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Quantitative-genetic variation in morphological and physiological traits within a quaking aspen (*Populus tremuloides*) population

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Abstract: Genetic diversity within populations is an important component of adaptive evolution, and recent research has demonstrated that genetic variation within plant populations can have important ecological effects. In this study, we investigate quantitative-genetic variation in several traits within a quaking aspen (*Populus tremuloides* Michx.) population. A common garden experiment was planted with replicates of 13 aspen genotypes collected from wet and dry sites within a population in southern Utah, USA. Ten growth, leaf, physiological, and structural traits were measured. There were significant, heritable phenotypic differences among genotypes in every measured trait and differences in 4 of the 10 traits among genotypes originating from wet and dry collection sites. The data were compared with other published studies, showing that aspen heritability (H^2) estimates and coefficients of genetic variation (CV_G) were comparable or higher than other *Populus* species and hybrid F_1 *Populus* genotypes, indicating a large amount of quantitative-genetic variation in aspen.

Résumé : La diversité génétique observée au sein même des populations constitue une importante composante du potentiel d'évolution adaptative. La recherche récente a démontré que cette variabilité génétique pouvait receler d'importants effets au plan écologique. Les auteurs ont étudié la variabilité génétique de plusieurs caractères quantitatifs au sein d'une population de peuplier faux-tremble (*Populus tremuloides* Michx.). Un dispositif de plantation comparative a été établi avec des réplicats de 13 génotypes de peuplier échantillonnés sur des stations sèches et humides au sein d'une population du sud de l'Utah, aux États-Unis. Les auteurs ont mesuré 10 caractères reliés à la croissance, aux feuilles, à la physiologie et à la structure. Des différences phénotypiques significatives et héritables ont été notées entre les génotypes provenant des milieux secs et humides pour 4 des 10 caractères. La comparaison des données avec la littérature a indiqué que les estimations de l'héritabilité (H^2) et du coefficient de variation génétique (CV_G) chez le peuplier faux-tremble étaient comparables ou plus élevées que celles d'autres espèces de peuplier ou de génotypes hybrides F1 de *Populus*. Ces résultats font ressortir la grande diversité génétique des caractères quantitatifs chez le peuplier faux-tremble.

[Traduit par la Rédaction]

Introduction

Genetic variation within populations is an important but often overlooked aspect of ecological studies. Genetic variation has two important consequences at the population level: heterozygosity tends to increase fitness of individuals, and genetic variation provides the evolutionary potential for populations to track environmental fluctuations and persist over time (Lynch and Lande 1993; Burger and Lynch 1997). There is also increasing evidence that quantitative-genetic variation in hybrid plants can strongly affect community composition of species, such as arthropods and nesting birds (Martinsen and Whitham 1994; Hochwender and Fritz 2004), and ecosystem-level processes, such as soil nutrient retention and decomposition (Driebe and Whitham 2000; Schweitzer et al. 2004). These studies suggest that genetic variation can have important effects; however, to fully understand the ecological and evolutionary implications of phenotypic variation, the heritable genetic component must be characterized.

Quaking aspen (*Populus tremuloides* Michx.) is a widely distributed and dominant tree species throughout North America and has important effects on community structure and wildlife diversity in the western United States (DeByle 1985). Studies characterizing genetic variation based on isozyme (Jelinski and Cheliak 1992) and microsatellite (Wyman et al. 2003; Cole 2005) markers indicate that aspen is one of the most genetically variable plant species. There is also marked variation in quantitative traits, and both field studies (Barnes 1975; Mitton and Grant 1996) and con-

Received 30 September 2007. Accepted 23 January 2008. Published on the NRC Research Press Web site at cjfr.nrc.ca on 28 May 2008.

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 doi:10.1139/X08-012

Table 1. Traits measured in the common garden experiment.

Trait	Units	Definition
Growth		
Initial height (H_i)	cm	Height of leading shoot at initiation of experiment
Total height (H_t)	cm	Height of leading shoot at end of growing season
Branch length (BL)	cm	Length of all branches
Total stem length (SL)	cm	H_t + BL
Relative growth rate (RGR)	cm/month	(SL – H_i)/3
Internode length (I)	cm	Mean internode length along stem
Leaf traits		
Leaf number (L_n)		No. of mature leaves
Leaf length (L_l)	cm	Mean leaf blade length of largest cohort of leaves
Leaf width (L_w)	cm	Mean leaf blade width of largest cohort of leaves
Leaf width/length ratio (L_w/L_l)		
Single leaf area (LA_s)	cm^2	π (L_l/2) (L_w/2)
Physiology		
UV-A transmittance (T)	%	UV-A protection of leaf epidermis
Water use ($\delta^{13}C$)	‰	$\delta^{13}C$ stable isotope ratio
Stem structure		Tree architecture based on branching

trolled experiments in a common environment (King et al. 1999; Donaldson and Lindroth 2004) reveal substantial phenotypic variation in aspen. Still, the degree to which the phenotypic variation is due to heritable genetic variation is not known.

In this study, we quantify within-population quantitative-genetic variation of quaking aspen to determine whether the high degree of phenotypic variation found in natural aspen stands has a significant heritable genetic basis. In a common garden study, broad-sense heritabilities (H^2) and coefficients of genetic variation (CV_G) were calculated to characterize genetic variation in 10 growth, leaf, physiological, and structural traits. Root stock was collected from both wet and dry sites, allowing the assessment of overall genetic variation as well as differences between genotypes that established on sites with differing levels of soil moisture. We hypothesized that aspen genotypes would exhibit substantial heritable genetic variation in phenotypic traits and that genotypes collected from wet sites and dry sites would exhibit heritable differences only in traits that strongly affect water relations. To provide perspective on the amount of genetic variation in western aspen relative to other related species, we compare our H^2 and CV_G values with other published studies that report quantitative-genetic variation in the genus *Populus*.

Materials and methods

Collection and propagation of aspen shoots

Aspen roots were collected for propagation from 60 genotypes within a 40 km^2 area of native aspen forest in Iron County, Utah, USA. A landform map for the area was developed combining elevation, slope, and aspect, and sites were selected from dry, south-facing slopes (dry sites) and moist, north-facing slopes (wet sites). Within the mapped sites, genotypes were collected from distinct stands with a very low probability of sampling the same genotype twice; however, in the event a genotype was resampled, our estimates of genetic variation would be conservative. Lateral root segments were collected from each genotype and planted horizontally in trays, where they sprouted vegetatively over the winter and early spring of 2006. Developing shoots were cut from the root segments, dipped in rooting hormone, and planted in trays under clear plastic covers to maintain high humidity and reduce plant water stress. Upon sprouting an independent root system, each shoot was planted in a mixture of potting soil and field soil and fertilized. All shoots were grown in a greenhouse until the late spring, when they were moved outside and planted in the common garden experiment. The 13 genotypes that produced the largest number of shoots were selected for the experiment, eight of which were collected from wet sites and five from dry sites.

Common garden experiment

A common garden experiment was set up in a 100 m × 200 m area of a flat, previously tilled field in Millville, Utah. Trees were planted among four blocks in a grid design in which each tree had six equidistant neighbors at 50 cm spacing. All blocks were watered equally as needed to minimize water stress. Note that references to dry-site and wet-site genotypes refer to the site of collection of root material in the field, not watering treatments implemented in the experiment.

Traits measured

Measurements were taken in mid-August 2006 to characterize phenotypic traits of each aspen genotype (Table 1). Between 14 and 20 ramets per genotype were sampled for leaf traits and internode length, and 31–135 ramets per genotype were measured for growth traits and leaf number. From phenotypic measurements, total stem length, relative growth rate, single leaf area, and leaf width/length ratio (L_w/L_l) were calculated as shown in Table 1, and a total of 10 traits were used in analyses. Stem structure was coded as a categorical variable, classified into branching and unbranching patterns. Leaf ultraviolet-A (UV-A) transmittance was measured using a portable UV-A-PAM chlorophyll fluorometer (Gademann Enterprises, Wuerzburg, Germany), which uses the calibrated ratio of UV-A-excited fluores-

Table 2. *F* values, degrees of freedom, and *p* values for aspen phenotypic traits (excluding structure) from analysis of covariance (ANCOVA) by genotype and ANCOVA by collection site (wet and dry site type).

	Genotype			Collection site		
Trait	*F*	df	*p*	*F*	df	*p*
Growth						
I	30.68	12	<0.0001	1.44	1	ns
RGR	20.51	12	<0.0001	0.71	1	ns
H_t	37.79	12	<0.0001	5.53	1	0.0188
SL	20.50	12	<0.0001	0.71	1	ns
Leaf traits						
L_n	26.35	12	<0.0001	46.37	1	<0.0001
L_w/L_l	18.95	12	<0.0001	0.13	1	ns
LA_s	28.06	12	<0.0001	2.85	1	ns
Physiology						
T	2.21	12	0.0158	0.28	1	ns
$\delta^{13}C$	4.79	12	<0.0001	7.03	1	0.0101

Note: See Table 1 for trait abbreviations.

cence (375 nm excitation) to blue–green-excited fluorescence (470 nm excitation) to determine the percent UV-A shielding provided by protective pigments in the leaf epidermis. The UV-A epidermal transmittance is 100 minus this ratio: lower values indicate higher mesophyll protection from UV radiation. UV-A transmittance was measured for 3–17 trees per genotype in a single morning prior to direct sunlight. Plant water use was inferred from $\delta^{13}C$ stable isotope ratios of leaf tissue, which provides a long-term indicator of stomatal conductance and plant water use (Hubick et al. 1988). More negative $\delta^{13}C$ values indicate high internal leaf concentration of CO_2 and greater discrimination against ^{13}C by rubisco, an enzyme essential in photosynthesis (Farquhar and Richards 1984). More negative $\delta^{13}C$ values are associated with high stomatal conductance (i.e., biochemical limitation on photosynthesis), whereas less negative values indicate lower stomatal conductance (i.e., carbon limitation). The $\delta^{13}C$ values were generated from desiccated leaf tissue of five or six individuals per genotype using an isotope ratio mass spectrometer. Carbon stable isotope values are expressed using the delta notation (‰) against the Pee Dee Belemnite standard.

Table 3. Trait means (with SEs given in parentheses), broad-sense heritability estimates (H^2) (with SEs given in parentheses), and coefficients of genetic variation (CV_G) for aspen phenotypic traits (excluding structure).

Trait	Mean (SE)	H^2 (SE)	CV_G (%)
Growth			
I	3.02 (0.03)	0.50 (0.10)	12.5
RGR	9.88 (0.47)	0.30 (0.10)	41.1
H_t	73.66 (1.40)	0.45 (0.09)	19.3
SL	78.08 (1.43)	0.32 (0.09)	16.1
Leaf traits			
L_n	29.59 (0.65)	0.29 (0.10)	18.3
L_w/L_l	0.83 (0.01)	0.47 (0.12)	8.2
LA_s	36.27 (11.63)	0.56 (0.11)	24.2
Physiology			
T	4.12 (0.08)	0.17 (0.07)	8.9
$\delta^{13}C$	–27.35 (0.10)	0.36 (0.12)	1.9

Note: See Table 1 for trait abbreviations.

Data analysis

All phenotypic traits (excluding stem structure) were analyzed using the SAS general linear model procedure (SAS Institute Inc. 2003) using a two-factor analysis of covariance (ANCOVA), with separate analyses for genotype and site effects. Block was included as a factor in both models and initial height was treated as a covariate because of significant variation among genotypes at the time of planting. Only traits that did not show significant departures from normality were used in analyses (limiting the number of traits to 10), and *p* values were reported based on type III sum of squares estimates. Because the experimental design included replication within clones, the within-clone and among-clone variance could be directly interpreted as the environmental and genetic variation, respectively. From the among-clone variance (genetic variance component, σ^2_G), coefficients of genetic variation (CV_G) for each trait were calculated as $CV_G = \sigma^2_G/\text{mean}$. Broad-sense heritability (H^2) estimates were calculated as $H^2 = \sigma^2_G/\sigma^2_P$, where σ^2_P is the total phenotypic variance for a trait (both genetic and environmental). H^2 was calculated with the program H2boot, using bootstrapping to generate standard errors (Phillips 2001). H^2 and CV_G estimates together provide a strong measure of population variation: H^2 gives a ratio of genetic to total variance, and CV_G provides a measure of the magnitude of variation standardized by the trait mean. Stem structure was analyzed as a two-level categorical variable using a χ^2 test for independence and, thus, is not included in Tables 2 and 3. The estimates of genetic variation are based on replicated clonal individuals derived from root stock taken from natural populations, and therefore maternal effects cannot be partitioned from genetic variation. These effects potentially inflate our estimates of genetic variation among clones, although many other quantitative-genetic studies in trees also have the same limitation.

Table 4. A comparison of broad-sense heritability estimates (H^2) (with SEs given in parentheses, except where not available) and coefficients of genetic variation (CV_G) for three traits among *Populus tremuloides* in the current study, published data from a natural population of *Populus deltoides*, and published data for F_1 *Populus* hybrids.

	Populus tremuloides		*Populus deltoides*		F_1 *Populus* hybrids	
Trait	H^2 (SE)	CV_G (%)	H^2	CV_G (%)	H^2 (SE)	CV_G (%)
I	0.50 (0.10)	12.5			0.43 (0.09)[b]	0.7[b]
LA_s	0.56 (0.11)	24.2			0.47 (0.09)[b]	17.7[b]
H_t	0.45 (0.09)	19.3	0.28[a]	6.7[a]	0.35 (0.03)[c]	9.1[c]

Note: See Table 1 for trait abbreviations.

[a]Values are from Wilcox and Farmer (1967).

[b]Values are from Marron and Ceulemans (2006).

[c]Values are from Marron et al. (2006).

Results

There were significant phenotypic differences among genotypes in every trait based on ANCOVA results (Table 2). Structural type also varied significantly among genotypes (χ^2 = 335.9, df = 12, p <0.001). Variation in all traits (except stem structure, a non-numerical variable) had a significant genetic component and a broad range of observed values. The H^2 estimates were significantly different from zero for all traits, with a range from 0.17 to 0.56 (Table 3). The H^2 was greatest for internode length (0.50), height (0.45), and leaf morphology (mean of L_w/L_l ratio and single leaf area: 0.52). The CV_G values, which ranged from 1.9% to 41.1% (Table 3), were high for all growth traits (mean 22.3%) and most leaf traits (mean 16.9%) but low for water use (mean 1.9%).

Total height, leaf number, leaf $\delta^{13}C$, and stem structure differed significantly between genotypes from wet and dry collection sites (Table 2). Total height was greater for wet site genotypes (mean 67.09 cm for wet sites; 64.18 cm for dry sites), but wet and dry site genotypes did not differ in measures incorporating growth of branches (relative growth rate and total stem length). In contrast, dry-site genotypes had significantly greater structural complexity (data not shown; χ^2 = 39.6, df = 1, p <0.001), tending to grow a greater number of branches rather than increasing their vertical height. Genotypes from dry sites also had a significantly greater number of leaves (mean 31.75 for dry sites and 27.41 for wet sites) that tended to be smaller in size (marginally nonsignificant trend, single leaf area p = 0.092). Genotypes from wet collection sites had greater discrimination for ^{13}C (more negative $\delta^{13}C$ values), reflecting greater stomatal aperture and plant water use (mean –27.49‰ for wet sites and –26.87‰ for dry sites). Block effects were not significant at α = 0.05 for any of the traits except L_w/L_l and UV-A transmittance in the ANCOVA by genotype, and total height in the ANCOVA by site.

Discussion

We show that western aspen populations can have high levels of phenotypic variation with a strongly heritable genetic component. Every measured trait, including growth, leaf, physiological, and structural characteristics, showed significant phenotypic differences among aspen genotypes. The measured traits had significant heritability estimates and a wide range of phenotypic variation as measured by coefficients of genetic variation, showing that aspen stands carry a substantial amount of heritable quantitative-genetic variation. Genotypes collected from wet and dry site types exhibited heritable differences in 4 of the 10 phenotypic traits (total height, leaf number, water use, and stem structure). Selection seems to favor genotypes with greater height growth and water use at wet sites while favoring genotypes with more conservative water use and highly branching growth forms at dry sites, consistent with local adaptation to variation in soil moisture.

It is important to note that the 13 genotypes in this study represent an extremely small subset of the actual population and, almost certainly, underestimate the levels of genotypic and phenotypic variation in western aspen stands. Furthermore, only genotypes that exhibited prolific suckering (clonal reproduction) ability in the greenhouse were used in the experiment, likely introducing selection that may bias the magnitude of variation downward. Phenotypic plasticity also can contribute to levels of phenotypic variation, and considerable phenotypic plasticity has been found in previous studies of *Populus* hybrids (Marron et al. 2006). Plasticity can add additional phenotypic variation through the effects of genotype × environment interactions, and thus, the levels of phenotypic variation in natural aspen stands may be higher than documented here.

To provide perspective on the amount of genetic variation among aspen genotypes, we compared the variation found in this study to published data from other *Populus* species. Three published studies that report quantitative-genetic variation for 1- or 2-year-old trees were used: a study of a natural population of *Populus deltoides* Bartr. (Wilcox and Farmer 1967) and two studies of a breeding population of *Populus trichocarpa* Torr. Gray × *Populus nigra* L. and *P. trichocarpa* × *P. deltoides* F_1 hybrids (Marron et al. 2006; Marron and Ceulemans 2006). The H^2 and CV_G values were compared for three traits common among studies: total height, internode length, and single leaf area. The H^2 and CV_G values for tree height were roughly twice as high in aspen as in *P. deltoides*, and aspen had higher CV_G and comparable H^2 values across three traits when compared with F_1 *Populus* hybrids (Table 4). Although we recognize that direct comparisons between our study and other published data are imperfect because of differences in population structure and breeding designs, we found that the genetic

variation among the 13 aspen genotypes was generally comparable with, or higher than, the variation observed in both a congener and F1 hybrid crosses.

Phenotypic variation within populations can have important functional consequences, and our study shows that there is a large amount of heritable genetic variation within an aspen population. Traits such as growth and plant water use can affect competitive interactions among plants (Cohen 1970), and structural characteristics are important for species such as nesting birds (Martinsen and Whitham 1994). We report data only for young aspen trees in the first year of growth but emphasize that early variation in traits such as structural and height characteristics will strongly influence subsequent years of growth. Recent work has brought plant hybrid zones to the attention of ecologists, showing that variation among plant genotypes can have important community and ecosystem effects (Whitham et al. 2003). Our study demonstrates high levels of within-population genetic variation among aspen genotypes, and more work is needed to determine the ecological and evolutionary implications of this genetic variation in natural aspen landscapes.

Acknowledgements

Thanks to dozens of people who helped in the establishment of the common garden experiment, Doug Ramsay for producing maps, Ryan Shaw for root collection, the USDA Forest Service Rocky Mountain Research Station for greenhouse space and the Utah State University (USU) College of Natural Resources for common garden space. Funding was provided by a USU College of Science and Engineering Fellowship and USU Ecology Center research award to the first author; the Utah Agricultural Research Station, USDA Forest Service Rocky Mountain Research Station, USDA UV Monitoring Program, USDA National Resources Conservation Service, and USU VP for Research to the second author; and the USU Community University Research Initiative and ADVANCE Program to the third author.

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