008), as well as in other forest trees (Howe et al., 2003; Savolainen et al., 2007; Jackson, 2009), suggesting this trait is a hallmark signal of tree adaption to growing season length.

Differences in the scale and strength of adaptation in bud flush and bud set may reflect the consistency of the selective environment, the genetic control of these traits, or both. Spring bud flush occurs in response to warming temperatures, whereas bud set in *Populus* is primarily controlled by photoperiod (Bohlenius et al., 2006; Luquez et al., 2008), which is thought to be a reliable cue of seasonality because it is a stable property of latitude and is less variable than temperature. Additionally, while variation in both traits is due in part to genetic variation in *Populus* (Farmer, 1993; Howe et al., 2003, Luquez et al., 2008), our marker-based heritability estimates for *P. balsamifera* were high for bud set ($\hat{h}^2 = 0.975$) but much lower for bud flush ($\hat{h}^2 = 0.252$; Fig. 2)—in line with previous reports for *P. balsamifera* (Howe et al., 2003). Similarly, bud flush in oaks was found to be mostly under environmental control (Klaper et al., 2001), though in some conifers such as Douglas-fir (*Pseudotsuga menziesii*) and spruce (*Picea* spp.), bud flush also can show high heritability (Howe et al., 2003). The combination of high heritability and a consistent selective environment is probably responsible for the strong adaptive divergence in bud set across regions and subpopulations, whereas adaptive divergence in bud flush in *P. balsamifera* may be weakened by lower heritability and greater temporal variation in growth cues. Similar trends in bud phenology have been reported for *P. tremula* in Sweden (Luquez et al., 2008).

In addition to bud set, petiole length, leaf mass area, and height increment also showed high Q_{CT} values, providing evidence of regional-scale adaptation (Fig. 4B). Populations from the North showed greater height increment than populations from the Center or East (Fig. 5), indicating that selection has favored genotypes with fast growth rates at high latitudes (Soolanayakanahally et al., 2009). North region populations also showed signs of past selection for a higher leaf mass area and shorter petioles compared with results for populations from the Center and East. High leaf mass per unit area and short petioles are features of plants adapted to environments where photosynthesis is not light limited, whereas the greater leaf area and longer petioles of plants from the Center and East suggest selection on features of leaf architecture that increase light acquisition (Niinemets et al., 2004). Thus, adaptation in these traits, as well as other aspects of photosynthesis (for example, *A* and leaf N, which show highly significant Q_{ST}) may reflect selection acting on suites of physiological traits that together control the maximal

Fig. 4. Empirical distributions of marker and trait divergence (A) among all 20 subpopulations (F_{ST}, Q_{ST}), (B) among regions (F_{CT}, Q_{CT}), and (C) among subpopulations nested within regions (F_{SC}, Q_{SC}). The *y*-axis refers to the numbers of single nucleotide polymorphism (SNP) loci in each divergence category (F_{ST}, F_{CT}, or F_{SC}). Points represent Q_{ST}, Q_{CT}, or Q_{SC} for each trait, and placement along the *y*-axis is arbitrary. Dotted lines indicate the location of the upper 1% tail of the distributions from the SNP loci. WUE, water-use efficiency.

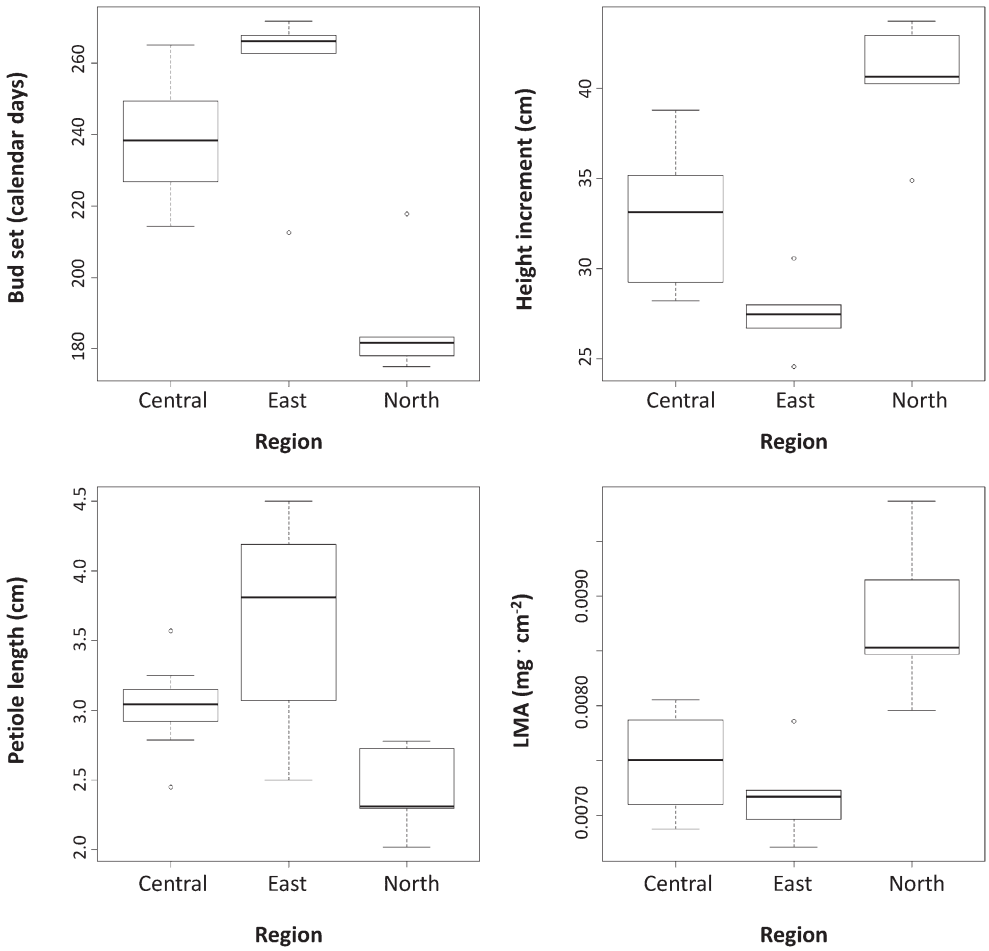


Fig. 5. Boxplots of traits showing evidence of regional adaptation ($Q_{CT} > F_{CT}$). LMA, leaf mass area.

rate of carbon assimilation under environments that impose different resource limitations on plant growth.

Our multivariate CCA analysis revealed that most of the variation among balsam poplar populations in physiology and phenology was correlated with a combination of environmental variables characterized by latitudinal variation in growing season length, temperature, and evaporative demand (Fig. 6). Populations occupying high latitude environments with short, dry growing seasons have evolved increases in traits associated with photosynthesis and growth rate, and earlier phenology. These traits also showed greater Q_{ST} than F_{ST}, indicating local adaptation of growth and phenology in relation to climate variation. In contrast, several traits associated with water balance (stomatal density and conductance, and leaf and wood $\delta^{13}C$) loaded less strongly onto CCA1 and also showed $Q_{ST} \approx F_{ST}$, as well as generally low levels of heritability (Fig. 2). Genetic ancestry also was correlated with CCA1, indicating the demographic history of drift among regions and subpopulations also occurred along the major axis of environmental variation. Strong covariance between historical demography and environmental gradients poses challenges for identifying the selective agents involved in local adaptation because neutral and selective divergence can be collinear (Coop et al., 2009; Keller et al., 2009; Eckert et al., 2010). Whereas the predominance of traits with

TABLE 2. Correlation matrix between genetic ancestry, geography, and climate for 20 balsam poplar subpopulations. SNP-PCs are from a principal components analysis of population single nucleotide polymorphism allele frequencies (Keller et al., 2010). Above the diagonal are Pearson correlation coefficients; below the diagonal are *P* values. Boldface values are significant after Bonferroni correction.

	SNP-PC1	SNP-PC2	Latitude	FFD	AST	SDI
SNP-PC1		0.0914	–0.6088	0.4672	–0.0178	–0.6155
SNP-PC2	0.7017		0.5846	–0.5080	–0.4615	**0.6270**
Latitude	0.0044	0.0068		**-0.8500**	–0.6030	**0.8898**
FFD	0.0378	0.0222	<0.0001		**0.8172**	**-0.6451**
AST	0.9408	0.0405	0.0049	<0.0001		–0.3295
SDI	0.0039	0.0031	<0.0001	0.0021	0.1560	

Note: AST, average summer temperature; FFD, frost-free days; SDI, summer dryness index.

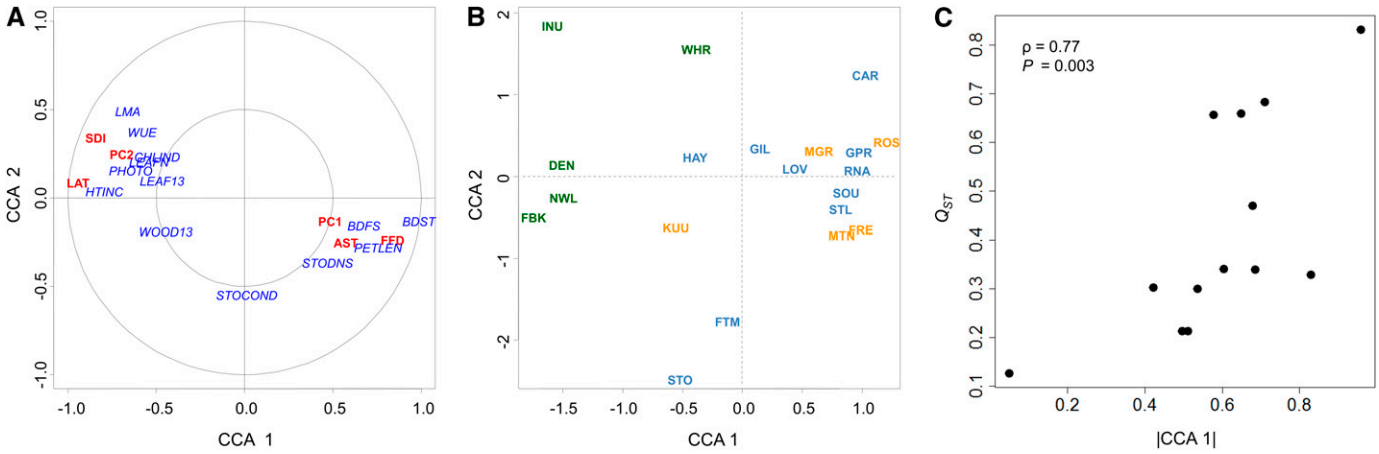


Fig. 6. Multivariate canonical correlation analysis (CCA) of associations between traits, climate, and ancestry. (A) Correlation of traits (in blue) and environmental variables (in red) with the first two canonical dimensions (CCA1 and CCA2). (B) Ordination of the 20 subpopulations (indicated by abbreviations) along the first two canonical dimensions. Colors denote membership of subpopulations to different regions (green = North, blue = Central, gold = East). (C) Correlation between quantitative trait divergence among all subpopulations (Q_{ST}) and the absolute value of the first canonical dimension (|CCA1|). (*Figure abbreviations*: AST, annual summer temperature; BDFS, bud flush; BDST, bud set; CHLIND, chlorophyll index; FFD, frost-free days; HTINC, height increment; LAT, latitude; LEAF13, leaf $\delta^{13}C$; LEAFN, leaf nitrogen; LMA, leaf mass area; PC1 and PC2, single nucleotide polymorphism principal components; PETLN, petiole length; PHOTO, photosynthetic rate; SDI, summer dryness index; STOCOND, stomatal conductance; STODNS, stomatal density; WOOD13, wood $\delta^{13}C$; WUE, water-use efficiency.

$Q_{ST} > F_{ST}$ and the correlation between Q_{ST} and CCA1 indicate that much of balsam poplar physiology reflects adaptation to climate across its range, we have likely underestimated the adaptive significance of some traits that fall within the empirical distribution of neutral divergence. It is also not yet known whether physiological adaptation to climate reflects a recent response to selection (i.e., after ice retreat ~18 kya) or a more prolonged history of adaptation to northern environments, as paleoecological and genetic evidence suggest that *P. balsamifera* may have been present in a northern refugium in Beringia during the last ice age (Breen and Olson, unpublished manuscript).

Hierarchical population structure and the scale of quantitative divergence—We observed different but partially overlapping sets of traits showing adaptive divergence at a large spatial scale among regions vs. at a smaller scale among local subpopulations within regions (Fig. 4B, C). Of the nine traits that had values of $Q_{SC} > F_{SC}$ consistent with local adaptation among subpopulations, only three (bud set, petiole length, and leaf mass area) also showed outlier values of Q_{CT} that signaled adaptive divergence among regions. Given the well-known formulation of response to selection (R) as a linear function of trait heritability (h^2) and selection strength ($R = h^2S$) (Lynch and Walsh, 1998), an adaptive response to weak selection is only possible when trait heritability is high. Accordingly, we observed a significant positive relation between phenotypic divergence among regions (Q_{CT}) and estimated trait heritability (Spearman's $\rho = 0.69$; $P = 0.009$), but this relation did not hold for Q_{SC} among local populations ($\rho = 0.18$; $P = 0.56$). This may indicate that regional scale selection is relatively weak once averaged across the environmental variability present at smaller scales, generating regional divergence (Q_{CT}) most effectively when traits have a high proportion of genetically based variation (e.g., bud set, height increment, leaf mass area). In contrast, even weakly heritable traits such as stomatal density showed $Q_{SC} > F_{SC}$, consistent with stronger or less variable selection at more local scales.

Inferring quantitative trait adaptation from empirical distributions of F_{ST}—The Q_{ST} vs. F_{ST} approach attempts to control for neutral phenotypic evolution by comparing the structure of additive genetic variance at quantitative traits (Q_{ST}) to that of a random sample of molecular loci (F_{ST}). The traditional method of comparison involves looking for overlap between point estimates of the mean and confidence intervals of Q_{ST} from a set of traits and F_{ST} from a handful of marker loci (usually 20 or fewer), or alternatively by simulating their expected distributions from a population genetic model (Whitlock, 2008). Because few marker loci and study populations are typically used, this method can be sensitive to whether confidence intervals produce the expected coverage for both estimators, and several methods can give misleading results (O'Hara and Merilä, 2005). In this study, we have taken an alternative approach of directly generating the empirical distribution of neutral divergence by estimating F_{ST} from a relatively large ($N = 310$) set of nuclear SNP loci. Doing so allowed us to directly evaluate where in the empirical distributions of F_{ST} our estimates of quantitative trait divergence fell and, when far enough in the tails (here, 1%), reject the null hypothesis of neutrality in favor of adaptation. Our approach is conceptually very similar to other methods of detecting selection by the presence of outliers in a distribution, such as genome scans of marker F_{ST} in hierarchically structured populations (Lewontin and Krakauer, 1975; Excoffier et al., 2009b). Empirical distributions may provide more robust null expectations for hypothesis testing than parametric models that assume a steady-state asymptomatic distribution (Garrigan et al., 2010). This may provide a particular advantage in situations like ours, where demographic history is known to have undergone a relatively recent and large-scale perturbation, and genome-wide data sets of marker diversity are available.

In generating the distribution of F_{ST}, we sampled one SNP per genomic region chosen haphazardly from a sequencing survey that spanned all 19 poplar chromosomes (Olson et al., 2010) and without regard to genomic location, gene identity, or the type of polymorphism (synonymous, nonsynonymous, noncoding).

Thus, whereas we cannot rule out the possibility that some of our SNPs might have been influenced by either direct or hitchhiking selection, our distributions of F_{ST} should be fairly representative of the genome-wide average because of recent demographic history. Further, the use of biallelic SNPs in Q_{ST} vs. F_{ST} comparisons has the advantage of employing a marker with a mutation rate that should be very similar to that of quantitative trait nucleotides (QTN) that underlie variation in phenotypic traits, whereas highly polymorphic markers such as microsatellites mutate more rapidly by orders of magnitude, which may lead to underestimating the magnitude of F_{ST} and to a higher probability of type I errors of local adaptation in comparisons with Q_{ST}.

Several issues deserve mention regarding our estimates of Q_{ST} for quantitative traits. First, while both Q_{ST} and F_{ST} possess large evolutionary variances among loci, estimates of Q_{ST} typically carry a larger sampling variance than do estimates of F_{ST}, even though their means are equal under neutrality and additivity. Whereas our study used 20 populations that provide reasonably good precision for estimating Q_{ST} (O'Hara and Merilä, 2005), the inherently large variance of Q_{ST} may have contributed to the wide range of values observed across our traits. Nevertheless, we note that the central tendencies of our Q_{ST} estimates were well above those for the corresponding F_{ST} distributions (Fig. 4). Second, we estimated the additive genetic component of quantitative trait variance indirectly based on phenotypic measurements made in common environmental settings (greenhouse or common garden) coupled with marker-inferred relatedness on an independent set of 100 SNP loci (Ritland, 2000). Environmental variance will tend to increase σ^2_{resid}; thus, to the extent that variance in phenotypic traits scaled by their heritabilities may be overestimates of the true additive genetic variance, our values of Q statistics will be conservative (Whitlock, 2008). It is also important to keep in mind that quantitative trait heritabilities are dependent on the environment under which they are measured (Lynch and Walsh, 1998), and therefore they may have taken different values if our measurements were conducted in other common environments. Experimental studies that have directly estimated heritability in nonhybrid North American *Populus* report values of 0.25–0.34 and 0.65–0.98, for bud flush and bud set, respectively, (Farmer, 1993; Bradshaw and Stettler, 1995; Howe et al., 2003), which accord very well with our estimates of 0.252 and 0.975, respectively. Regardless, sensitivity analyses that evaluate Q_{ST} across the range of h^2 show this is unlikely to change our overall conclusion of prominent adaptation (Appendix S2). Lastly, while our Q_{ST} vs. F_{ST} comparisons have identified traits that are candidates for climate-driven adaptation, the identity and number of genes underlying these adaptations are still unknown. Genome scans using candidate gene SNPs or quantitative trait loci (QTLs) have the potential of addressing this issue, but for highly polygenic traits, the contribution of any single SNP or QTL may explain only a small part of the total phenotypic variance and thus have less statistical power to identify divergent selection than do scans based on whole phenotypes (Latta, 2003). Nevertheless, recent and ongoing genome scans in forest trees suggest this approach is a promising way forward for studying the genetic basis of local adaptation (Eckert et al., 2010; Holliday et al., 2010b; Ma et al., 2010; Keller et al., unpublished data).

Conclusions—Tree migrations following historical climate change were rapid, occurring on the order of a few hundred generations. We have shown that the postglacial expansion of a widespread boreal tree, balsam poplar, was accompanied by pronounced patterns of adaptation in ecologically important traits to climate and growing conditions that exceed neutral expectations on the basis of demographic history.

The prevalence of ecophysiological and phenological traits with $Q_{ST} > F_{ST}$ suggests that balsam poplar possessed an impressive capacity for rapid local adaptation, even under co-occurring shifts in demography. The response of forest trees to future climate changes may take myriad forms, including adaptation, migration, plasticity, reductions in population size, or local extinction, among others (Aitken et al., 2008). Much of the evolutionary response will depend on the temporal and spatial scales of environmental change, the availability of standing genetic variation, and the magnitude of dispersal (Chevin et al., 2010). Although more detailed studies are needed that predict possible responses and constraints to selection (Davis and Shaw, 2001), the current results indicate that balsam poplar is both highly variable and capable of a broad range of adaptive physiological responses to a changing climate.

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