DNA Markers at Meadowview: A Progress Report, or, How can we determine what genes confer resistance to chestnut blight?


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Mapping Genes 1: Genetic Maps

Genes that are close together in the DNA tend to be inherited together. We can map genes relative to detectable attributes, including DNA features (see sidebar, “DNA Markers”), based on the frequency with which they occur together in related individuals.

First genetic map of Hebard (1994) showing relative positions of genes specifying twig hairs (Tah1), leaf venal hairs (LmhL), leaf intervelar hairs (Lmh), stipules (StpL), and stem color (Red1). Subsequent maps (Kubisak et al. 1997, 2013) have many DNA markers and show regions associated with resistance to chestnut blight. Genetic mapping is continuing, both to refine mapping of the ‘Mahogany’-derived resistance investigated in the published work, and to investigate resistance from other sources. Refinement requires looking at more trees with more DNA markers.

Mapping Genes 2: Physical Maps

The ultimate physical map is a complete DNA sequence, but another, lower-resolution physical map is generated by “molecular cloning” of the DNA. The first physical map of Chinese chestnut was constructed using Bacterial Artificial Chromosomes, or BACs, for short.

BAC inserts are typically a bit over 100,000 bases long. Since the chestnut genome is about 800 million bases, 800 of these fragments would contain all the DNA — if we could cut them and splice them systematically. But we can’t do that, and we also can’t tell which individual fragments go together, unless there is some overlap. So, practically, we need 10- to 20x the 8000 predicted fragments of chestnut DNA for our BAC library.

The physical map is generated by “fingerprinting” the BAC inserts and reconstructing the location of each insert in the genome relative to the other BAC inserts.

Mapping Genes 3: Integrating Genetic and Physical Maps

Because DNA is composed of two complementary strands, we can identify BAC inserts that contain a DNA marker by radioactively labeling a short segment of that DNA and permitting it to anneal to an array of BAC DNA. The integrated map enables us to sequence DNA from regions of interest, like the chestnut blight resistance “Quantitative Trait Locus” (QTL) chr1 on Linkage Group B.

A segment of the physical map containing a DNA marker mapping to chr1. BAC "puzzle" suggests presence of repetitive DNA.

Mapping Genes 4: Sequencing DNA

We cannot presently take a chromosome and sequence its DNA from one end to the other. Rather, with current "next-gen sequencing" technology, the DNA is fragmented and sequenced in small segments, or "reads" on the order of 100-1000 bases. As with the construction of a physical map from fingerprinted BACs, the sequenced segments are random, and overlapping reads are needed to assemble a sequence. It's like trying to assemble a puzzle with 8 million pieces, and on top of that, not all the pieces are present in the box, so you have been handed a big box with dozens of incomplete copies of the puzzle all mixed together! And the repetitive sequence is like having lots of pieces of blue sky. So it really helps to be able to narrow down the region of interest using the integrated genetic and physical maps. Assembling a sequence is an iterative process, as new methods and new data are available to fill in the gaps.

Bulk sequence analysis

Last fall (2013) we made two “bulks,” or pools, of DNA, one from 11 resistant F1 trees, and the other from 14 susceptible trees. The bulks were sequenced, and we used the assembled Chr1 QTL v1 sequence to identify individual bases that differed between the two bulks. The following table summarizes information about 11 of these that we consider most likely to be involved with blight resistance, because they are in predicted genes, and are predicted to alter the protein that is encoded by the gene.

What next?

We are proceeding to test these SNPs on more trees to see if the association with blight resistance holds up.

If any of them still looks good, use the gene to transform American chestnut and see if it affects the tree’s susceptibility to chestnut blight.

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References

Abstract:
Since its founding 31 years ago, The American Chestnut Foundation (TACF) has pursued a strategy of backcross breeding to transfer blight resistance from Asian to American chestnut trees. During that time, TACF has partnered with university and federal researchers, notably in projects funded by the National Science Foundation and the Forest Health Initiative, which generated chestnut DNA sequence data in large quantities, chestnut DNA markers, and maps of the chestnut. While the ultimate goal of such research is the identification of the genes that confer resistance to blight and Phytophthora root rot, DNA markers also are useful for characterizing genetic diversity in both Asian and American chestnuts, for detecting ms-identified trees and pollination contamination, and for monitoring the elimination of unwanted Asian chestnut sequences during backcrossing. DNA markers linked to blight resistance can be used in conjunction with progeny testing to identify trees that are "true-breeding" for resistance. This year at Meadowview we screened a subset of trees from a Nanking backcross family, together with the parents and Nanking, to identify Simple Sequence Repeat (SSR) markers that will be useful for constructing a genetic map for this family and investigating whether the genomic regions associated with resistance are the same for this source of resistance as the ones previously identified for Mahogany/Graves. As reported last year, using a collection of 5000 Single Nucleotide Polymorphisms (SNPs) on an Illumina Array, we evaluated an expanded Mahogany F2 mapping population and a collection of Graves B3 families that had been screened for blight resistance. Unfortunately, there was a high rate of failure of SNP assays on the collection, so we did not see the increase in marker density that we had hoped for. Nonetheless, the existence and positions of two quantitative resistance loci (QTL) were confirmed in the expanded F2 mapping population. SSR genotyping of the F2 population is under way to increase marker density.

The SNP data were also used to exclude outcrosses from sets of F2 trees. F2 trees highly resistant or susceptible to blight were also extracted and bulked for DNA sequence analysis, resulting in numerous SNPs. The resulting sequences have been aligned to the assembled blight-resistance-QTL sequences v6.0 to identify and annotate SNPs associated with blight resistance. Genome-wide analysis is in progress.