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RATE OF RECOVERY OF THE AMERICAN CHESTNUT PHENOTYPE THROUGH BACKCROSS BREEDING OF HYBRID TREES

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Abstract: We describe a study in which the morphological characteristics of several chestnut populations – American chestnut, Chinese chestnut, first-generation hybrids, and first-, second-, and third-generation backcross hybrids – were quantified and compared. Twenty-four morphological variables known to distinguish American and Chinese chestnuts were used to develop a composite Index of Species Identity. The aggregate morphology of the first hybrid generation was almost exactly intermediate (mean ISI = 0.50) between Chinese (0.11) and American chestnut (0.85). The first backcross generation resembled American chestnut more than expected, but the second and third backcross generations conformed closely to expectations. Although some degree of "Chinese" character could be found within the third backcross generation, 96 percent of the trees in this population fell within the range of ISI values for pure American chestnut and none fell within the range of Chinese chestnut.

Keywords: *Castanea dentata* / backcross breeding / morphology / morphometrics / Index of Species Identity

INTRODUCTION

Nearly a quarter of a century ago, Burnham (1981) proposed the backcross breeding system that is currently the basis for The American Chestnut Foundation's (TACF) chestnut restoration efforts. In a few years, TACF expects to produce a third intercross generation from the third generation of backcrosses to American chestnut (Hebard 2002). This BC_3-F_3 generation is expected to be highly blight resistant yet essentially "American" in all other characteristics, and it is expected that these trees will be the basis for the first serious efforts to restore American chestnut to its former habitats in the Appalachian region. Although experience with breeding other plants suggests that the third backcross is sufficient to recover the characteristics of the recurrent parent (American chestnut in this case), no one has yet quantified how well this will work in chestnut breeding. This is an important question for those whose interest is ecological restoration of *American* chestnut. How truly "American" will these trees be?

In this paper we summarize a study that was designed to answer this question by comparing the morphological characteristics of American chestnut, Chinese chestnut, their first-generation hybrid (F_1), and three successive backcross generations to American chestnut (BC_1, BC_2, and BC_3) (Diskin 2003). The morphology of the third backcross generation will be discussed in particular detail because this generation has the same relative proportion of the American chestnut genome as those trees that are currently proposed for use in restoration trials.

OVERVIEW OF THE METHODOLOGY

Twenty-four morphometric variables based on leaf, twig, bud, and stipule characteristics that distinguish American chestnut from Chinese chestnut were measured on trees sampled from TACF's Glenn C. Price Research Farm in Meadowview, Virginia. Approximately 50 trees, ranging in age from two to six years old, were sampled from each of the following generations: American chestnut, Chinese chestnut, and F_1,

BC_1, BC_2, and BC_3 hybrid generations. All 24 variables were measured on each tree, and the results of the individual measurements were analyzed using standard statistical methods.

The overall morphology of each tree was summarized in an "Index of Species Identity" (ISI). The ISI is the score of the first principal component, transformed to a scale from 0 to 1.0, from a principal components analysis of the 24 original variables. Essentially, ISI is a composite index of the best of the variables typically used by taxonomists to distinguish *Castanea dentata* (Marsh.) Borkh. from *C. mollissima* Blume. ISI score frequencies were plotted for each population, and the degree of overlap or separation in frequency distributions was used to compare the aggregate morphologies of the hybrid generations and their parental species. Mean ISI scores were also calculated for each generation and analyzed using standard statistical methods.

MORPHOLOGY OF THE HYBRID GENERATIONS COMPARED TO THEIR PARENTAL SPECIES

Because we measured only variables with proven utility in distinguishing Chinese and American chestnut specimens, the two species occupied the extremes of morphologies observed in the study. Chinese and American chestnuts scored at opposite ends of the scale for each individual variable as well as the composite variable, ISI (mean scores of 0.11 and 0.85 for Chinese and American chestnut, respectively).

Comparative morphologies of the four hybrid generations and their parental species are most easily summarized by ISI scores. The morphology of the F_1 generation was almost exactly intermediate between American and Chinese chestnut, with a mean ISI of 0.50. The first-, second-, and third-generation backcross hybrids were different from the F_1 hybrid but, surprisingly, similar to one another, with ISI means of 0.78, 0.77, and 0.79, respectively.

Expected ISI scores can be calculated for each backcross generation assuming that observed ISI scores for the two species are accurate and assuming a straightforward 50 percent dilution of Chinese alleles in each backcross generation and quantitative, additive inheritance of ISI values. Under these assumptions, the F_1 should have an ISI that is exactly intermediate (0.48) between the parental species, and the observed value of 0.50 is not significantly different from expectation. The BC_1, BC_2, and BC_3 backcross hybrids should have ISI means of 0.67, 0.76, and 0.81, respectively, or halfway toward the American species value of 0.85 in each successive generation.

Although ISI values for the BC_2 and BC_3 populations were similar to one another, they did not differ significantly from the values expected under the above assumptions (0.77 vs. 0.76 and 0.79 vs. 0.81 for BC_2 and BC_3 populations, respectively). The small difference between these two generations simply reflects the fact that backcrossing yields diminishing returns with each generation. However, the BC_1 population was anomalously more similar to pure American chestnut than expected (0.78 vs. 0.67). Among other things, the anomaly may be attributable to the fact that the 48 trees representing this generation were derived from crosses between only one Chinese and two American chestnut parents. Just as one or two individuals may not be representative of an entire species, their progeny may not be representative of a typical hybrid population. (It should be noted that the hybrid populations used in this study are not the same as those used by TACF to produce its BC_3F_2 hybrids, nor are they directly related to one another in the sense, for example, that the particular BC_1 population in this study was used to produce the BC_2 population that we measured.)

Based on ISI values, 90 percent of the BC_2 trees and 96 percent of the BC_3 trees had aggregate morphologies within the range of American chestnut values. None of the trees in these two populations had the highest ISI values found in a very few American chestnut trees, and a small percentage had values

lower than observed in any American chestnut trees. However, no backcross hybrid trees had values even close to the highest ISI values recorded for Chinese chestnut.

American chestnut morphology was fully recovered in the BC_3 generation for 15 of the 24 individual morphological characteristics that were measured. In each of these variables, there was no statistically significant difference between American chestnut and BC_3 trees: leaf relative length, tooth length, tooth depth, leaf length to tooth length ratio, leaf width to tooth depth ratio, lenticel width, bud length, bud yaw angle, tooth hooking, leaf apex shape, interveinal leaf hairs, stipule size, twig color, twig hair density, and bud color. The BC_3 generation did not fully resemble American chestnut in distance from base to maximum leaf width, twig diameter, bud width, bud relative length, bud appression, bud pitch angle, leaf base shape, leaf veinal hair density, and bud tip shape. However, for all but one of these variables (bud relative length), the third backcross generation more closely resembled American chestnut than Chinese chestnut.

CONCLUSIONS

Progress towards American chestnut morphology generally conformed to expectations based upon the proportion of American chestnut genome in the various hybrid generations: the F_1 was almost exactly intermediate between the parental species and the BC_3 was very close to 15/16ths "American" on the composite index scale. Thus, backcross breeding appears to substantially recover American chestnut morphology in the backcross generations. Each of the three backcross generations was distinct from Chinese chestnut in that no individuals fell within the range of Chinese chestnut morphology, but each generation overlapped in morphology with American chestnut. Although the morphology of the third-generation backcross hybrids was largely similar to American chestnut, some Chinese-like characteristics remained. These could probably be further removed through selection for particularly "American" individuals in TACF's BC_3-F_2 generation before the production of BC_3-F_3 seed.

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