Peroxidase Allozyme Phenotypes in *Castanea* and Their Segregation among Progeny

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Interest in growing and breeding chestnut trees has increased with the recent publication of Bumham (1988) suggesting a back-cross breeding program to improve chestnut blight resistance of American chestnut [*Castanea dentata* (Marsh.) Borkh.]. American chestnuts can now be kept alive for breeding by use of a biological control based on a virus that invades the blight fungus (Anagnostakis, 1990). Santamour (1988) showed that graft compatibility in *Castanea* depended on the similarity of three anodal peroxidases, A, B, and C.

I have modified his technique and checked 10 trees of *C. mollissima* Bl., 11 *C. dentata*, three *C. sativa* Mill., three *C. crenata* Sieb. & Zucc., two *C. seguinii* Dode, and one *C. henryi* (Skan) Rehd. & Wils. The hybrids investigated were F_1 s, with American as one parent, F_2 s, and first generation backcrosses (BC1) to American. All trees were growing at the Sleeping Giant Chestnut Plantation, or at the Lockwood Farm, Connecticut Agricultural Experiment Station, both in Hamden, Conn. The plot maps are available from the director, Connecticut Agricultural Experiment Station.

Twigs with dormant buds were harvested

and stored in plastic bags in a freezer at -20°C until used. For extraction, three buds were removed from the twigs and placed in a glass homogenizer with 0.5 ml of a solution containing 0.1 M N-2-hydroxyethylpiperazine-N'-3-propanesulfonic acid (EPPS; Sigma Chemical Co., St. Louis), 5 mM potassium metabisulfite, 10 mM cysteine, and 1% Tween 80, adjusted to pH 7.0. The buds were ground and transferred to microcentrifuge tubes (Eppendorf, Fremont, Calif), centrifuged (at 4°C) to a firm pellet, and the supernatants transferred to new tubes. Electrophoresis was performed on 7% native polyacrylamide gels in Tris-glycine buffer, pH 8.9, for ≈ 7 h at 30 mA per gel (vertical electrophoresis apparatus cooled to 10°C). Gels were stained to detect peroxidase activity by allowing them to react with H_2O_2 (0.5 ml of a 3% solution in 50 ml) and 3-3'-diaminobenzidine (Sigma, 0.25 mg in 50 ml) in 0.2 M (2-(N-morpholino)ethanesulfonic acid (MES; Sigma), pH

Many anodal bands were seen, but the consistent bands, referred to as bands A and B by Santamour et al. (1986), near the origin were easily identified. None of the slower-moving bands that Santamour called C were found. All 11 trees of *C. dentata* and three trees of *C. sativa* had only the A allozyme. Three trees of *C. crenata*, two of *C. seguinii*, and the only *C. henryi* had only the B allozyme. Among 10 trees of *C. mollissima* one had only A, five had only B, and four

had A and B. This distribution is consistent with previous observations of Santamour et al. (1986).

Hybrids of *C. crenata* x *C. dentata* (two trees) and two of *C. dentata* x *C. seguinii* had both A and B. Two of *C. dentata* x *C. mollissima* had both A and B, two of *C. mollissima* x *C. dentata* had A and B, and one had only A. Two hybrids [(*C. crenata* x *C. dentata*) x (*C. mollissima* x *C. dentata*)] had only A.

Two clones of the BC1 tree 'Clapper' had only A, and BC1 tree 'Graves' had both A and B. These trees are both (*C. mollissima* x *C. dentata*) x *C. dentata*.

New BC1 trees (seed planted Mar. 1989, and twigs harvested Dec. 1990) that had Asian hybrid parents with both A and B crossed with American trees with only A would have segregated 1:1 for AA : AB if A and B were allozymes determined by alleles at the same locus. Both kinds of crosses yielded more progeny with the peroxidase phenotype AA than expected. The peroxidase phenotypes of (*C. mollissima* x *C. dentata*) x *C. dentata* progeny from 10 crosses were 37 AA and 26 AB, but the deviation from 1:1 was not significant (chi-square analysis yielded $P = 0.16$). Five crosses of *C. dentata* x (*C. crenata* x *C. dentata*) yielded 19 AA and 12 AB, which is also not significantly different from 1:1 (chi-square analysis yielded $P = 0.21$). These results support the prediction of Santamour et al. (1986) that these important peroxidases are allelic.

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