Breeding Blight Resistant American Chestnut

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ABSTRACT. The objective of the breeding program of The American Chestnut Foundation is to utilize the blight resistance of Chinese chestnut in a backcross program to introduce resistance into American chestnut. Some Chinese chestnuts have high resistance to chestnut blight, and usually cross well with American chestnut and the progeny usually show high viability. Several different sources of disease resistance are used, and several different American chestnut trees are used as the recurrent parent. The published results and our own observations on the genetic variability observed in the progenies would suggest that the progenies represent a reasonably complete random sample of recombinants. The basis of The American Chestnut Foundation's breeding program is to test Charles Burnham's hypothesis that states: 1) resistance to blight is controlled by a relatively small number of genes; and, 2) by backcrossing for several generations to American chestnut, it will be possible to develop trees that possess all features of the American chestnut but have resistance to blight.

The American Chestnut Foundation was begun with the hope of restoring the American chestnut *(Castanea dentata* [Marsh.] Borkh.) to a position of importance as a timber tree and as a source of chestnuts for human and animal consumption. A requirement for restoration of the American chestnut in North America is the attainment of resistance to the fungal pathogen, *Cryphonectria (Endothia) parasitica* (Murr.) Barr, the causal of chestnut blight. The impetus behind The American Chestnut Foundation is a breeding strategy presented by Burnham (1) and Rutter and Burnham (6). The essence of their strategy is to use blight resistance of the Chinese chestnut *(C. mollissima* BO and other *Castanea* species in a breeding program with American chestnut, and then continually backcross to American chestnut.

The proposed breeding strategy assumes that there is a simpler inheritance to blight resistance than there is to the morphology and growth habit of a tree. The assumption seems to be reasonable, based on experience of breeding for resistance in field crops. Disease resistance commonly looks very complex in its inheritance, particularly if the resistance is obtained from an interspecific cross, but, upon more systematic analysis, it often is shown to have a rather simple inheritance (5). Clapper also had shown that the segregation for resistance among progeny from a cross of Chinese x American backcrossed to Chinese approximated a 3:1 resistant to susceptible ratio (3, 4). The ratio suggests that two pairs of genes control resistance to blight. The results also showed that the resistance to blight was only partially dominant because the presumed homozygotes were more resistant than the heterozygotes.

The backcross program was suggested because the principal need is to transfer only one trait, that is, blight resistance, into American chestnut (1, 2, 6). The American chestnut is the recurrent parent. The objective is to get an American chestnut type tree (i.e., a timber-type tree) with resistance from the Chinese parent. Theoretically, a Chinese x American hybrid backcrossed twice to an American parent should give a population that is genetically 7/8 American, except for the chromosomal regions that contain the genes for blight resistance. A population that has 7/8 from its genes of American chestnut, hopefully would be primarily of American type in morphology and growth characteristics. However, the population would not be expected to breed true. The American chestnut has a self-incompatibility system and outcrossing is essentially obligatory (2). Intercrossing among the individuals of a backcross population may reveal considerable segregation for morphology and growth characteristics. The amount of segregation would be expected to be greatest in the F_2 population, and less after each generation of backcrossing to American chestnut. The number of backcrosses to American chestnut needed to establish a population that is sufficiently like an American chestnut is not known. The question can be asked two ways. How similar to American chestnut in morphology and growth characteristics must a tree be to be an acceptable American chestnut? What proportion of the population fits the description of being an American-type tree? If 3/4 of the progeny from BC_2 plants are acceptable American-type trees, it will be necessary to prepare plans as to what should be done with 1/4 of plants that do not have the desirable American type. Will they be self-eliminating in a dense stand or will they have to be removed? Plants that are a more traditional Chinese type, with form similar to an apple tree, may be shaded by the timber type trees and be unsuccessful competing in dense stand. Rogueing of undesirable trees also may be necessary.

A generation of intercrossing within a population created by backcrossing also will be necessary to make plants homozygous for one or more genes for blight resistance. Plants with 2 genes for resistance are expected to show a higher level of resistance than plants with only one gene for resistance. Since resistance does not appear to be dominant, heterozygous plants are not expected to have as high a level of resistance as homozygous plants. The proportion of plants that have resistance and the level of resistance of individual plants and populations of plants in a reasonably sized planting that is adequate to yield timber and/or nuts will have to be determined. It also is expected that the level of resistance necessary in a population for successfully growing American chestnuts may not be the same in different ecological niches.

Sources of resistance. The American Chestnut Foundation is using Chinese chestnut as a source of resistance to be backcrossed into American chestnut. As much as possible, the program uses existing F1s and Fes of Chinese x American crosses and then uses this material to be backcrossed to American chestnut. The `Clapper,' `Graves,' and `Douglas' trees and/or their descendants have been used in backcrosses to American chestnut. The chestnut trees at the Lockwood Farms and Sleeping Giant State Park in Connecticut also have been available as a source of breeding materials. The Connecticut germplasm represents an array of interspecific hybrids that have differing potentials as parents in the backcrossing programs. Not all *C. mollissima* collections are resistant to *C. parasitica* and different collections of Chinese chestnuts may have different genes for blight resistance. The tentative conclusion is that there is an array of germplasm that has resistance to blight, and some of the crosses already have been made with American chestnut so that F1 hybrids and BC1 plants exist for use in further backcrossing.

An interesting question is whether there is some low level of resistance in scattered, surviving large American chestnuts. The survival of these trees also could be due to either low levels of resistance or to the presence of hypovirulence in the cankers on these trees. Based on experiences with breeding other plant species for disease resistance, it is expected that there will be some low level of resistance present in these large survivors. The Amherst tree in Virginia, for example, contains many cankers, many of which are quite superficial. Other trees seem to have few cankers, even if the few cankers are quite large. It may be possible to combine the trait of few cankers with the trait of superficial cankers to develop a level of resistance that is adequate to grow an American chestnut to a large enough size that is useful as a timber tree and a reasonably dependable producer of nuts. Such a program already underway is described elsewhere in this proceedings (see Elkins et al., abstract). The intercrossing of surviving American chestnuts is not a high priority program for The American Chestnut Foundation. We do intend to use large surviving, but diseased American chestnut trees (with low levels of resistance?) as parents in the backcrossing program for a comparison to crosses with American chestnut trees for which we have no indications of resistance. The comparison will be of the blight resistance of the progeny populations produced.

As the Foundation's program has progressed, it has been possible to cross plants earlier than anticipated. Some hybrid plants have been induced to produce male catkins at only 2 yr of age. Hybrids that have flowered have been crossed to American chestnuts even though the individual plants have not been tested for resistance. This is an interesting situation in which the breeding program is moving faster than the resistance evaluation of the progenies. Several plants from one generation are backcrossed to American chestnut trees. Later, tests for resistance will show that some of the progenies used for backcrossing were susceptible, and their progenies will be discarded, or at least not used further.

THE BACKCROSS BREEDING PROGRAM

The breeding and evaluation program is presented in Figures 1 and 2. F1 plants are backcrossed to American chestnut to produce the backcross population (BC_1). Plants that show some level of resistance are then backcrossed again to American chestnut to yield the BC2 population, and the cycle is repeated in additional backcross generations. Of concern in a backcross program is the number of genes necessary to give resistance. If resistance is controlled by few genes (e.g., 1-4 resistance genes), it should not be too difficult to carry the resistance along through several backcross generations. All genes may not be present in a single plant after several generations of backcrossing but there should be a good possibility that several genes for resistance can be maintained in the population of plants carried through each generation. As the genes are transferred from a Chinese genetic background into an American genetic background , there is the possibility that the expression resistance may change; the level of expression of resistance may go up or down.

The BC1 population should theoretically be 3/4 American (Figure 1). Resistant plants in this population can be intercrossed to evaluate the segregation for resistance and plant type in the BC1F2 population (Figure 2). The proportion of plants that express resistance and the levels of resistance expressed by different plants can be evaluated. It should be possible to get an estimate of the number of genes that contribute to resistance to blight based on the frequency of plants expressing resistance and the level of resistance expressed. Among the plants that show some blight resistance there will be segregation for plant morphology and growth characteristics. The proportion of

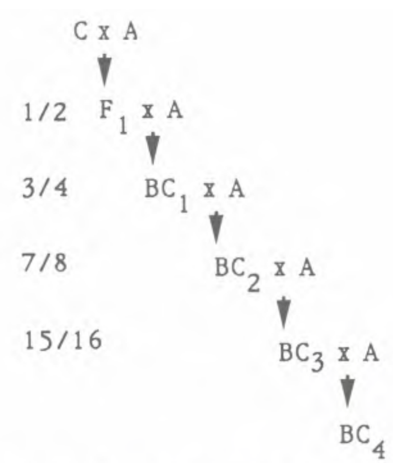

Figure 1. The program of crossing Chinese × American and backcrossing to American chestnuts, and the theoretical proportion of American chestnut genes in each generation.

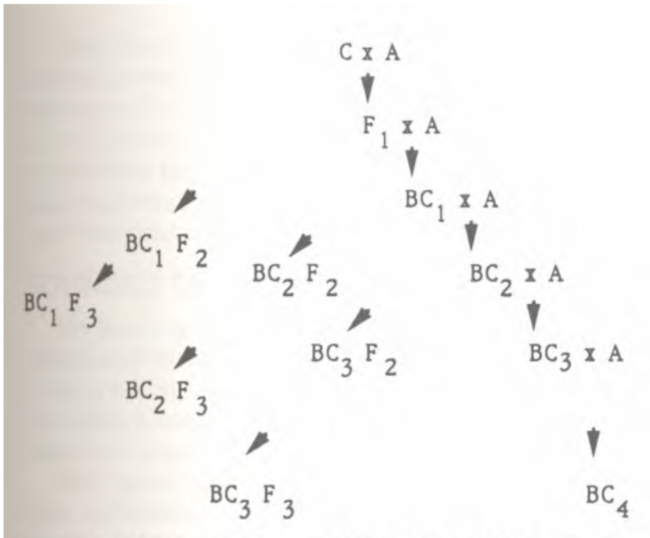

Figure 2. The backcross program and the evaluation of the proportion of American type trees with blight resistance in each BC population.

resistant plants that resemble American chestnuts in morphology and growth characteristics will be very important. The observations should give an indication of the genetic complexity of form and function between Chinese and American chestnut species.

The BC2 population theoretically should have 7/8 of its genes from American chestnut. When resistant plants from this population are intercrossed to establish the BC2F2 population it seems reasonable to expect that there will be less segregation for morphology and growth type among the blight resistant plants than in the BC1F2 population. In the selection of plants of the BC1 population to be backcrossed to American, it is conceivable that some genes that confer resistance, or are involved in the expression of resistance, may be lost. The BC2F2 population may or may not show the same segregation for the proportion of resistant plants or the level of resistance of individual plants observed in the BC1F2 population. The BC2F2 population should show less segregation for plant morphology and growth characteristics than the BC1F2 population. Extensive backcrossing has both advantages and disadvantages.

The comparisons among populations BC1F2, BC2F2 and BC3F2 should be useful in developing breeding strategies. The performance of the BC_1F2, BC_2F2 and BC_3F2 populations will need to be determined in several geographic locations. An adequate level of resistance in one location may not be adequate in another. The ecological niche in which plants are growing also may affect the growth habit of plants. Plants with the same combination of Chinese and American genes may be more similar to the Chinese parent in one location and more similar to the American parent in a second location.

Selection within BC_1F_2, BC_2F_2 and BC3F2 populations can take many forms. For example, one might intercross all plants of the BC1F2 population that are resistant to blight and primarily like American chestnuts in morphology and growth characteristics. The segregation in the BC1F3 population will indicate whether such a population will breed reasonably true to form, that is, resistant to blight and possessing an American chestnut form. There should be increasing uniformity from BC1F3 to BC3F3 populations.

USE OF RFLPS IN BREEDING

The ability to develop genetic maps of organisms took a quantum leap forward with the development of DNA restriction fragment length polymorphism (RFLP) technology. A requirement for this technology is that there exists a considerable amount of polymorphism at the DNA level. High levels of polymorphism exist between the two parent species used in the breeding programs, and the segregation of these polymorphic markers in the backcross generations will make possible the construction of a high-density RFLP map of the *Castanea* genome. There are two types of information that The American Chestnut Foundation wishes to get from the use of RFLP maps. We would like to find RFLP markers that bracket chromonsomal regions that contain genes for blight resistance. Since we can breed plants at a younger age than will give reliable evaluations of blight resistance, we presently make crosses with a large number of plants in segregating generations with the expectation that only a few of the parents have resistance to blight. After the tests for blight resistance are made, progenies obtained by backcrossing susceptible plants to American are discarded. With RFLP markers that bracket disease resistance genes, selection of plants with the marker from the nonrecurrent parent in the backcrossing scheme is likely to select for plants with the genes for blight resistance. For example, in Figure 3, in 4 BC1 plants that are resistant to blight, only the C3 marker from Chinese is in common. If the genes that give resistance to blight are linked to C3, then selection of plants with C3 should give a high probability of having identified the plants with the disease resistance genes that can be selected for the next generation of backcrossing.

A second use of the RFLP technology is to select plants in each generation with the greatest similarity to the recurrent parent for each generation of backcrossing. An example is given of four resistant plants in Figure 3. One plant has the marker from the American parent for only two of the RFLPs. One has the marker from the American parent for seven of the RFLPs. Obviously, the latter is closer to the American parent than the former. In each generation, selection is for the marker from the nonrecurrent parent for DNA sequences flanking the chromosomal regions with genes for blight resistance and for the markers from the recurrent parent for the rest of the DNA sequences.

In a typical backcross program, the proportion of the genes from the recurrent parent is theoretically expected to be 1/2, 3/4, 7/8, 15/16 etc. for the F1, BC1, BC2, BC3, generations, respectively (Figure 1). Whether this proportion of genetic material from the recurrent parent is achieved has been difficult to determine. Analyses of segregating generations using RFLPs have shown that the proportion of RFLP markers for the recurrent parent is less than predicted in each population (Figure 4). Therefore, backcrossing does not apparently move a population

BC_1 - 4 Resistant Plants			
C_1	C_1	C_1	A_1
C_2	C_2	A_2	A_2
C_3	C_3	C_3	C_3
C_4	A_4	A_4	A_4
A_5	C_5	C_5	A_5
A_6	A_6	A_6	A_6
C_7	A_7	C_7	A_7
C_8	C_8	A_8	A_8
C_9	C_9	A_9	A_9
C_{10}	A_{10}	C_{10}	A_{10}
2 A	4 A	5 A	7 A

Figure 3. An example of the combination of RFLPs with the markers from Chinese (C) and American (A) parents in the BC_1 generation.

toward homozygosity with the recurrent parent as rapidly as predicted from theory. By selecting individuals in each generation that have the highest percentage of markers for the recurrent parent, and using these individuals for the next generation of backcrossing, it is possible to approach homozygosity with the recurrent parent in fewer generations than predicted by theory for random selection of individuals for backcrossing in each generation. The potential use of RFLP technology for hastening achievement of goals in breeding of plants with long generation times becomes quite obvious.

ECOLOGICAL ADAPTATION

The American chestnut was adapted to a wide geographic range from Maine to Georgia and west to the Mississippi River; it was a dominant species in the Appalachian Mountains. Whether trees adapted to one geographic area will perform well in another area is of interest to the breeding program. Much of the ACF's backcross program involves crossing American chestnuts that are in the vicinity of the ACF research farm in Meadowview, Va. The Foundation has a large membership that includes a considerable number of people who wish to be actively involved in the breeding program. The ACF is planning to make pollen available from the backcross program for the purpose of crossing onto American chestnuts in different parts of the country. Pollen from plants segregating for resistance (of some generation of the backcross program) could be used to pollinate surviving American chestnuts in New York, Georgia, or Kentucky. Crosses made with pollen from the breeding program with regionally adapted American trees should lead to the production of regionally adapted populations that have blight resistance.

Chinese Parent		American Parent
C_1		A_1
C_2		A_2
C_3		A_3
C_4		A_4
C_5	X	A_5
C_6		C_6
C_7		C_7
C_8		A_8
C_9		C_9
C_{10}		A_{10}

F1			
C_1	A_1		A_1
C_2	A_2		A_2
C_3	A_3		A_3
C_4	A_4		A_4
C_5	A_5	X	A_5
C_6	A_6		A_6
C_7	A_7		A_7
C_8	A_8		A_8
C_9	A_9		A_9
C_{10}	A_{10}		A_{10}

BC_1			
C_1	A_1		A_1
C_2	A_2		A_2
C_3	A_3		A_3
C_4	A_4		A_4
A_5	A_5	X	A_5
A_6	A_6		A_6
A_7	A_7		A_7
C_8	A_8		A_8
A_9	A_9		A_9
C_{10}	A_{10}		A_{10}

BC_2	
C_1	A_1
C_2	A_2
C_3	A_3
C_4	A_4
A_5	A_5
A_6	C_6
A_7	C_7
A_8	A_8
A_9	C_9
A_{10}	A_{10}

Figure 4. The segregation of RFLPs from the non-recurrent parent in backcrossing.

There also is the possibility that intercrosses among surviving American chestnuts, that may have low levels of resistance, will yield populations with higher levels of resistance. If some of these trees do, in fact, possess low levels of resistance, they may be useful to incorporate into the backcross breeding program as one of the sources of American chestnut to be used as the recurrent parent.

SOURCES OF RESISTANCE

Any breeding program for disease resistance must be cognizant of the fact that the entity to which resistance is sought is a living entity. Living entities can change (mutate, recombine genes, etc). Therefore, there must be several sources of resistance used in any breeding program.

With regard to the American chestnut breeding progam, each source of resistance must be backcrossed with American chestnuts to develop blight resistant chestnuts that are similar in morphology and growth characteristics to American chestnuts (Figure 5). These lines also will have to be tested for their levels of resistance and growth characteristics in several geographic locations.

There also must be a constant search for new sources of resistance. Resistance obtained from different collections of Chinese chestnut may or may not have the same genes for resistance to blight. Resistance obtained from Japanese and European chestnut may be due to different genes that can be used separately or together with resistance obtained from Chinese chestnut. Resistance to blight occurs in several species of *Castanea* but it is not known if the resistance is due to different genes. The molecular mapping of resistance in interspecific crosses may be very informative and help determine whether resistance in Chinese, Japanese, European, and possibly in American chestnut is in the same chromosomal locations (which suggests they may contain the same genes) or in different chromosomal locations (which suggests they may have different genes for resistance).

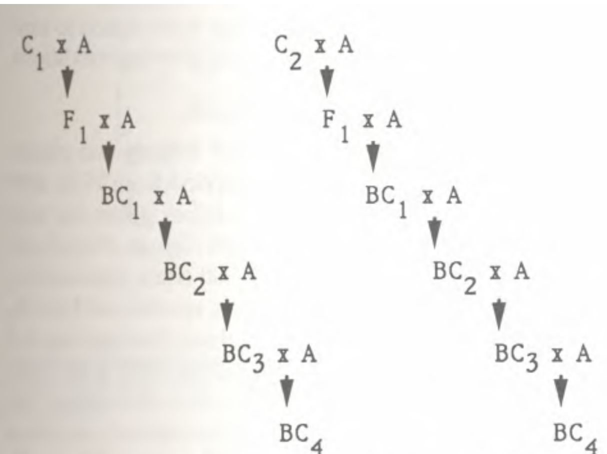

Figure 5. The use of two sources of blight resistance, C1 and C2, in parallel backcrossing programs.

The search for new sources of resistance is based on the assumption that the breeding program will put selection pressure on the pathogen population and that new races of the pathogen will be recovered. It also is important to recognize that breeding may inadvertently develop populations more susceptible to pests other than blight that will then become important problems for growing American type chestnuts. There must be constant observation for changes in all problems associated with growing chestnuts. Testing populations at all stages of the breeding program in many geographic locations should give warning signs of other parasites of chestnuts, and give opportunities to adjust the breeding program accordingly. Production of a blight resistant plant that is essentially indistinguishable from an American chestnut is only the beginning of a program to restore American chestnuts as a major component of the North American forest regions.

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