THE SHAPE OF CHESTNUTS TO COME: EVALUATING MORPHOLOGY IN BLIGHT-RESISTANT LINES OF AMERICAN CHESTNUT

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Introduction

Variability in tree architecture is heritable (e.g. Bradshaw and Stettler 1995, Solar and Stampar 2003, 2005) and has distinct consequences for interspecific competitive (Horn 1971) and ecological associates, such as nesting birds (Martinsen and Whitham 1994). Tree shape is also adaptive, as geographic differences in community composition and climatic variables have been found to favor different morphological traits in isolated populations (Friend and Woodward 1990). Recent work indicates that adaptive variation in morphology is common to chestnuts, *Castanea* sp., as well. For instance, Villani and colleagues (1992) have demonstrated that across a climatic gradient, genetically distinct populations of Turkish chestnuts, *Castanea sativa*, are spatially correlated with morphological and physiological characters. A similar gradient may have been present in the once widely distributed American chestnut, *C. dentata*; however morphological variation in *C. dentata* has not been quantified. As chestnut restoration progresses, there has been increasing interest in quantifying and preserving the natural variation in a wide variety of chestnut traits, including morphology.

Quantification of architectural variation relies on multivariate analysis of basic morphological measures, often termed morphometrics (Solar and Stampar 2003). These include internodal length, branch angle and basal circumference. We used morphometric analysis to quantify the variation between two advanced-backcross lines from the American Chestnut Foundation’s (TACF) Meadowview Research Farm that originate from New Hampshire and Virginia, respectively. Our intent was to determine if morphological variation between the two lines is significant, and to identify which traits contribute to the variation between lines. Importantly, if architectural variation persists within a backcross-selection program that is focused on blight-resistance, we can be hopeful that TACF national and state chapter efforts to include diverse *C. dentata* populations have been fruitful in conserving heritable genetic variation in traits not associated with blight-resistance.

Methods

Specimens and morphometric techniques

Trees were randomly selected from plots of two- and four-year-old progeny growing at the TACF's Wagner Research Farm, Meadowview, VA, in 2008; all trees were from the generation B_3F_2 of the breeding program. A total of 165 trees were chosen from six plots, four 4-year-old plots and two 2-year-old plots. All trees were chosen from two different cultivars representing two major tree forms—Bu3C3C and Paul Galloway. The Bu3C3C line is a form that is representative of trees from the oak-hickory forests within the Ridge and Valley and Blue Ridge Province and originates from Iron Mountain in southwest Virginia. The Paul Galloway line is representative of the form found in New England, presumably with narrower branch angles and longer internode length (Fig. 1), and originates from Wapole, New Hampshire. Further information regarding the progeny found in each plot can be found in Table 1.

Morphological features included in this study included 1) the circumference of trunk at 25 cm above the ground, 2) distance to first branch, 3) first branch angle, 4) first internode length, 5) second branch angle, 6) second internode length, and 7) third branch angle. Branches less than 2 cm in diameter were not considered for measurement. Measurements of 2-year-old trees occurred in July 2008, while 4-year-old trees were measured in October 2008.

Figure 1. A post-selection plot of 4-year-old B_3F_2 progeny of mother BG310 at the Wagner Research Farm demonstrating the Paul Galloway morphology.

Statistical analysis

Multivariate analysis of variance (MANOVA) was used to evaluate the total morphometric variation between lines, with line as the main effect. One-way ANOVA was used to examine variation in each morphometric between lines, with line as the main effect. Variation among 4-year-old progeny was additionally evaluated using principal components analysis (PCA). All statistical analyses were performed separately for each age class (except PCA), using JMP statistical software (SAS Institute, Cary, NC).

Results

2-year-old trees from the two lines differed significantly in their morphology (MANOVA: $F_{7, 41} = 13.97$, $p < 0.001$). The Paul Galloway line exhibited a narrower first ($F_{1, 49} = 47.56$, $p < 0.001$), second ($F_{1, 49} = 21.54$, $p < 0.001$), and third ($F_{1,49} = 12.05$, $p = 0.001$), branch angle and longer first ($F_{1,49} = 13.91$, $p < 0.001$) and second ($F_{1,49} = 9.97$, $p = 0.003$) internodes (Table 1). Basal circumference ($F_{1,49} = 0.622$, $p = 0.434$) and distance to first branch ($F_{1,49} = 2.92$, $p = 0.094$) were not different between the two lines.

4-year-old trees from the two lines differed significantly in their morphology (MANOVA: $F_{7,32} = 4.38$, $p = 0.0017$). Similar to the 2-year-old trees, 4-year-old Paul Galloway trees exhibited a narrower first ($F_{1,39} = 12.81$, $p = 0.001$), second ($F_{1,39} = 11.62$, $p = 0.002$), and third ($F_{1,39} = 6.84$, $p = 0.013$) branch angle. In contrast to the 2-year-old trees, at 4 years of age, the first ($F_{1,39} = 0.1479$, $p < 0.7027$) and second ($F_{1,39} = 0.1608$, $p = 0.6907$) internodes were indistinguishable between lines (Table 1). Basal circumference ($F_{1,39} = 0.622$, $p = 0.434$) and distance to first branch ($F_{1,39} = 2.92$, $p = 0.094$) were also not different between the two lines.

Table 1. The cross, line, and age of trees selected from TACF Wagner Research Farm, Meadowview, VA.

Age (yrs)	Line	# of Trees	Mother	Basal Circumference	Distance to First Branch	First Branch Angle	Second Branch Angle	Third Branch Angle	First Internode	Second Internode
2	Bu3C3C	14	BG393	11.71±0.79	28.5±1.69	72.93±3.53	63.85±2.8	57.71±2.72	27.86±3.3	23.86±3.20
2	Paul Galloway	24	BG310	11.14±0.5	42.5±5.72	45.68±2.33	45.23±2.05	46.32±2.53	42.18±4.38	42.86±5.06
2	Paul Galloway	14	BG531	14.65±0.91	33.7±3.52	51.36±2.71	52.86±3.00	44.31±2.99	75.37±5.52	46.62±6.10
4	Bu3C3C	8	BG393	11.69±1.04	31.52±2.57	59.13±5.02	59.63±2.53	53.5±3.15	46.76±8.48	31.19±5.45
4	Bu3C3C	10	BG413	13.03±1.20	32.48±3.76	68.1±4.63	64±4.38	54±4.46	30.09±6.34	36.45±4.50
4	Paul Galloway	6	BG531	19.5±1.14	46.83±2.8	52.5±7.42	52.83±6.30	45.66±4.40	30.33±4.40	36.33±7.34
4	Paul Galloway	10	BG310	13.50±1.17	28.45±1.60	44.5±2.57	43.3±2.48	43.6±1.80	28.30±5.19	37.08±3.10
4	Paul Galloway	6	BG323	15.92±0.40	45.58±10.46	52.5±4.23	57.5±2.14	49.16±3	71.5±13.35	33.67±7.65

The first two eigenvectors accounted for approximately 56% of the total morphometric variation among the 4-year-old chestnuts in our study. The first principal axis was defined by increasing branch angle. The mother BG310 (Paul Galloways) was most negatively associated with the first principal axis (decreased branch angle) while the mother BG413 (Bu3C3C) was most positively associated with the same axis (Fig. 2).

Discussion

Morphometric analysis easily distinguished 2-year-olds of the New Hampshire line from

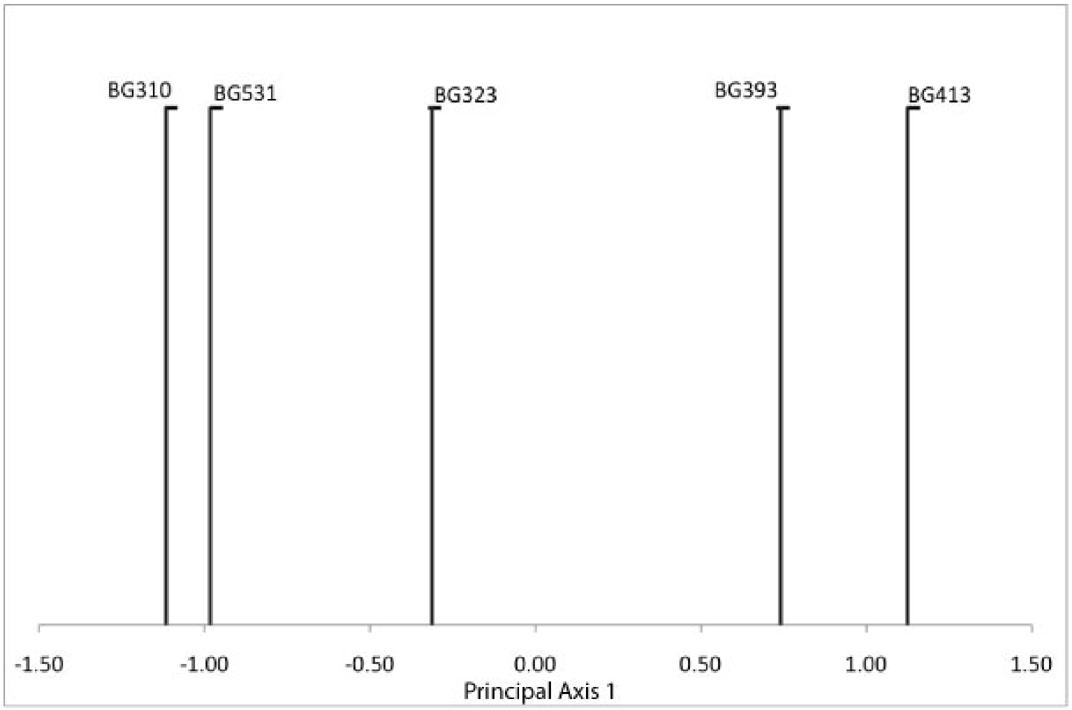

Figure 2. Ordination of 4-year-old B_3F_2 lines along the first principal component axis of morphological variables. All progenies with negative scores are Paul Galloway lines, all others are Bu3C3C lines.

the Virginia line. In comparison to the trees of the Bu3C3C line, young trees of the Paul Galloway line exhibited significantly longer internodal lengths and narrower branching angles, which were maintained until, at least, age 4 (Table 1). These data are consistent with each line's predicted morphology as related to visual estimation of morphology (F. Hebard, pers. comm.).

Morphometrics was able to identify architectural variation in chestnuts and distinguish between backcross lines of different geographic origin. The morphological measurements that contributed most to distinguishing the lines in this study were those characters that define the unique morphology of each line. For example, faster growing trees exhibit longer internodal lengths, which visually define the Paul Galloway line; slower-growing, less efficient light competitors are expected to have a relatively larger branching angle, which was characteristic of the Bu3C3C line. American chestnut trees appear to have evolved distinct morphologies, presumably the result of interspecific competition, and it is likely that different forms convey a competitive advantage under specific ecological conditions. Therefore, the ultimate success of chestnut restoration will depend partly on the morphological diversity of blight-resistant American chestnuts used during reforestation. Morphometric analysis indicates that through the inclusion of diverse germplasms within its backcross-breeding program, the TACF Research Farms has successfully preserved at least two distinct geographic variants that have the potential for restoring the American chestnut to North American forests. Considering the geographic and environmental range of *C. dentata*, additional morphologies undoubtedly remain to be identified and the methods outlined here could be easily applied to additional lines. However, the vast scope of TACF's programs is likely to encompass much of this diversity within the populations tended by TACF state chapters and the Meadowview Research Farms.

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