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Variation in American Beech (Fagus grandifolia Ehrh.): Isozyme Analysis of Genetic Structure in Selected Stands[1])

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Summary

The genetic structure of *Fagus grandifolia* EHRH. stands in Massachusetts (MA) and West Virginia (WV) was studied by analysis of isozyme variation at nine loci. The stands were found to be substructured into mosaics of putative clones and trees of seedling origin. The level of genetic diversity was high: observed per locus heterozygosities averaged 0.382; number of alleles per locus averaged 2.9. Significant deviations from Hardy-Weinberg equilibrium were detected for up to five of the nine loci studied. Deviations resulted from an excess of heterozygotes at the 6PG-2, MDH-1, and CTO-1 (MA only) loci, and a deficiency of heterozygotes at the CTO-2 and PER-3 loci. Overall, positive mean F_{IS} values indicated slight deficits (2.3%) of heterozygotes within populations. Positive assortative mating, as a result of crossing within clonal and/or family patches in stands, may be responsible. A mean F_{ST} value of 0.064 provided evidence for moderate differentiation between the two populations. Clonal structure had a small effect on the computation of population genetic statistics for these two stands.

Key words: *Fagus grandifolia*, isozyme, clone, stand structure, population structure, F-statistics.

FDC: 165.3; 165.5; 176.1 *Fagus grandifolia*.

Introduction

American beech (*Fagus grandifolia* EHRH.) is a widespread, highly shade-tolerant species occurring in 20 forest types throughout its range (*Fig. 1*) (BURNS and HONKALA, 1990). It is one of relatively few tree species which reproduce both sexually by seed and vegetatively by production of root sprouts (BORMANN and LIKENS, 1979; WARD, 1961). Although seeding must be the mechanism for initial establishment, root sprouting appears to be the main mode of regeneration on specific sites and in certain areas of its range (BORMAN et al., 1970; FORCIER, 1975; HELD, 1980).

The role of root sprouting in hardwood trees as a successional mechanism has received some attention (FORCIER, 1975; HELD and WISTENDAHL, 1977). Sprouting ensures the continued development of stems in species such as beech where seed production may be low, erratic, subject to heavy predation by animals, or where embryos may be non-viable. Production of root sprouts appears also to be a process by which beech maintains its dominance in the community. Root sprouting is also an obvious means by which specific genomes are perpetuated and where conditions are favorable, increased and spread through the forest community. This characteristic undoubtedly has an effect on the genetic structure and composition of populations within a species, but has been little studied in this regard for woody species other than *Populus* (CHELIAK and PITEL, 1984; HYUN et al., 1987) and *Alnus* (HUENNEKE, 1985).

Beech bark disease is a major dieback-decline disease that causes significant mortality and defect in American beech. It results when bark, attacked and altered by the beach scale, *Cryptococcus fagisuga* LIND., is invaded and killed by the fungi, primarily *Nectria coccinea* var. faginata LOMAN, WATSON, and AYERS, and *N. galligena* BRES. (EHRLICH, 1934; LOHMAN and WATSON, 1943; COTTER, 1977). Some trees remain insect- and disease-free in stands long-affected by beech bark disease, and challenge trials have shown them to be resistant to *C. fagisuga* (HOUSTON, 1982, 1983). Resistant trees are found in relatively low numbers (ca. <1% of all beech trees in stands examined), and frequently occur in discrete groups.

As part of a study of the distribution of genotypes within stands of American beech relative to patterns of beech bark disease, a number of stands of this species located from Prince Edward Island, Canada, to West Virginia, U.S.A. were sampled, and beech trees within

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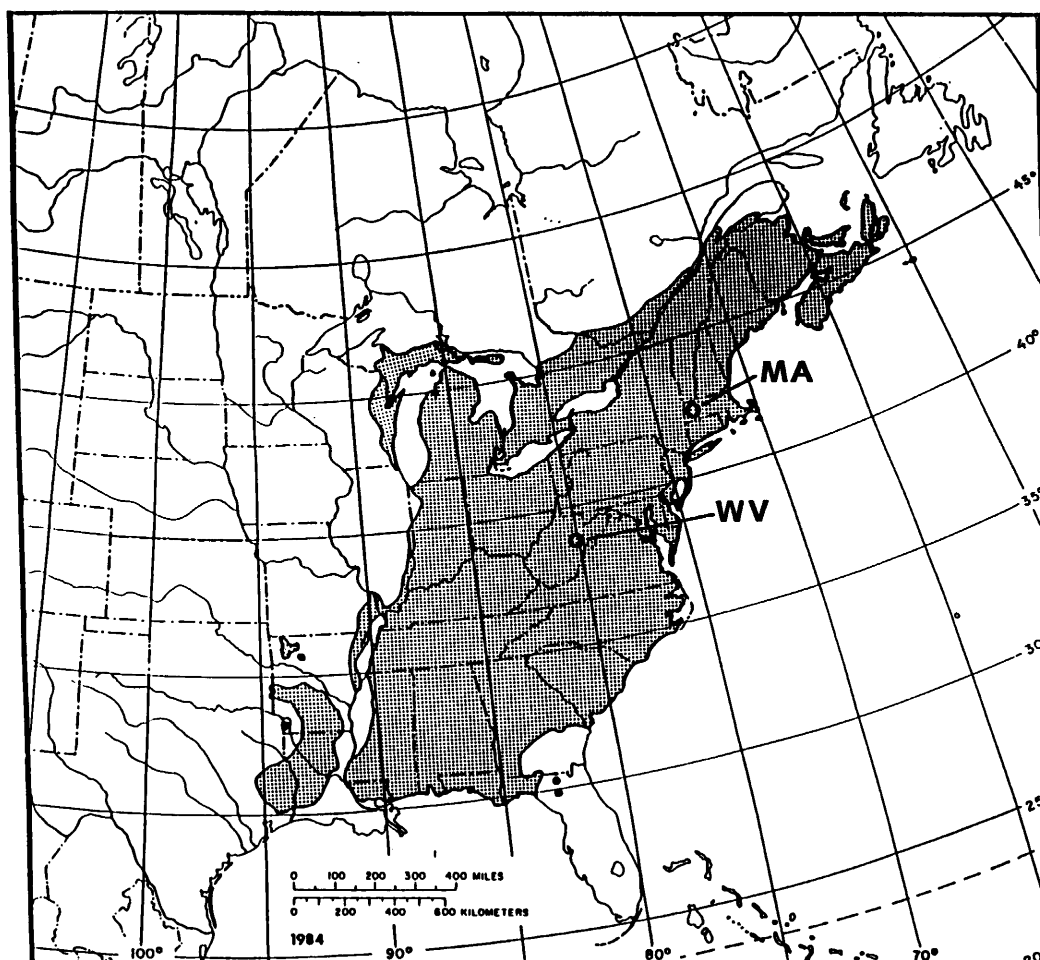

Figure 1. — Natural range of American beech (*Fagus grandifolia* Ehrh.), with locations of sampled stands in Massachusetts (MA) and West Virginia (WV).

stands were genotyped using isozymes. The results of isozyme analysis of the genetic structure in two such stands, located in the Monroe State Forest, Massachusetts (MA) and the Monongahela National Forest, West Virginia (WV) are reported here. Preliminary analyses of data collected in these and several other smaller stands were reported previously (Houston and Houston, 1987, 1990, 1991).

Materials and Methods

The locations of beech trees, $\geq$5 cm DBH (VW) and $\geq$10 cm DBH (MA), in each stand were mapped using a hand compass and rangefinder. Trees were classed as either resistant or susceptible to beech bark disease based on the absence or presence of the initiating insect, *Cryptococcus fagisuga,* and/or cankers caused by the species of *Nectria* associated with this complex disease. Dormant buds were collected from the upper crown of each tree, stored under ice, frozen in liquid N_2 within 72 hours, and held at —70 °C until bud tissue extracts could be prepared for electrophoresis. Enzymes were extracted from bud tissue by removing bud scales and grinding single buds in 0.75 ml grinding buffer modified slightly from Cheliak and Pitel (1984).

Using starch gel electrophoresis (Houston and Houston, 1987), seven polymorphic enzymes, including nine putative loci (phosphoglucose isomerase (PGI-2), phosphoglucomutase (PGM-1), 6-phosphogluconate dehydrogenase (6PG-1, -2), alcohol dehydrogenase (ADH-2), malate dehydrogenase (MDH-1), cytochrome oxidase (CTO-1, -2), and peroxidase (PER-3) from dormant bud tissue were analyzed for 173 trees in the MA stand, and 152 trees in the WV stand.

It was not possible to confirm patterns of inheritance for these enzymes due to the lack of control-bred or half-sib progenies. Genetic control of polymorphic enzymes was inferred, however, from observation of functional subunit numbers in heteromultimer types of putative heterozygotes, and from published reports of work with these enzymes in other angiosperms.

Buffers used to separate isozymes are listed in *table 1.* All systems used 12.4 % gels except for PER (12 % gel). Following electrophoresis, enzymes were stained in 1.5 mm gel slices using protocols reported by Cheliak and Pitel (1984) for CTO, MDH and PGM, Siciliano and Shaw (1976) for ADH, 6PG and PGI, and Yen and Sadanaga (1977) for PER. Isozyme banding patterns for the nine loci were characterized by measuring r_f values for each isozyme band of each sample. Patterns for each enzyme system were verified by repetitive electrophoresis and by co-electrophoresis of individual tree samples.

An additional 12 enzyme systems (or loci), including phosphoglucose isomerase (PGI-1), alcohol dehydrogenase (ADH-1), (PER-1, -2), triosephosphate isomerase (E. C. 5.3.1.1[4]), isocitrate dehydrogenase (E.C. 1.1.1.42), alpha-

[4]) Enzyme Commission designation.

galactosidase (E.C. 3.2.1.22), menadione NADH reductase (E. C. 1.6.99.2), superoxide dismutase (E. C. 1.15.1.1), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12), adenylate kinase (E.C. 2.7.4.3), fumarase (E.C. 4.2.1.2), aldolase (E.C. 4.1.2.13), and glycerate-2-dehydrogenase (E.C. 1.1.1.29) demonstrated no polymorphism and were judged to be monomorphic, or did not stain clearly or reproducibly and were excluded from the analyses.

Each tree was assigned an alphabetic (and corresponding numeric) isozyme genotype for each enzyme locus based on its banding patterns. The nine numerical genotypes were then integrated to construct a unique multi-locus enzyme genotype for each tree. Assignment of these alphabetic and numerical genotypes to each mapped tree provided for statistical analysis of population structure, and permitted a visual assessment of the stand genetic architectures, including putative clonal patterns.

Data reduction and calculations of most population genetic statistics were accomplished using BIOSYS-1 (SWOFFORD and SELANDER, 1989), as modified by BLACK (BIOSYS-2; 1992). Three common gene diversity measures were calculated for each stand (population): (i) percent polymorphic loci, (ii) average number of alleles per locus, and (iii) observed and expected heterozygosities (NEI, 1978).

Percent polymorphic loci were calculated by dividing the number of polymorphic loci by the total number of loci scored. A locus was considered polymorphic if the frequency of the most common allele was ≤ 0.99. The average number of alleles per locus was obtained by dividing the total number of alleles over all loci by the number of loci. The expected heterozygosity at HARDY-WEINBERG equilibrium, H_{exp} (the within-population genetic diversity) (NEI, 1978) and the observed heterozygosity, H_{obs} (number of observed heterozygous genotypes divided by total number of genotypes), were calculated for each locus and averaged over all loci. Monomorphic loci were not included in the above calculations.

Heterogeneity χ^2 analyses were performed for each locus to detect significant differences in allele frequencies among stands. Not significant χ^2 values indicate that allele frequencies drawn from all stands are not different from samples drawn at random from the total population. Significant χ^2 values indicate that stand heterogeneity exists (WORKMAN and NISWANDER, 1970).

NEI's (1977) parameters of gene diversity were used to investigate the distribution of genetic diversity within and among populations. Total gene diversity (H_T, the expected heterozygosity when all populations are treated as a panmictic unit), calculated according to NEI and CHESSER's (1983) formula for small population size, was partitioned into within- (H_S) and among-population (D_{ST}) components, and the overall coefficient of differentiation (G_{ST}) was calculated as D_{ST}/H_T. The mean G_{ST} value over all populations was obtained by averaging across all polymorphic loci.

Genetic structure was also assessed from WRIGHT's F-statistics (fixation indices; F_{IS} and F_{IT}) (WRIGHT, 1943, 1951, 1965, 1969) using the methods of WEIR and COCKERHAM (1984). These methods account for both sample size and variation in sample size among populations, and use jackknifing to estimate F-statistic variances. These fixation indices describe the effect of population structure on heterozygote frequencies. NEI's (1978) unbiased genetic identity, I, was also calculated as a measure of interpopulational variation.

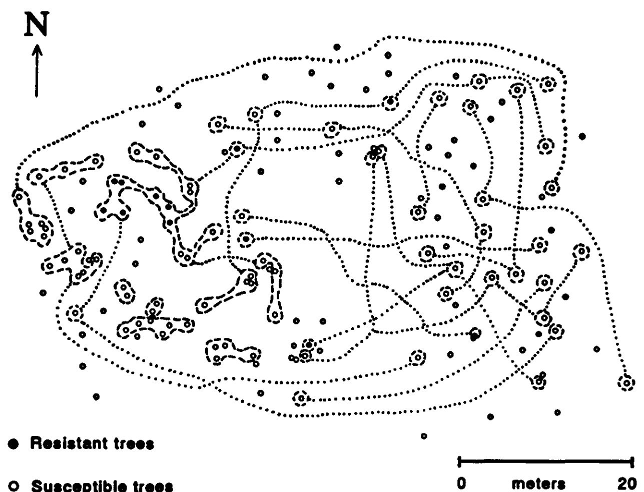

Figure 2. — Genetic relationships in the Massachusetts stand. Trees with identical, but unique, multi-locus isozyme genotypes are enclosed with a dashed line, and/or connected by a dotted line. Boundaries for putative clones were drawn arbitrarily, and are not meant to imply knowledge of actual patterns of clonal extension. All other individuals have a unique genotype.

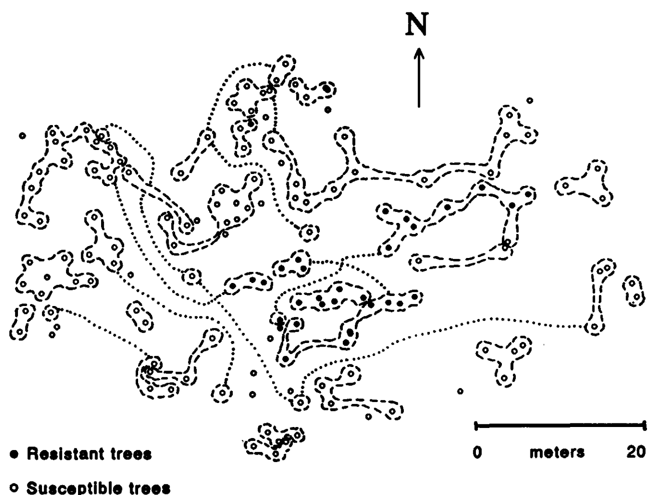

Figure 3. — Genetic relationships in the West Virginia stand. Trees with identical, but unique, multi-locus isozyme genotypes are enclosed with a dashed line, and/or connected by a dotted line. Boundaries for putative clones were drawn arbitrarily, and are not meant to imply knowledge of actual patterns of clonal etension. All other individuas have a unique genotype.

Designations of trees as resistant or susceptible to beech bark disease are included (*Fig. 2,3*) because this trait served as the initial phenotypic "marker" of genetic similarity, i.e., clonal/family structure, and mosaicism in the study stands.

Results and Discussion

Genetic relationships within stands based on isozyme genotypes are shown in *figure 2* for the Massachusetts (MA) stand, and *figure 3* for the West Virginia (WV) stand. The MA stand contained 90 unique genotypes, while the WV stand included 40 unique genotypes. The data suggest that many of the trees in each stand are ramets of existing clones, i.e., have identical genotypes, or have very similar genotypes and are related by descent. Map designations of putative clones were drawn arbitrarily to enclose trees of identical isozyme composite genotype, and are not meant

Table 1. — Electrophoresis and gel buffers used to characterize isozymes of dormant beech bud tissues.

6PG-1,-2; PGI-2[1]	Electrode: pH 8.0, 0.05M Tris, 0.016M EDTA, 0.65M Borate; Gel: pH 8.0, 1:10 dilution of electrode buffer (Siciliano and Shaw 1976)
PGM-1, MDH-1	Electrode: pH 7.0, 0.125M Tris-citrate; Gel: pH 7.0, 0.05M Histidine-HCl, 0.0014M EDTA (Cheliak and Pitel 1984)
ADH-2	Electrode: pH 7.0, 0.135M Tris, 0.043M Citrate; Gel: 0.009M Tris, 0.003M Citrate (Siciliano and Shaw 1976)
CTO-1,-2	Electrode: pH 8.1, 0.06M Lithium hydroxide, 0.3M Borate; Gel: pH 8.5, 0.03M Tris, 0.005M Citrate, 1% electrode buffer (Ridgeway et al. 1970)
PER-3	Electrode: pH 8.7, 0.3M Borate, 0.1M NaOH; Gel: pH 7.5, 0.014M Tris, 0.004M Citrate (Kristjansson 1963)

[1]) Enzyme Commissions designations:
PGI-2, phosphoglucose isomerase, E.C. 5.3.1.9; *PGM-1*, phosphoglucomutase, E.C. 2.7.5.1; *6PG-1, -2*, 6-phosphogluconate dehydrogenase, E.C. 1.1.1.44; *ADH-2*, alcohol dehydrogenase, E.C. 1.1.1.1; *MDH-1*, malate dehydrogenase, E.C. 1.1.1.37; *CTO-1, -2*, cytochrome oxidase, E.C. 1.9.3.1; and *PER-3*, peroxidase, E.C. 1.11.1.7.

to imply actual patterns of clonal extension. No individual isozyme genotypes or composite tree genotypes were associated with resistance to beech bark disease. In fact, there were a few instances in which resistant and susceptible trees were characterized by identical multi-locus isozyme genotypes.

In the absence of control-bred progenies with which to confirm relationships, it was assumed that the existence of identical tree isozyme genotypes in close proximity implies a clonal relationship, while those genotypes which differ between trees by only one or two isozyme banding patterns imply a high degree of familial relatedness, i. e., are probably half- to full-sibs. However, it is also possible that trees with identical isozyme genotypes may also be siblings rather than ramets of a given clone. Similar spatial relationships were evident in both populations.

In the WV stand (*Fig. 3*), clustering of like genotypes was generally well defined, and putative clones were easily delineated. In the MA stand (*Fig. 2*), however, a number of genotypes classified as identical, but not in close association with each other, were evident, some of which were separated by as much as 50 meters. Such trees may represent (1) closely related individuals, e.g., full- or half-sibs, which might be resolved as different genets if a more intensive sampling of their genomes could be accomplished, or (2) ramets of the same clone, but more widely spaced than ramets of some other putative clones observed in these stands.

If the first case is true, the distribution patterns of these trees are well within the range of seed dispersal observed for known seed predators of beech nuts such as blue jays and squirrels (Johnson and Adkisson, 1985, 1986). Given the limited number of genes being sampled in this study by the isozyme technique, the existence of siblings with identical genotypes would not be totally unanticipated. It is possible (but less probable) that identical genotypes (for the nine genes sampled in this study) might have been produced in the same, or different, seed years.

Yet, the possibility that more widely separated individuals with identical genotypes are of clonal origin cannot be ruled out. Several studies of the linear and areal distribution of root systems for conifer and hardwood species have been reported. The linear spatial distributions of many of the putative clones identified in these stands exceed the linear distributions measured for beech clones by Jones and Raynal (1986). They found that 99% of the sprouts identified as originating from a specific tree were located no more than 10 meters from that tree. However, in their study, only sprouts still attached to the parental root system were considered, probably to insure that stems included in the study were truly ramets of the same clone. They also identified sprouts, usually greater than 10 years old, which had become functionally independent from the parent tree. They concluded that the potential for clones to spread is limited. This report did not, however, describe the stand conditions under which studied clones had developed, or take into consideration the reported ability of this species to develop in, and colonize, gaps in canopy structure (Ward, 1961; Held, 1980). Also, no attempt was made to definitively ascertain the genetic composition of independent sprouts.

Some reported studies have involved the actual excavation of complete root systems of mature trees. Yeager (1935) determined the radial root extension of a 43-year-old *Quercus macrocarpa* Michx. to be 12.5 meters. Radial root lengths of approximately 17 meters for *Pinus palustris* Mill. and 15 meters for *Quercus laevis* Walt. were reported by Hough et al. (1965), and Weaver and Kramer (1933) measured lateral root extension in *Quercus macrocarpa* Michx. as far as 18 meters from the root collar. Studies of root system development in *Quercus rubra* L. (Lyford, 1980) and *Acer rubrum* L. (Lyford and Wilson, 1964) revealed that woody lateral roots were as long as 17 meters and 25 meters, respectively.

In the aspens, root extension and clone development may be even more extensive; Buell and Buell (1959) excavated one lateral root 32 meters long. Single *Populus grandidentata* Michx. clones over 60 meters long and exceeding 0.4 ha in size are commonly found in the eastern United States (Barnes, 1966), and over 80 ha in size in

Table 2. — Allele frequencies for selected American beech populations in Massachusetts and West Virginia.

Locus	Allele	Massachusetts N = 173	West Virginia N = 152	X^2
PGI-2	A	0.090	0.079	0.237
	B	0.910	0.921	
PGM-1	A	0.358	0.145	60.4**[1]
	B	0.590	0.855	
	C	0.052	0.000	
6PG-1	A	0.075	0.082	0.113
	B	0.925	0.918	
6PG-2	A	0.358	0.312	17.5**
	B	0.639	0.632	
	C	0.003	0.056	
ADH-2	A	1.000	0.993	2.28
	B[2]	0.000	0.000	
	C	0.000	0.007	
MDH-1	A[2]	0.000	0.000	2.72
	B	0.431	0.493	
	C	0.347	0.319	
	D	0.222	0.188	
CTO-1	A	0.350	0.371	13.8**
	B	0.494	0.372	
	O[3]	0.156	0.257	
CTO-2	A	0.251	0.135	155.5**
	B	0.130	0.546	
	C	0.000	0.043	
	D	0.009	0.000	
	E	0.009	0.000	
	O[3]	0.601	0.276	
PER-3	A	0.633	0.641	72.8**
	B	0.142	0.000	
	C	0.121	0.293	
	D	0.000	0.003	
	E	0.000	0.004	
	O[3]	0.104	0.059	

[1]) ** = Significant difference between stands at the 0.01 level.
[2]) Allele detected in beech stands not included in this report.
[3]) "Null" allele.

Table 3. — Genetic variability at nine loci in American beech populations in Massachusetts and West Virginia.

	Massachusetts	West Virginia
No. of trees	173	152
Mean # alleles/locus	2.9	2.9
% loci polymorphic[1]	88.9	88.9
Mean H_{exp}/locus[2]	0.407	0.383
Mean H_{obs}/locus[3]	0.382	0.392

[1]) A locus was considered polymorphic if the frequency of the most common allele was $\leq$ 0.99.
[2]) Unbiased estimate of heterozygosity (Nei, 1978).
[3]) Direct count estimate of heterozygosity.

P. tremuloides Michx. clones in the western United States (Kemperman and Barnes, 1976). The greatest linear distances observed between identical genotypes in this study are thus of the same magnitude as total root extension distances observed for other species, including some in which root sprouting is a recognized means of reproduc-

tion. Reports of root extension in the literature have recently been summarized by STONE and KALISZ (1991). Based on published data for rates of lateral root elongation in hardwoods, our data suggest that, over long time periods, clonal expansion in *Fagus grandifolia* EHRH. may indeed result in the distribution of genotypes throughout a stand. Analyses of genetic structure in additional beech stands in. Maine, Nova Scotia and Prince Edward Island are in progress, and may help to clarify this point.

In general, the genetic architecture of the MA stand appeared to be less structured (i.e., displayed less clonal development) than that of the WV stand, and this difference undoubtedly reflects past stand history, including cultural treatments. Past conditions and/or treatments in the WV stand may have promoted stronger clonal development by stimulating root sprouting. In fact, evidence of past logging activity, suggested by the presence of old stumps, was much more apparent in the WV stand than the MA stand. Logging disturbance is only one factor which can promote root sprouting in beech. Injuries to roots caused by freezing and thawing of surface soils and by animals may also induce sprout production (JONES and RAYNAL, 1987, 1988).

Allele frequencies in each stand are given in *table 2*. Significant differences in allelic frequencies were detected at several loci both within and among stands, most noticeably for PGM-1, 6PG-2, CTO-1 and -2, and PER-3.

The mean number of alleles per locus was the same for both stands (2.9), as was the proportion of polymorphic loci (P = 89 %). The latter exceeds that reported for *Quercus macrocarpa* and *Q. gambelii* (avg. P = 56 %; SCHNABEL and HAMRICK, 1990b), but is consistent with those reported for *Populus tremuloides* (P = 81 %; JELINSKI and CHELIAK, 1992) and *Gleditsia triacanthos* (P = 81 %; SCHNABEL and HAMRICK, 1990a), two clone-forming species. The direct count estimate of mean heterozygosity per locus was only slightly lower in the MA stand (H_O = 0.382, S.E. = 0.103) than in the WV stand (H_O = 0.392, S.E. = 0.113) (*Table 3*). These values are somewhat higher than those described for *Gleditsia* (H_O = 0.119; SCHNABEL and HAMRICK, 1990a) and *Quercus* spp. (H_O = 0.201, 0.218; SCHNABEL and HAMRICK, 1990b; SCHWARZMANN and GERHOLD, 1991), but similar to that for *Populus* (H_O = 0.320; JELINSKI and CHELIAK, 1992). NEI's (1978) unbiased genetic identity for these two stands was 0.956.

Chi-square analyses detected significant deviations from HARDY-WEINBERG equilibrium for 5 of the 8 variable loci (ADH-2 was fixed) in the MA population, and for 5 of 9 loci in the WV stand. Analysis of coefficients for heterozygote deficiencies or excesses indicated these deviations resulted from an excess of heterozygotes at the 6PG-2, MDH-1 and CTO-1 loci in both stands. Heterozygote deficiencies at the CTO-2 and PER-3 loci also contributed to significance. For the latter two loci, however, the presence of putative "null" alleles may have served to skew calculations, resulting in inflated expectations for heterozygotes.

Observed heterozygosity over all loci only slightly exceeded HARDY-WEINBERG expectations in the WV stand, but was somewhat lower than the expected heterozygosity calculated for the MA stand (*Table 3*). Chi-square tests for conformance to HARDY-WEINBERG equilibrium for individual loci were significant for the 6PG-2, MDH-1, CTO-1, CTO-2 and PER-3 loci in both populations.

Deviations from HARDY-WEINBERG equilibria and accompanying heterozygote deficiencies or excesses have been reported for other hardwood species, including *Robinia pseudoacacia* L. (SURLES et al., 1989) and European beech, *Fagus sylvatica* L. (CUGUEN et al., 1988). In the latter report, a heterozygote deficit was also found for two peroxidase loci as sampled in 250 stands across Europe. The level of genetic variation observed in this sample of American beech is higher than that reported for many conifer species and most angiosperms, e.g., *Alnus crispa* (AIT.) PURSH. (BOUSQUET et al., 1987), *Quercus* species (MANOS and FAIRBROTHERS, 1987; SCHWARZMANN and GERHOLD, 1991) and *Eucalyptus obliqua* L'HERIT. (BROWN et al., 1975). Observed heterozygosities were also higher than the mean (0.21) reported for 16 species of tropical trees and shrubs by HAMRICK and LOVELESS (1989).

Table 4. — Fixation indices and chi-square analyses for nine polymorphic loci in two stands of American beech.

	Fixation Index[1]			
Locus	Total (F_{IT})	Within stand (F_{IS})	Among stand (F_{ST})	Chi-square analysis[2]
PGI-2	-.013	-.010	.002	n.s.
PGM-1	.246	.134	.128	**
6PG-1	-.085	-.082	-.002	n.s.
6PG-2	-.503	-.508	.004	**
ADH-2	.000	-.004	.004	n.s.
MDH-1	-.447	-.451	.003	n.s.
CTO-1	-.107	-.127	.017	**
CTO-2	.653	.567	.198	**
PER-3	.628	.612	.042	**
Mean =	.085	.024	.063	
Jacknife estimates over loci:				
Mean =	.090	.023	.064	

[1]) WRIGHT, 1978; WEIR and COCKERHAM, 1984.
[2]) ** = significant at the 0.01 level.

Table 5. — NEI's (1973) statistics of genetic diversity for nine polymorphic loci in two populations of American beech.

Locus	H_T	H_S	D_{ST}	G_{ST}
6PG-1	0.145	0.145	0.000	0.000
6PG-2	0.483	0.481	0.001	0.003
ADH-2	0.007	0.007	0.000	0.004
CTO-1	0.640	0.633	0.006	0.010
CTO-2	0.656	0.582	0.074	0.112
MDH-1	0.633	0.632	0.001	0.002
PER-3	0.540	0.527	0.013	0.024
PGI-2	0.155	0.155	0.000	0.000
PGM-1	0.414	0.384	0.030	0.071
Mean[1]	0.408	0.394	0.014	0.034

[1]) Mean values do not include monomorphic loci.

Several life-history traits have been associated with high allozyme diversity, including large ranges, high fecundities, outcrossing modes of reproduction, wind pollination, long generation times, and occurrence in later successional phases (LOVELESS and HAMRICK, 1984). American beech exhibits all of these life-history traits, as well as the ability to regenerate vegetatively. This latter trait provides the species with additional flexibility to respond, both spatially and temporally, to environmental variation (and perturbation), and may thus allow it to maintain higher levels of individual heterozygosity than species which are limited to sexual reproduction alone (ELLSTRAND and ROOSE, 1987).

Estimates of fixation indices averaged over both stands are listed in *table 4*. F_{ST} values (avg. = 0.064) indicated moderate differentiation (WRIGHT, 1978) between the two populations, i.e., approximately 93 % of the observed variation resides within stands. This value is similar to those reported for most coniferous species (avg. = 0.076, data from GOVINDARAJU, 1988; avg. = 0.068, HAMRICK and GODT, 1990). CUGUEN et al. (1988) calculated an F_{ST} value of 0.060 for *Fagus sylvatica* L. based on analysis of three isozyme loci sampled in 250 natural stands. COMPS et al. (1990) reported a slightly lower value (F_{ST} = 0.054) for five isozyme loci in 99 European beech stands throughout central and southern Europe. A lower value (F_{ST} = 0.030) was reported for *Populus tremuloides* MICHX. (JELINSKI and CHELIAK, 1992), another clone-forming species, but one with several life history characteristics which are quite different from those of American beech.

F_{IT} (the deviation in genotype frequencies from those in a hypothetical population in which mating is random and no genetic structure is present), and F_{IS} (the deviation of heterozygote frequencies from those expected within stands) values were negative for several loci (most notably PGI-2, 6PG-1, 6PG-2, MDH-1, and CTO-1), although overall means were positive (*Table 4*). However, significant (and large) positive F_{IS} values for CTO-2 and PER-3 contributed, perhaps disproportionately, to the positive mean. A significant excess of heterozygotes was observed for several loci, as indicated above. This is in contrast to results noted in an isozyme analysis of population structure in *Quercus rubra* L. (SCHWARZMANN and GERHOLD, 1991), in which observed heterozygosities did not differ significantly from those expected under random mating, and in population studies of *Fagus sylvatica* L. (CUGUEN et al., 1988; COMPS et al., 1990), in which F_{IS} and F_{IT} values were generally positive, indicating a heterozygote deficit relative to panmixia. European beech is not a root-sprouting species, however, and differences between the two species in population genetic structure may reflect differences in their reproductive strategies which result in differing mating patterns and existing stand structures. If the positive mean F_{IT} and F_{IS} values observed for American beech in this study are valid, however, this implies an overall deficit of heterozygotes, perhaps caused by assortative mating between ramets of the same clone and/or near neighbors related by descent. The average estimate of F_{IT} was almost four times larger than the mean F_{IS}, indicating moderate genetic differentiation among stands.

NEI's (1978) statistics of genetic diversity (*Table 5*) were also calculated to analyze the distribution of allelic diversity. The average G_{ST} (coefficient of differentiation) value indicated that approximately 3.4 % of the observed variability was found between populations, less than that estimated by F_{ST}. This is similar to values computed for *Fagus sylvatica* L. (G_{ST} = 0.036 to 0.052; COMPS et al., 1991) and *Quercus petraea* LIEBL. (G_{ST} = 0.036; MÜLLER-STARK and ZIEHE, 1991), but somewhat lower than that reported for *Quercus macrocarpa* (G_{ST} = 0.076; SCHNABEL and HAMRICK, 1990).

Measurements of population genetic structure in a species where random mating among unique genets is assumed may lead to incorrect interpretation of results if a "mixed mode" of reproduction, including vegetative propagation, is operative (COOK, 1983). Stand data were thus adjusted to examine the effects of clone (ramet) numbers on the analyses, and to explore whether the inclusion of all ramet genotypes was biasing the analyses by skewing genotype frequencies. For this analysis, trees with identical genotypes were considered to be ramets of the same clone if they exhibited a linear separation (in any direction) of not more than 5 meters from an adjacent identical genome, and each clone so identified was counted as a single genotype. The mean sample size per locus (i. e., number of genotypes) was reduced by 29 % (to n = 123) in the MA stand, and by 60 % (to n = 61) in the WV stand, an indication of the more extensive clonal structuring in the latter stand as reflected by the genotype mapping.

The net effect of this reorganization of the data was to increase slightly (4 % to 5 %) the observed and estimated average heterozygosities per locus in the MA population (H_{obs} = 0.402, H_{exp} = 0.425). In the WV stand, both the direct count and the unbiased estimate estimates of heterozygosity increased by 3 % (H_{obs} = 0.403, H_{exp} = 0.395). The mean number of alleles per locus in each population remained the same, as did the percentages of loci which were polymorphic. NEI's unbiased genetic identity was unchanged.

Average values of F-statistics were generally increased by recognition of clonal entities. The F_{ST} value (a measure of population subdivision, or among-stand differentiation) was increased slightly (avg. = 0.061, + 6 %), as were F_{IT} values (a measure of overall inbreeding) (avg. = 0.096, + 7 %), while the average F_{IS} value (the inbreeding coefficient within populations) was increased the most (avg. = .032, + 39 %). For the limited population samples included in this report, it appears that clonal development may have a significant effect on some (but not all) of the statistics produced in the analyses.

Analyses completed thus far lend strong support to the concept that populations of American beech are comprised of well-developed genetic mosaics. A general concordance of (various) multi-locus isozyme genotypes with observed patterns of resistance and susceptibility to beech bark

disease reinforces the existence of clonal development as observed in both stands. The data also suggest that recognition of clonal structure may have a significant effect on the computation and interpretation of population genetic structure statistics. Spatial analysis of the effects of clonal structure on the organization of genetic variability in larger population data sets is in progress.

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