The American Chestnut Foundation Breeding Program

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Introduction

Chestnut blight, incited by *Cryphonectria parasistica*, devastated American chestnut (*Castanea dentata* (Borkh.) Marsh) in the first half of the 20th century, killing approximately 4 billion dominant and codominant trees. Millions of small sprouts still persist throughout the botanical range of *C. dentata*. Most are not infected and do not flower, except for short periods, before the shoot is killed by blight. Around the fringes of the botanical range, isolated trees can escape infection for prolonged periods, reaching diameters of about 50 cm at breast height (dbh). In the heart of the range, fewer than 20 large (>33 cm dbh) trees are known to persist that have survived blight infection for longer than 10 years (Griffin et al. 1983). Those trees are termed large, surviving American chestnut trees. Some have low levels of blight resistance, but not enough for very many of their progeny to persist. They currently are being bred for higher levels of blight resistance (Griffin 2000).

Without active control measures, such as breeding for resistance and introduction of hypoviruses, it generally has been assumed that American chestnut will become extinct in its native range due to blight and the paucity of reproduction. However, the rate of extinction is quite low and numerous sprouts persist throughout the range (Scrivani 2011). These often flower for short periods when exposed to high light levels and serve as a reservoir of germplasm. However, the persistence of this reservoir is not assured in the face of the changing environment, and continued monitoring is necessary. Increasing deer populations currently are a major threat to long-term survival of American chestnut sprout clumps (Burke and Wilber, unpublished [2]).

Breeding for blight resistance was initiated around 1930, but those early programs were abandoned as hopeless in the early 1960s because they had been unable to combine the forest competitiveness of American chestnut with the chosen sources of blight resistance, oriental chestnut species. However, the early breeding programs did identify species with blight resistance and develop methods for making crosses, cultivating seedlings, and screening them for blight resistance.

Charles Burnham (1981) first hypothesized that the blight resistance of Chinese chestnut, *C. mollissima* Blume, could be backcrossed into American chestnut. Backcrossing is the method of choice for introgressing a simply inherited trait into an otherwise acceptable cultivar. One of Burnham's assumptions in 1981 was that blight resistance is controlled by a single factor. Subsequently, in 1986, Burnham, French, and Rutter (Burnham et al. 1986) accepted Clapper's (1952) conjecture that blight resistance is controlled by two incompletely dominant factors. Burnham, French, and Rutter were the principals who started the American Chestnut Foundation (TACF) in 1983 to facilitate testing of the Burnham hypothesis.

Burnham recruited Lawrence Inman to help with design of the program. Inman (1987) proposed breeding populations of chestnut at multiple locations throughout the American chestnut range to preserve local adaptation and increase genetic diversity. He also proposed using multiple sources of blight resistance. Inman (1989) suggested restricting local collections to within a radius of 16 kilometers. Following Namkoong (1991), Hebard (1994) proposed breeding each source of blight resistance with 20 different American chestnut trees for each cycle of backcrossing; on average, 20 individuals would capture alleles occurring at frequencies greater than 0.05 (=1/20).

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[2] Burke, K.L.; Wilbur, H.M. Unpublished. Effects of white-tailed deer on growth and mortality of *Castanea dentata* and *Acer pensylvanicum*.

Hebard (2006) specified that intercrossing after backcrossing, whose purpose is to restore true breeding by creating and identifying segregants homozygous for resistance alleles, should be restricted to single sources of blight resistance. It would be impossible to eliminate alleles for susceptibility at the F_2 stage if different donor trees had different loci with alleles for resistance.

Hebard (2006) presented results to date for the TACF breeding program, and discussed methods in depth. The purpose of the present report is to update the overall results and to discuss population management aspects of the program. While the TACF breeding program occurs in multiple locations, the most advanced crosses are located at our principal research facility in Meadowview, Virginia (fig.1). Results given below were gathered at Meadowview.

Figure 1—View of the American Chestnut Foundation's Price Research Farm from its Bryan Research Farm. The Foundation has four farms in Meadowview covering about 150 acres; the other two farms are B_3-F_2 seedling seed orchards of about 15 acres each.

Results and Discussion

Variation in Pathogenicity Between Virulent Strains of *Cryphonectria parasitica*

Blight resistance is evaluated using two strains of the blight fungus, both virulent, but one, SG2-3, of mild pathogenicity and one, Ep155, of high pathogenicity. Cankers incited by the highly pathogenic Ep155 differ in size most prominently between chestnut trees with high and intermediate levels of blight resistance; whereas cankers incited by the mildly pathogenic SG2-3 differ in size most prominently between trees with intermediate and non-existent levels of blight resistance (fig. 2). Thus, a wider range of resistance can be distinguished using both strains rather than just one alone.

The experiment summarized in fig. 2 employed a set of trees of various ages and levels of blight resistance that were planted in a randomized, complete-block design over several years and

inoculated in 1 year, using Ep155 and SG2-3. The three main effects were highly significant (table 1).

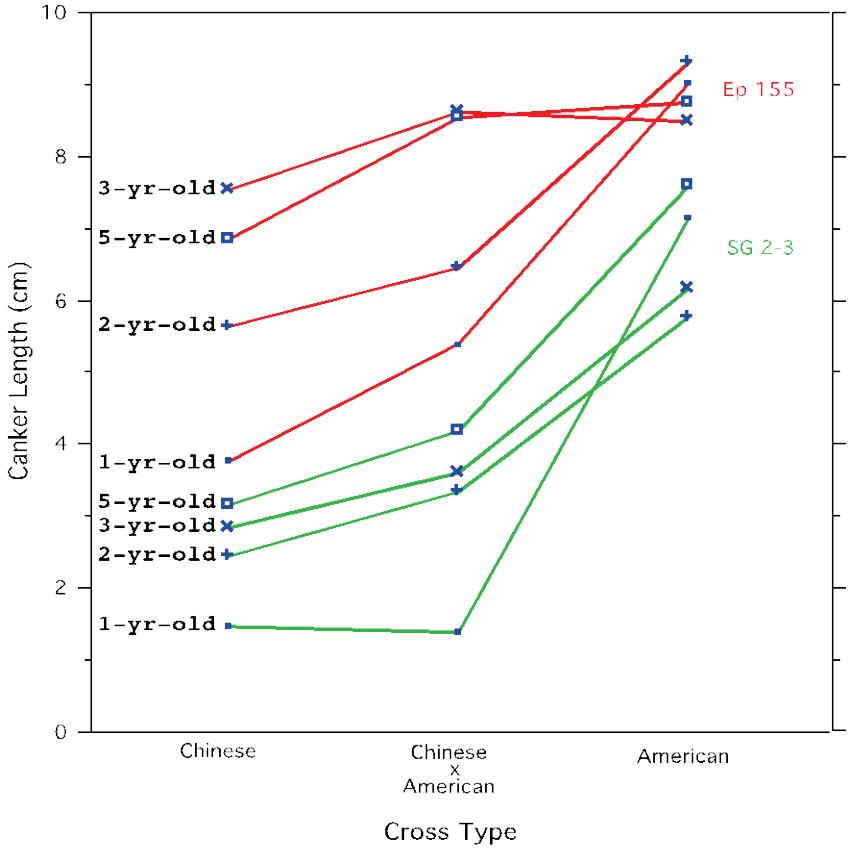

Figure 2—Mean canker length versus resistance for chestnut trees of different ages inoculated with two virulent strains of *Cryphonectria parasitica*, one highly pathogenic (Ep155) and the other slightly pathogenic (SG 2-3).

Table 1—Analysis of variance of chestnut blight canker length 2 months after inoculation, testing the effect of blight resistance (cross type), tree age, and inoculum pathogenicity

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Block	5	45.99630	9.1993	3.9453	0.0026
Cross Type	2	286.67139	143.3357	61.4732	<.0001
Age	3	60.11460	20.0382	8.5939	<.0001
Cross Type*Age	6	39.19499	6.5325	2.8016	0.0144
Inoculum	1	346.72540	346.7254	148.7021	<.0001
Cross Type*Inoculum	2	19.39003	9.6950	4.1580	0.0183
Age*Inoculum	3	7.23204	2.4107	1.0339	0.3808
Cross Type*Age*Inoculum	6	14.61847	2.4364	1.0449	0.4007
Model	28	889.5520	31.7697	13.6253	
Error	105	244.8262	2.3317		
C. Total	133	1134.3782			<.0001

The effect of age in the experiment was confounded with shading of young trees by older trees. This occurred because spacing (3.0 m x 1.5 m) had not been set widely enough to avoid shading. So "age" was treated as a fixed effect in analysis of variance. Cross type and inoculum pathogenicity also were

treated as fixed effects. Nevertheless, the "age" factor is an environmental effect, and illustrates (once again) that environment, broadly considered, has a major effect on expansion of chestnut blight cankers, and thus assessments of blight resistance.

The significant (p=0.014) interaction of cross type with age (table 1) occurred because age did not affect canker size on American chestnut as much as on Chinese chestnut and on their F_1 hybrid (fig. 2). In this experiment, canker sizes were closer overall to American rather than Chinese chestnut. The reverse occurred in another experiment conducted in another year (described below), again illustrating effects of environment on canker expansion.

Inoculum pathogenicity affected canker size more in the F_1s than the pure species (p=0.018), which is to be expected as rates of canker expansion reach lower and upper limits in Chinese and American chestnut, respectively. An idealized shape of canker expansion versus resistance is a sigmoid curve, where the plateaus are the upper and lower limits of canker expansion. Thus, even though rates of linear canker expansion have fairly uniform variances throughout their range, and are appropriate metrics for resistance, host variety x fungus strain interactions can occur (Huang et al. 1996); however, this is not host specificity associated with avirulence genes, which has not been detected yet for chestnut blight. Likewise, the overall non-linearity of canker size with resistance can also lead to association of quantitative trait loci (QTL) for blight resistance with only one strain of the blight fungus. For instance, in fig. 2, 3-year-old trees might show a resistance QTL with SG2-3 but not Ep155. That same locus might show a lack of "virulence" for SG2-3 but not Ep155 in 1-year-old trees. I conclude that care must be exercised in analyzing variety x strain interactions for chestnut blight.

Current Stage of Backcrossing Program

At Meadowview, we have almost completed advancing two sources of blight resistance, derived from the 'Clapper' and 'Graves' first backcrosses (Hebard 2006) into 30 B_3-F_2 lines each of American chestnut. We have planted about 58,600 such nuts in two seedling seed orchards; about 35,400 remain after rogueing (table 2). As selection and rogueing continue, these will be reduced to about 500 plants, which should occur within 3 to 5 years.

Hopefully we will be able to select for true breeding for blight resistance in these B_3-F_2 trees. Simulations based on patterns of inheritance of resistance in straight F_2s and B_1-F_2s indicate that it would be difficult to select for homozygous resistance to blight caused by two loci and impossible with three loci, due to overlap of phenotypic resistance classes. Analysis of those populations and others suggested that two or three loci control blight resistance in Chinese chestnut (Hebard 2006, Kubisiak et al. 1997). Three QTLs for blight resistance were found in the F_2 mapping population originally genotyped by Kubisiak et al. in 1997 when it was regenotyped with several thousand new markers (Kubisiak et al., unpublished[3]). Additional populations have been genotyped and phenotyped and data are being analyzed. Markers fairly close to these loci might facilitate identification of trees homozygous for blight resistance. In the meantime, the most blight-resistant individuals are being selected based on results from inoculation of their open-pollinated B_3-F_3 progeny in orchard settings, after initial selection in the parents, also based on inoculation. B_3-F_3 progeny additionally are being evaluated in wooded settings for forest performance. It currently is unclear whether those forest tests will contribute to selection of B_3-F_2 parents.

The Rationale of Screening B_3-F_2s for Blight Resistance

We use a gradual process to select for blight resistance in our B_3-F_2 seed orchards. A more rapid process would have to be more stringent, which would kill too many good trees.

[3] Kubisiak, T.L.; Nelson, C.D.; Staton, M.E.; Zhebentyayeva, T.; Smith, C.; Olukolu, B.A.; Fang, G.C.; Hebard, F.V.; Anagnostakis, S.; Wheeler, N.; Sisco, P.; Abbott, A.G.; Sederoff, R.R. Unpublished. A transcriptome-based genetic map of Chinese chestnut, (*Castanea mollissima*), and identification of regions of segmental homology with peach (*Prunus persica*).

We start by inoculating 2-year-old seedlings with the SG2-3 strain of the blight fungus and selecting those with small cankers. Although virulent and capable of killing American chestnut trees, the SG2-3 strain is considerably less pathogenic than the Ep155 strain, as shown above. Previous tests, including that summarized in fig. 2 and table 1 above, indicated that even 2-year-old Chinese chestnut trees are most likely killed by Ep155, whereas most 2-year-old trees survive inoculation with SG2-3 if they possess levels of blight resistance equal to or greater than that of F_1s between Chinese and American. Such F_1s typically are intermediate in blight resistance between the two parents. The results of the SG2-3 inoculations enable us to eliminate about 60 to 70 percent of the B_3-F_2s, depending on the size of the tested trees and the year.

We do not include controls such as Chinese chestnut in the B_3-F_2 seed orchards. The small SG2-3 cankers after the first season of canker expansion on the selections could not get any smaller on Chinese chestnut, so their inclusion as controls for that period would not be informative. Chinese chestnut trees could be informative in later years if left in the orchard, but would then start to produce undesired pollen. The B_3-F_2 orchards also are designed to produce seed with maximum genetic diversity over long periods by maximizing distance between sibs, and the design appropriate for that goal cannot provide statistically and experimentally sound evidence of blight resistance in the B_3-F_2s.

We make additional selections over the next few years following inoculation by examining cankers on the preliminary selections. However, as outlined above, we have been intending to make the final selections among the B_3-F_2 trees by testing the blight resistance and performance of their B_3-F_3 progeny in orchard and, possibly, forest settings.

Results of the First Season of Canker Expansion in an Orchard Test of Blight Resistance in B_3-F_3 Chestnut Trees Planted Using a Formal Experimental Design

In 2008, we harvested our first crop of B_3-F_3s large enough to test formally in both the orchard and forest using an experimental design and control plants. The orchard test was planted in 2009 using a completely randomized design. In retrospect, an incomplete block design would have enabled more accurate selection of superior parents, and we subsequently changed to that. In June 2011, we inoculated that first test planted in 2009 and measured canker lengths in December 2011, using methods described by Hebard (2006).

Table 3 shows statistics for cankers incited by both Ep155 and SG2-3 on the different cross types in the experiment. The controls for canker sizes, ranked from susceptible to resistant, were American, B_2, F_1 plus B_1-F_2, B_1xChinese, and Chinese. (The backcross controls are imperfect resistance standards, as mistakes in selection can lessen their resistance). We expected the B_3-F_2 selections in the seed orchard that produced these progeny to have blight resistance equal to or greater than that of F_1s and B_1-F_2s, since those B_3-F2$_s$ had been screened only using strain SG2-3. If this first expectation were met, one would then expect their B_3-F_3 progeny from open pollination to have mean canker lengths intermediate between the F_1/B_1-F_2s and the B_1xCs. However, instead, cankers on the B_3-F_3s were similar to or slightly longer than those on B_1-F_2s. Reasons this may have occurred include that pollen is still being produced by unselected as well as selected B_3-F_2 parents in the seed orchards, that selection is not complete, and/or that there was some degradation of factors for blight resistance during backcrossing.

Out of 583 trees tested, there were 95 B_3-F_3s with small cankers for both strains Ep155 and SG2-3, where small cankers are those less than 5 cm in length. Normally, one would expect trees with such small cankers to have a high level of blight resistance. However, there was dominance toward small cankers in this test, leading to a higher frequency of trees with small cankers in the B_1-F_2 progenies than is observed in most tests of F2s. We observed 35 out of 171 B_1-F2$_s$ with small cankers, where usually we would expect to observe about 10. We expect many of these small cankers on the B_1-F_2s to start expanding in 2012. How much that occurs also in the 95 B_3-F3s with small Ep155 cankers will be interesting to follow.

The phenotypic dominance toward small cankers, after the first season of canker expansion in this test, also is reflected in the bimodal distribution of canker sizes. The bimodality is most evident for the Ep155 cankers in the B_3-F_3 and B_1-F_2 crosses in table 3, where there is a peak in the 0-5 cm class and another in the 10-15 cm class. The peak at 0-5 cm occurred because cankers in the 0-5 cm class cannot get any smaller, so their numbers pile up when phenotypic dominance is toward small cankers. There was even more clustering in the small canker class for the SG2-3 cankers, which is expected given its lesser pathogenicity. However, the ranking of mean canker size for cross type was the same for both strains.

The predominance of small cankers led to a significant fungus strain by cross type interaction, since the SG2-3 and Ep155 cankers on Chinese chestnut were similar in size, while in the other cross types, SG2-3 cankers were about 6-8 cm shorter than Ep155 cankers. There was no interaction between strain and families nested within cross type. Another effect of very small cankers was that the variance of canker length was reduced in Chinese chestnut compared to the other crosses, especially for strain SG2-3. The unequal variances (heteroscedasticity) for strain SG2-3 increased the likelihood that differences between cross types and families would be declared statistically significant, making declarations of significance suspect for SG2-3.

Table 4 shows canker length statistics for individual families within the various cross types. The Ep155 cankers yielded significant differences in canker size for the more resistant trees while the SG2-3 cankers yielded significant differences for the more susceptible trees (although, again, the significance of the SG2-3 differences is suspect). Twenty-one of 36 B_3-F_3 families had significantly ($p<0.05$) smaller SG2-3 cankers than the American chestnut family. The B_3-F_2 parents of the remaining 16 families are candidates for rogueing.

Discussion of B_3-F_3 Canker Results

This crop of B_3-F_3s was significantly more blight resistant than American chestnut, roughly comparable in resistance to Chinese x American F_1s or backcross F_2s, in the aggregate. None of the families had as high a level of blight resistance as Chinese chestnut, but many contained highly blight-resistant individuals as of the end of the first season of canker expansion. This is roughly in accord with expectation, given that selection is not finished in the B_3-F_2 orchards from which the nuts were harvested. The best families had resistance roughly comparable to that of crosses of selected straight backcrosses with Chinese chestnut, which is what we would expect in crosses of Chinese chestnut with the pollen being produced in the orchards. However, we have not eliminated the possibility that some of the blight resistance of Chinese chestnut has been lost during backcrossing.

Characterization of Breeding Populations

The effective population size, denoted N_e, is used commonly to estimate minimal sizes needed to maintain long-term viability of populations, such as species. Using quantitative genetic considerations, Franklin (1980) estimated that an N_e of 50 is needed to avoid immediate collapse of a population due to inbreeding depression, and an N_e of 500 is needed to offset loss of alleles by genetic drift with recruitment of new alleles by mutation. N_e can be estimated as the harmonic mean of population sizes, given the other assumptions of Hardy-Weinberg equilibrium.

To compute N_e as a harmonic mean, we need the number of individuals at each generation. The size of the original American chestnut population can be considered infinite for this computation; whereas, the population goes through a bottleneck at the straight backcross stage. The population size at straight backcross was set at 20 using Namkoong's considerations, as mentioned above in the introduction. Hebard (2002) estimated from first principles that obtaining nine F_2 progeny from a straight backcross mother tree gives a 95 percent chance of capturing all her alleles. Thus the size of the F_2 generation is nine multiplied by 20 American chestnut lines per source of blight resistance, or 180. The size of backcross F_3 and subsequent filial generations also can be considered infinite, but were limited to several thousand in simulations of inbreeding to be discussed momentarily. Using these numbers of individuals, the N_e of 20 lines of American chestnut, computed from the harmonic

mean, is about 72. It would increase if more than one individual per line were retained as a parent of the backcross F_2 generation (fig. 3), which is generally the case.

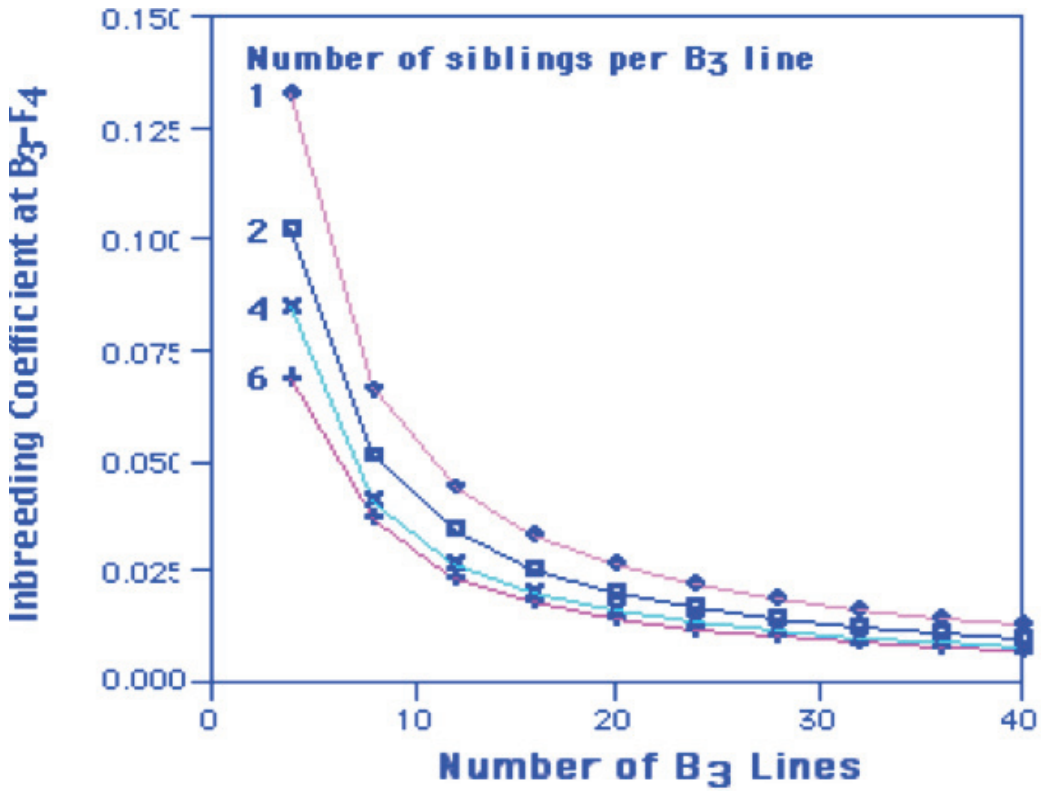

Figure 3—Effect of the number of siblings per B_3 line on the inbreeding coefficient at B_3-F_4 versus the number of American chestnut lines at B_3, for partial diallel mating at B_3 with four lines per diallel, ten B_3-F_2 offspring per line, and for random mating thereafter.

The inbreeding effective population size can be estimated independently using the inbreeding coefficient of a population. For some breeding scenarios, a harmonic mean cannot be used to compute N_e, but inbreeding coefficients can. Hebard (2002) computed inbreeding coefficients by 'brute force' simulation. The results of similar simulations are shown in table 5, which illustrate the effect of TACF's Chapter breeding program on the overall N_e of the breeding population at B_3-F_4 (each chapter has a goal of breeding 20 American lines for at least one source of blight resistance).

Two sources of blight resistance, derived from the 'Clapper' and 'Graves' trees, constitute most of the breeding stock in the TACF program. We probably will be able to breed at five locations for each source, which would make the N_e of the overall breeding stock about 500 (table 5). Of course, selection at loci for resistance will decrease N_e, as will other violations of Hardy-Weinberg assumptions, such as skewed numbers of progeny per parent. This degradation will be offset somewhat by the chapters using more advanced breeding stock than that simulated for table 5. We plan to measure N_e in the breeding stock to compare with these predictions and to guide further breeding. We have some baseline data from the native population of American chestnut from a rather thorough sampling of the species (Kubisiak and Roberds 2006). DNA from that sampling also is available for further probing.

A third source of blight resistance, derived from the Nanking cultivar of Chinese chestnut, is being advanced in 20 American lines at Meadowview and a few other chapters. This will increase the N_e of

our breeding population. We also hope to expand other sources of blight resistance to an N_e of 72 at various chapters, as outlined by Hebard (2004).

The decision to introgress blight resistance into American chestnut from other species was based in part on the thought that no blight resistance existed in the native population. This does not appear to be the case (Griffin 2000). Burnham (1990) outlined a plan for utilizing this native resistance which we have been following in a breeding program at Meadowview separate from the backcrossing effort. Ironically, the relatively small progeny sizes required by the backcross method make it a more efficient means of generating a breeding population with large N_e than recurrent selection of large, surviving American chestnut trees after introgressing them into a broad enough base of American chestnut with no resistance to blight.

As the backcross breeding program has developed, we have come to realize that our goal is to enable the American chestnut to resume evolving on its own, as a wild species. Our B_3-F_3 'Restoration' chestnut trees should have sufficient blight resistance to produce numerous viable offspring in natural settings, although it is still unclear whether they will have levels of blight resistance similar to Chinese chestnut and whether even that level of blight resistance will enable them to be dominant forest trees. Their blight resistance could be increased further, if need be, by allowing them to intercross in forested settings with other sources of blight resistance, such as from additional backcross lines produced by TACF, from large, surviving American chestnut trees, or from other sources, such as transgenics or cisgenics. The intercrossing and further increase of progeny could be facilitated as needed by silvicultural interventions, but further intensive breeding in an orchard setting would not occur with the new base population, only with these supplemental populations. Hypovirulence may also play a role in re-establishing a self-sustaining breeding population, as Griffin (2000) and others have pointed out. The important point is that we are close to developing a self-sustaining breeding population of predominately American chestnut with a large enough N_e to constitute a viable species.

Table 2—Type and number of chestnut trees and planted nuts at TACF Meadowview Research Farms in May 2011, with the number of sources of blight resistance and the number of American chestnut lines in the breeding stock

Type of Tree	Number of		
	Nuts or Trees	Sources of Resistance	American Lines
American	1575		223
Chinese	1014	30	
Chinese x American: F_1	417	18	57
American x (Chinese x American): B_1	646	11	24
American x [American x (Chinese x American)]: B_2	1316	13	43
American x {American x [American x (Chinese x American)]}: B_3	2158	10	92
Am x (Am x {Am x [Am x (Ch x Am)]}): B_4	888	4	14
(Ch x Am) x (Ch x Am): F_2	213	5	5
[(Ch x Am) x (Ch x Am)] x [(Ch x Am) x (Ch x Am)]: F_3	5	1	1
[Am x (Ch x Am)] x [Am x (Ch x Am)]: B_1-F_2	625	7	10
{Am x [Am x (Ch x Am)]} x {Am x [Am x (Ch x Am)]}: B_2-F_2	667	9	12
B_2-F_3	31	1	1
(Am x {Am x [Am x (Ch x Am)]}) x (Am x {Am x [Am x (Ch x Am)]}): B_3-F_2	35394	2	51
B_3-F_3	3826	2	22
Clapper B_3 x Graves B_3: B_3-I_1	110	2	9
Chinese x [American x (Chinese x American)]: Chinese x B_1	167	3	7
Ch x {Am x [Am x (Ch x Am)]}: Chinese x B_2	72	1	2
Ch x (Am x {Am x [Am x (Ch x Am)]}): Chinese Test Suite x B_3	286	5	16
Chinese Test Suite x Chinese	1471	67	67
Chinese Test Suite x Japanese	46	2	2
Chinese Test Suite x European	43	1	1
Chinese Test Suite x Large, Surviving American	149	7	7
European x American: F_1	2	1	1
Japanese	3	1	1
Japanese x American: F_1	8	1	1
[(Japanese x American) x American]: B_1	5	1	1

(Table 2 continued)	142	1	1
Japanese x European	80	1	1
Japanese x Large, Surviving American	27	5	5
Castanea ozarkensis	21	1	2
Castanea pumila	44	3	3
Castanea seguinii	48	3	3
Seguin x American: F_1	54	2	2
Large Surviving American: F_1	486	12	37
Large Surviving American: B_1	506	8	14
Large Surviving American: B_2	72	3	4
Large Surviving American: B_3	161	1	1
Large Surviving American: F_2	266	13	11
Large Surviving American: F_3	270	1	2
Large Surviving American: I_1	1666	32	13
Large Surviving American: I_2	303	11	1
Large Surviving American: I_3	104	2	32
Large Surviving American advanced: F_1	804	12	25
Other	3		
Total	**56194**		

Table 3—Mean, standard deviation, and distribution of canker size classes (length in cm) for cankers incited by two strains of the blight fungus on cross types of American and Chinese chestnut in 2011

Cross Type	Fungus Strain	N	Least Squares Mean*		Standard Deviation	Length Class					
						0-5	5-10	10-15	15-20	20-25	25-
American	Ep155	20	17.4	A	3.4		1	4	11	4	
B_2	Ep155	44	14.5	AB	6.6	4	2	19	15	2	2
B_3-F_3	Ep155	583	11.7	BC	6.3	95	71	219	151	32	15
B_1-F_2	Ep155	171	10.9	BC	6.4	35	23	70	30	10	3
F_1	Ep155	8	10.0	ABC D	3.6		4	3	1		
B_1xC	Ep155	39	8.3	CD	5.0	12	9	16	2		
CxC	Ep155	38	3.2	D	2.3	27	11				
American	SG2-3	17	11.0	A	7.8	3	5	7			2
B_2	SG2-3	45	5.3	B	5.6	32	5	6		2	
B_3-F_3	SG2-3	592	4.9	B	4.3	402	111	65	10	3	1
B_1-F_2	SG2-3	172	3.0	BC	3.9	126	26	18	2		
F_1	SG2-3	7	3.3	BC	1.7	6	1				
B_1xC	SG2-3	39	1.8	BC	2.6	35	3	1			
CxC	SG2-3	38	1.6	C	0.6	38					

* Means followed by the same letter are not significantly different at $p<0.05$ by a Tukey HSD test. The declarations are suspect for strain SG2-3 due to heteroscedasticity.

Table 4—Means for length (in cm) of cankers incited by two strains of the blight fungus on individual families of American and Chinese chestnut in 2011

Cross Type	Mother*	Father	Source of Resistance	N	Grand Mean	Least Square Means**			
						Strain Ep155		Strain SG2-3	
American	PL1-08	op	none	16	14.4	17.4	A	11.4	A
B_3-F_3	D5-26-54	op	Clapper	14	12.5	17.0	AB	8.0	ABC
B_2	B2208	AN17	Nanking;none	5	11.7	16.4	ABCD	7.0	ABCD
B_3-F_3	D5-29-124	op	Clapper	1	11.2	12.1	ABCDE	10.4	ABCD
B_3-F_3	D3-28-10	op	Clapper	11	11.0	16.2	ABC	5.8	ABCD
B_2	TM474	A1530	Nanking;none	8	10.8	15.0	ABC	6.6	ABCD
B_3-F_3	D5-17-89	op	Clapper	20	10.6	13.8	ABC	7.5	AB
B_3-F_3	D5-19-72	op	Clapper	22	10.2	14.2	ABC	6.1	ABCD
B_3-F_3	D5-18-95	op	Clapper	7	10.0	11.4	ABCDE	8.6	ABCD
B_3-F_3	D5-18-50	op	Clapper	10	9.8	14.1	ABCD	5.4	ABCD
B_3-F_3	D5-27-108	op	Clapper	17	9.3	13.8	ABC	4.7	BCD
B_3-F_3	D5-27-101	op	Clapper	29	9.3	14.1	ABC	4.5	BCD
B_3-F_3	D5-26-131	op	Clapper	32	9.3	13.1	ABC	5.4	BCD
B_3-F_3	D5-29-50	op	Clapper	10	9.1	11.9	ABCDE	6.3	ABCD
B_2	B210	A1117	Nanking;none	15	9.1	13.4	ABC	4.9	BCD
B_3-F_3	D5-25-49	op	Clapper	18	9.1	12.2	ABCD	6.1	ABCD
B_3-F_3	D5-18-101	op	Clapper	12	8.9	14.1	ABC	3.8	BCD
B_3-F_3	D5-29-3	op	Clapper	23	8.9	13.5	ABC	4.2	BCD
B_1-F_2	TM538	TM158	Nanking;Nanking	7	8.8	14.8	ABCD	2.7	BCD
B_3-F_3	D5-22-17	op	Clapper	15	8.8	13.1	ABCD	4.5	BCD
B_3-F_3	D5-27-36	op	Clapper	24	8.8	13.0	ABC	4.6	BCD
B_3-F_3	D5-26-94	op	Clapper	28	8.6	13.0	ABC	4.2	BCD
B_3-F_3	D6-26-27	op	Clapper	37	8.6	12.9	ABC	4.3	BCD
B_3-F_3	D5-18-25	op	Clapper	26	8.4	13.0	ABC	3.7	BCD
B_3-F_3	D5-17-122	op	Clapper	1	8.2	10.4	ABCDE	6.0	ABCD
B_2	CY554	MB190	Nanking;none	16	8.0	13.1	ABC	2.8	BCD
B_3-F_3	D2-29-44	op	Clapper	24	8.0	11.6	ABCD	4.3	BCD
B_1-F_2	B2354	TM672	Nanking;Nanking	117	7.9	11.7	ABC	4.2	BCD
B_3-F_3	D5-30-24	op	Clapper	20	7.7	11.7	ABCD	3.6	BCD
B_3-F_3	D5-27-95	op	Clapper	32	7.4	11.7	ABCD	3.1	BCD
B_3-F_3	D4-28-31	op	Clapper	4	7.2	12.0	ABCDE	2.4	ABCD
B_1xC	JB478	MuChin1	MuChinX;MuChin1	1	7.1	13.3	ABCDE	0.8	ABCD
B_1-F_2	TM158	TM538	Nanking;Nanking	21	7.1	10.5	ABCDE	3.6	BCD
B_3-F_3	D2-26-72	op	Clapper	23	6.9	10.3	ABCDE	3.6	BCD
B_3-F_3	D1-27-25	op	Clapper	12	6.8	11.7	ABCDE	2.0	BCD
F_1	TA3	MB190	Kuling;none	1	6.7	9.5	ABCDE	3.8	ABCD
F_1	KY106	MB190	Meiling;none	6	6.7	10.5	ABCDE	2.9	BCD
B_3-F_3	D5-22-86	op	Clapper	17	6.7	11.2	ABCDE	2.2	BCD

(Table 4 continued)

B_3-F_3	D5-25-147	op	Clapper	11	6.6	9.9	ABCDE	3.4	BCD
B_3-F_3	D5-18-2	op	Clapper	7	6.5	9.1	ABCDE	3.8	ABCD
B_3-F_3	D5-30-11	op	Clapper	22	6.4	10.5	ABCDE	2.3	CD
B_3-F_3	D9-26-36	op	Clapper	3	5.9	7.4	ABCDE	4.4	ABCD
B_1xC	TM672	GR119	Nanking;Nanking	18	5.7	9.1	ABCDE	2.4	BCD
B_1-F_2	B2275	B293	72-211;72-211	10	5.5	9.3	ABCDE	1.7	BCD
B_1-F_2	B2430	B2275	72-211;72-211	13	5.5	8.4	ABCDE	2.6	BCD
B_3-F_3	D2-28-76	op	Clapper	14	5.3	7.9	BCDE	2.8	BCD
B_3-F_3	D5-17-130	op	Clapper	9	5.2	7.9	ABCDE	2.5	BCD
B_3-F_3	D8-26-69	op	Clapper	16	5.2	8.0	CDE	2.4	BCD
B_3-F_3	D2-26-66	op	Clapper	3	5.0	8.2	ABCDE	1.7	ABCD
B_3-F_3	D2-28-52	op	Clapper	3	4.3	5.9	ABCDE	2.7	ABCD
B_1xC	B2239	GR119	Nanking;Nanking	6	4.2	6.4	ABCDE	1.9	BCD
B_1xC	JB5	MuChin1	MuChinX;MuChin1	3	4.1	5.9	ABCDE	2.3	ABCD
B_1xC	B2426	GR119	Nanking;Nanking	10	4.1	6.8	CDE	1.4	BCD
CxC	GR119	SLR1T15	Nanking;Mahogany	20	2.6	3.7	E	1.5	D
CxC	KY106	op	Meiling;unknown	5	2.4	3.3	CDE	1.4	BCD
CxC	KY75	SLR1T15	Meiling;Mahogany	10	2.4	3.2	DE	1.6	BCD
CxC	TA3	op	Kuling;Unknown	3	2.2	2.8	ABCDE	1.7	ABCD

* The first letter of the code for the B3-F3 crosses identifies the farm containing its B3-F2 parent. The first number identifies the block of trees containing the B3-F2 parent. The middle number identifies the plot within a block, and corresponds to the American great, great grandparent of the B3-F2. The last number is the tree number within a plot, within a block. In this set of crosses, there is only one open-pollinated B3 grandparent of the B3-F3s in a plot, except that the B3 grandparent of D5-18-95 and D5-18-101 differs from the B3 grandparent of the other trees in plot 18.

** Within a column, means not followed by the same letter are significantly different at $p<0.05$ by a Tukey HSD test. The declarations are suspect for strain SG2-3 due to heteroscedasticity.

Table 5—Effect of adding sets of 20 B_3-F_2 progeny from the American Chestnut Foundation's Chapter breeding program on inbreeding and effective population size for the same source of blight resistance (inbreeding effective population size doubles with each additional chapter if different sources of blight resistance are used)

Number of Chapters	Inbreeding Coefficient	Inbreeding Effective Population Size
1	0.0207	72
2	0.0115	130
3	0.0085	176
4	0.0070	214
5	0.0060	248

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