

## ALLOZYME FREQUENCIES IN NEW JERSEY AND NORTH CAROLINA POPULATIONS OF ATLANTIC WHITE-CEDAR, *CHAMAECYPARIS THYOIDES* (CUPRESSACEAE)<sup>1</sup>

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The present study was conducted to assess the distribution of genetic variation within and among populations of *Chamaecyparis thyoides* in both marginal and centrally located populations. Allozyme frequency analyses of ten loci from foliage of four New Jersey populations and two North Carolina populations of *C. thyoides* showed polymorphic loci = 50%, mean number of alleles per locus = 2.8, effective number of alleles per locus = 1.17, and expected heterozygosity = 0.14. Diversity was highest in two populations from southern New Jersey. The isolated population at High Point, New Jersey had only two polymorphic loci and expected heterozygosity of 0.03. There was no correlation between genetic and geographic distances among populations, implying that cedar must have possessed some means of long-distance dispersal at the end of the last glacial period, rather than advancing northward step by step.

**Key words:** allozyme; cedar swamp; *Chamaecyparis*; Cupressaceae; isozyme; white-cedar.

In developing sampling strategies for conservation efforts, or genetic improvement of tree species, it is useful to take into account overall geographic patterns of genetic differentiation (National Research Council, 1991). Such patterns may reveal localized pockets of diversity based either on relatively short-term dispersal patterns or more long-term migration patterns across several generations. Analysis of the current distribution of genetic variation within and among local populations of a species can shed light on the distribution of genetic diversity and can also help us to understand the historical processes underlying that genetic diversity (e.g., Schnabel, Hamrick, and Wells, 1993; Sheely and Meagher, 1995). Knowledge of patterns in the distribution of genetic diversity can also help us determine whether to focus on local stock materials or more geographically distant stock for reestablishment efforts, provenance trials, and breeding purposes.

Atlantic white-cedar, *Chamaecyparis thyoides* (L.)B.S.P. has been regarded as a possible candidate for restoration efforts. It is an obligate wetland species occupying swamps along the Atlantic and Gulf coasts of the United States. Most cedar swamps lie along the Coastal Plain from New Jersey southward, but a few are perched atop mountains at some distance inland, as at High Point, New Jersey (457 m elevation, 145 km inland). The largest natural areas containing cedar swamps

are in eastern North Carolina, southeastern New Jersey, and northwestern Florida. The western Florida population is sometimes regarded as a separate subspecies, *ssp. henryae* (Li, 1962; Little, 1966, 1979). This population extends into Alabama around Mobile Bay, and there are two isolated populations to the west in Mississippi (Fig. 1).

As the climate warmed at the end of the last glacial period ~12 000 yr ago, *C. thyoides* moved northward to occupy its present range. According to Belling (1977), its route of migration was along a low-lying area between the coastal plain and the Appalachian Mountains, and establishment at other sites, in the mountains and on the coastal plain, took place later. However, Laderman (1989) reports pollen evidence indicating white-cedar's presence on the New Jersey coastal plain 9500 and 7700 yr ago, vs. less than 300 yr ago at High Point; and Watts (1979) states that *C. thyoides* macrofossils (leaves, cones) at Helmetta Swamp (Monmouth county, NJ) date back to 10 000 yr ago.

Conkle (1992) reported that allozyme diversity in four major western conifers was greatest in the south, and less in populations that had migrated north to their present areas as the climate warmed after the Pleistocene. In 1995, Eckert sampled eight cedar swamps at the north end of cedar's range (New Hampshire and Maine, 20 trees/swamp), estimated heterozygosity in the species at 0.12 there, and suggested that it might be greater toward the center of the range. We were curious to find out whether *C. thyoides* would exhibit greater diversity southward, with lowest values at High Point, intermediate in southern New Jersey, and highest in North Carolina (indicating a possible glacial refugium).

There has been much recent interest in restoring white-cedar swamps, particularly in North Carolina and New Jersey (Summerville, 1995; Kuser, 1996). Production of propagules for planting cedar in areas lacking natural seedbanks is well underway; a substantial portion of this consists of rooted cuttings (Kuser and Zimmermann, 1995). A commercial nursery in North Carolina currently

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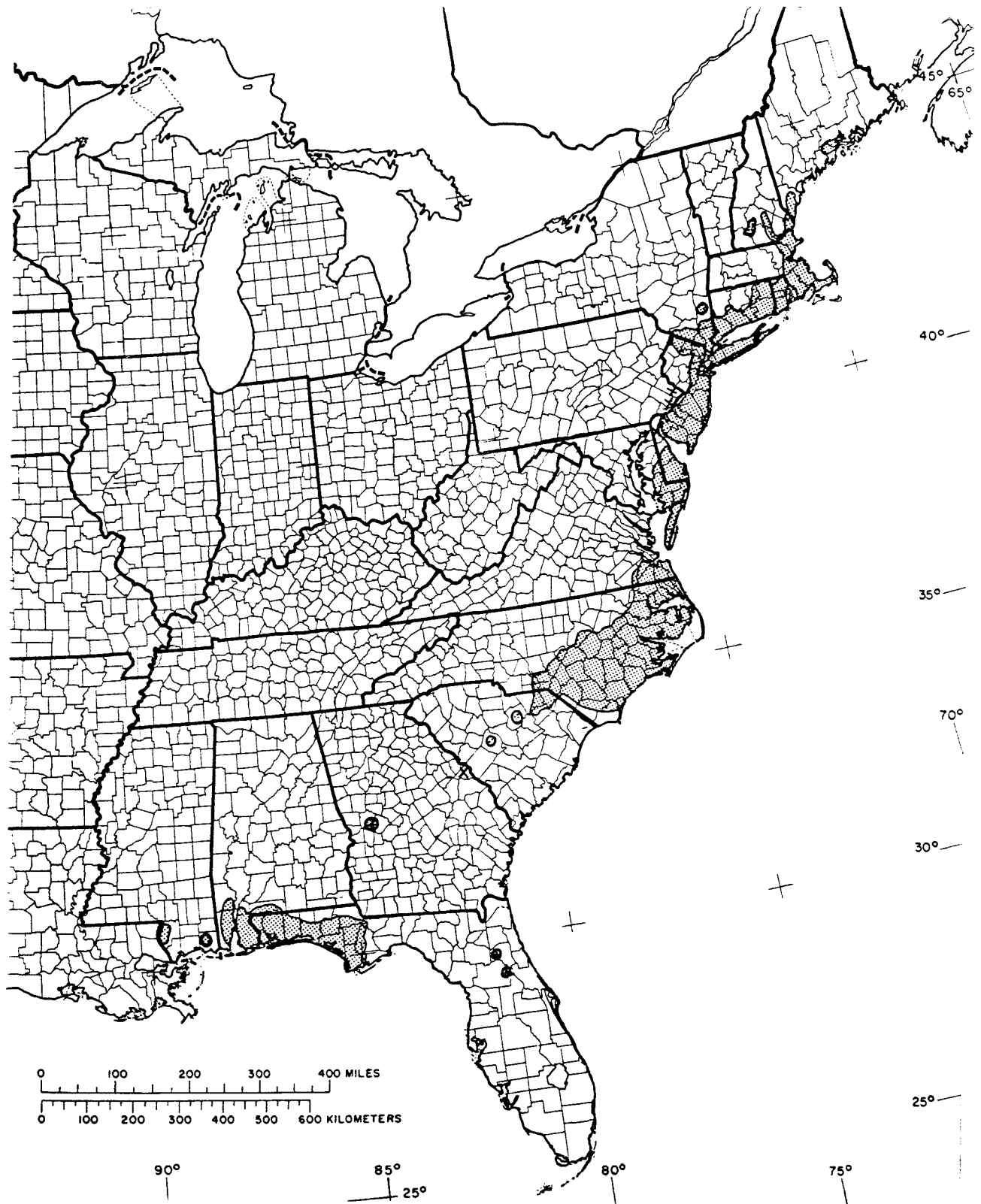


Fig. 1. Range of Atlantic white-cedar (Little, 1971).

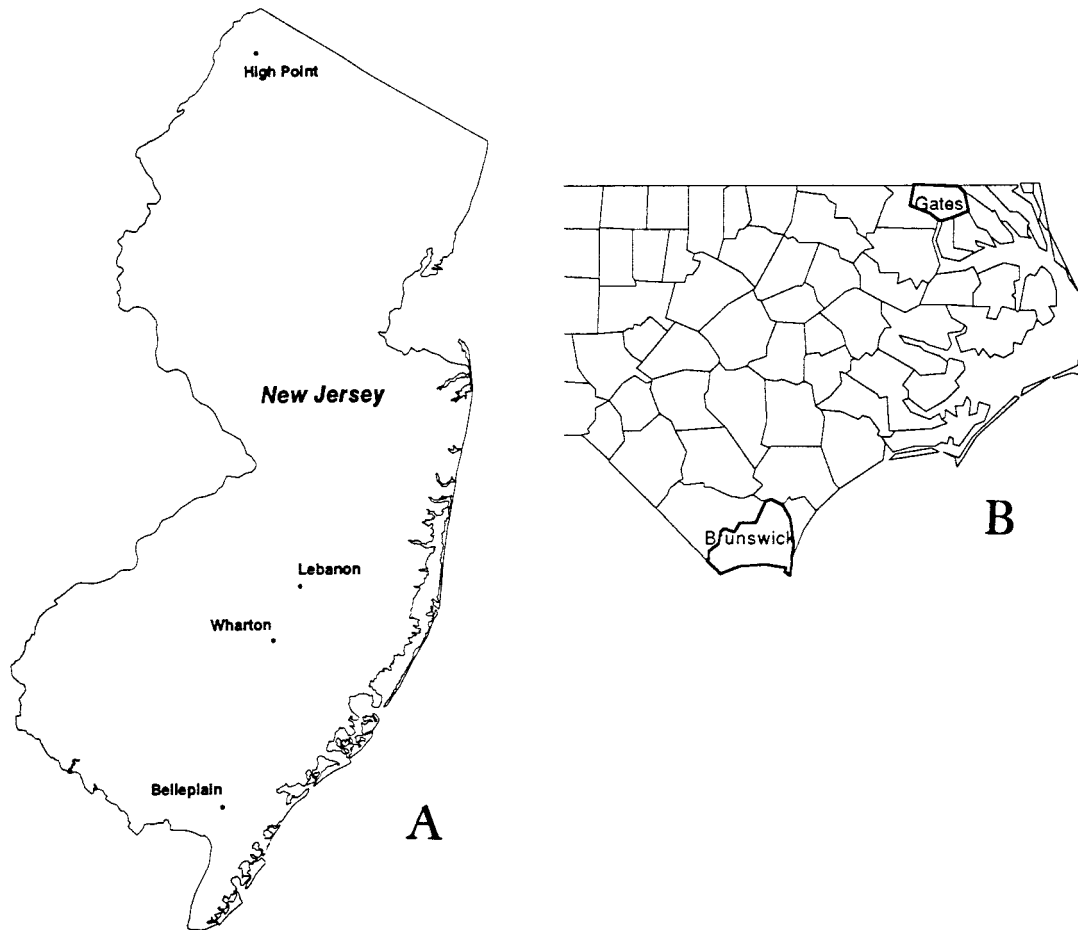


Fig. 2. Cedar swamps sampled in New Jersey (A) and North Carolina (B).

produces ~400 000 cuttings a year, (North Carolina, Delaware, and New Jersey genetic stock); and New Jersey's state nursery produces 20 000 cuttings a year. Before artificial planting of cedar swamps goes much further, we need to learn more about the genetic architecture of white-cedar: amount of genetic variation, degree of local adaptation, and how far propagules can be successfully moved from sites of origin. While answers to some of these questions await results of provenance tests underway in North Carolina and New Jersey (Kuser and Zimmermann, 1995), comparative amounts of variation found by allozyme studies of six populations in those states are reported in this paper. Thus we have: (1) measured overall levels of genetic variation in New Jersey and North Carolina populations, (2) assessed the relative distribution of genetic variation within and among populations, and (3) conducted a regression analysis of the relationship between patterns of genetic variation and presumed historical migration patterns, as reflected in current geographic distance, for populations of this species in New Jersey and North Carolina.

#### MATERIALS AND METHODS

**Sample sites**—We sampled six populations of *Chamaecyparis thuyoides*, four in New Jersey and two in North Carolina (Fig. 2). From each of the six populations, fresh foliage was collected from 40 trees during

the period November 1994–April 1995. Where cedar stands were extensive, as at Lebanon State Forest, New Jersey, several subpopulations 1–5 km apart were included in the 40-tree sample to insure good coverage and avoid vicinism. This was not possible at High Point where the cedar swamp covers only 16 ha, so we sampled as evenly as possible throughout the swamp. Trees were tagged and foliage samples were numbered, placed in small ziploc bags over ice in a picnic cooler, then transferred to the laboratory coldroom at 0°–5°C until processed. The North Carolina samples were packed in ice and sent to New Brunswick, New Jersey by next-day-delivery mail. Because we have been able to root cedar cuttings after several months of cold storage in ziplocs, we did not anticipate any loss in freshness.

**Sample collection and electrophoresis**—Population genetic surveys were based on horizontal starch gel electrophoresis performed on foliar samples (needles) ground in liquid nitrogen and prepared using the extraction buffer described by Mitton et al. (1979), as modified by Eckert (1995). Samples were subsequently evaluated for scorable allelic composition for the following allozymes: fluorescent esterase (FE-1, FE-2), glucose-6-phosphate dehydrogenase (G-6-PDH), glutamate oxaloacetate transaminase (GOT-1, GOT-2), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (PGD), and phosphogluco isomerase (PGI). Buffer systems and enzyme assays followed Wendel and Weeden (1989).

**Genetic analysis**—Standard measures of genetic variation were estimated, including percentage polymorphism, mean number of alleles per locus, effective number of alleles per locus, and expected hetero-

TABLE 1. Allozyme frequencies in six populations of *Chamaecyparis thyoides* in New Jersey and North Carolina.

Locus/ allele	Populations					
	New Jersey			North Carolina		
	High Point	Lebanon	Wharton	Belleplain	Gates	Brunswick
FE-1						
1		0.15		0.16	0.14	0.02
2	1.00	0.85	0.98	0.78	0.86	0.98
3			0.02	0.01		
4				0.09		
FE-2						
1		0.08	0.06	0.02	0.08	0.09
2	1.00	0.91	0.94	0.91	0.76	0.82
3		0.01		0.06	0.16	0.09
G-6-PDH						
1	0.04	0.19	0.20	0.20	0.08	0.22
2	0.95	0.41	0.80	0.75	0.91	0.78
3	0.01	0.40		0.04	0.01	
4				0.01		
GOT-1						
2	1.00	1.00	1.00	1.00	1.00	1.00
GOT-2						
1				0.01		
2	1.00	1.00	1.00	0.99	0.99	0.94
3					0.01	0.06
IDH						
1		0.05		0.05	0.01	
2	1.00	0.95	1.00	0.95	0.99	1.00
MDH						
1					0.01	
2	1.00	1.00	1.00	1.00	0.99	1.00
ME						
1		0.04		0.31	0.02	
2	0.91	0.72	0.94	0.59	0.62	0.90
3	0.09	0.24	0.06	0.10	0.35	0.10
3		0.01	0.01			
PGD						
1		0.04				
2	1.00	0.95	0.99	1.00	1.00	1.00
3		0.01	0.01			
PGI						
1	0.04	0.04	0.26	0.15	0.05	0.11
2	0.96	0.95	0.72	0.84	0.94	0.85
3		0.01	0.01	0.01	0.01	0.04

zygosity at the population and species levels (Hamrick and Godt, 1990). A locus was considered polymorphic if the most common allele was present at a frequency of 0.95 or less. For each of these statistics, we calculated both species-level and population-level estimates. Data from all six populations were pooled to calculate species-level estimates. To obtain population-level measures, estimates were calculated for each of the six populations and then averaged over populations. Wright's  $F$  statistics measuring the hierarchical distribution of genetic variance within and among populations (Wright, 1978) were estimated from variance components obtained from a nested ANOVA of gene frequencies (Weir, 1990). The fixation index,  $F_{ST}$ , measures the extent of differentiation among populations, often attributed to genetic drift and limited gene flow. The inbreeding coefficient,  $F_{IS}$ , measures the effects of nonrandom mating within populations. For example, in populations with high levels of selfing, a resulting high inbreeding coefficient is reflected by a high frequency of homozygotes. The overall departure from Hardy-Weinberg equilibrium over all populations is reflected by  $F_{IT}$ . Finally, isolation

TABLE 2. Measures of genetic diversity for *Chamaecyparis thyoides* at the species and population levels and for each population. P = percentage polymorphic loci, A = mean number of alleles per locus,  $A_e$  = effective number of alleles per locus, and  $H_e$  = expected heterozygosity.

	P	A	$A_e$	$H_e$
Species level	50.0	2.80	1.17	0.145
Population level	48.3	1.97	1.15	0.128
High Point State Park, NJ	20.0	1.40	1.03	0.033
Lebanon State Forest, NJ	70.0	2.20	1.25	0.200
Wharton State Forest, NJ	40.0	1.80	1.11	0.099
Belleplain State Forest, NJ	60.0	2.40	1.22	0.180
Gates County, NC	50.0	2.20	1.15	0.133
Brunswick County, NC	50.0	1.80	1.14	0.125

by distance measures as defined by Slatkin (1985) were used to determine the correlation between population proximity and genetic similarity. To do that, estimates of gene flow between pairs of populations were calculated using Slatkin's (1993) method. Those estimates were plotted against geographic distances separating the population pairs, for which regression analysis was performed.

RESULTS

Allozyme frequencies in the six populations are shown in Table 1. Among the ten loci analyzed, GOT-1 was monomorphic in all six populations; all other loci contained 3–10 genotypes (1–4 alleles) in one population or another. The High Point, New Jersey population was monomorphic at eight loci; G-6-PDH yielded three alleles and ME yielded two alleles. Three populations possessed unique alleles: only Gates had MDH allele 1, only Lebanon had PGD allele 1, and only Belleplain had G-6-PDH allele 4 and FE-1 allele 4. Both North Carolina populations had GOT-2 allele 3; the four New Jersey populations were without this allele.

Percentage polymorphic loci, mean number of alleles per locus, number of effective alleles per locus, and expected heterozygosity were greatest at Belleplain State Forest, NJ and Lebanon State Forest, NJ, intermediate at Brunswick and Gates counties, NC and Wharton State Forest, NJ, and lowest at High Point State Park, NJ (Table 2). Different loci show varying amounts of inbreeding and differentiation among populations (Table 3). Positive estimates of  $F_{IT}$  and  $F_{IS}$  indicate nonrandom mating of individuals relative to the total population and to their subpopulations, respectively. The small estimate of  $F_{ST}$  shows little genetic differentiation, with only 9% of total

TABLE 3. Hierarchical  $F$  statistics analysis of population genetic structure for *Chamaecyparis thyoides* from six populations: four in New Jersey and two in North Carolina.

Locus	$F_{IT}$	$F_{ST}$	$F_{IS}$
FE-1	0.076	0.076	0.000
FE-2	0.103	0.049	0.057
G-6-PDH	0.354	0.159	0.233
GOT-2	0.030	0.230	0.000
ME	0.543	0.089	0.499
PGD	0.019	0.019	0.000
PGI	0.109	0.060	0.052
Jackknife estimate	0.276	0.094	0.200
SD	0.097	0.024	0.099

TABLE 4. Estimates of gene flow between all population pairs of *Chamaecyparis thyoides* in New Jersey and North Carolina calculated by Slatkin's (1993) method, and geographic distances (km) separating all population pairs. Be = Belleplain State Forest, NJ; Br = Brunswick County, NC; Ga = Gates County, NC; Hp = High Point State Park, NJ; Le = Lebanon State Forest, NJ; and Wn = Wharton State Forest, NJ.

Population pairs	Gene flow	Geographic distance (km)
Le/Wn	8.05	27
Be/Wn	9.78	58
Be/Le	10.6	85
Hp/Le	3.59	166
Hp/Wn	9.40	188
Be/Hp	5.71	242
Br/Ga	26.6	310
Be/Ga	15.2	350
Ga/Wn	10.4	410
Ga/Le	6.45	425
Ga/Hp	11.2	563
Be/Br	11.9	655
Br/Wn	54.3	715
Br/Le	8.36	730
Br/Hp	13.4	868

allozyme variation found among populations. Table 4 contains calculated estimates of gene flow between all population pairs and the geographic distances separating them. Somewhat surprisingly, genetic resemblance and geographic proximity do not appear to be significantly related in this study, as shown by the positive slope and nonsignificance [ $P (>F) = 0.20$ ] of the regression in Fig. 3.

#### DISCUSSION

Expected heterozygosity in *C. thyoides* varied from 0.20 to 0.03 in the six populations sampled. It was lowest in the outlying swamp at High Point, New Jersey, highest in two of the three populations in southern New Jersey, and intermediate in the third southern New Jersey population and the two North Carolina populations. The lack of correlation of geographic and genetic distances between swamps suggests that cedar had some means of long-distance dispersal.

Based on the six populations, the expected heterozygosity of *C. thyoides* at the species level is 0.145, close to the 0.133 found by Millar and Marshall (1991) in *C. lawsoniana*, and higher than the 0.094 in *Thuja occidentalis* (Perry, Knowles, and Yeh, 1990) or 0.04 in *T. plicata* (Yeh, 1988). At the population level, our 0.128 is slightly higher than the 12% reported by Eckert (1997), who suggested that heterozygosity might be greater toward the center of the range than at the northern edge, which he sampled. We had thought that the same might be true of heterozygosity levels in New Jersey vs. North Carolina, i.e., that heterozygosity would be greater in North Carolina (where the largest cedar-swamp area is located), but this was not the case. It would be interesting to know the heterozygosity levels in the western Florida-Alabama-Mississippi area populations of *C. thyoides*, and see whether they indicate a possible cedar refugium in the Pleistocene possessing genes that did not travel north when the ice thawed.

Allozyme frequency analyses of the isolated mountaintop population at High Point, New Jersey show that cedar in

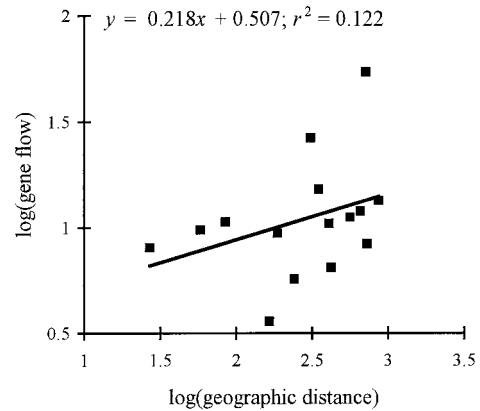


Fig. 3. Log (gene flow) by log (geographic distance) plotted for all pairs of populations of *Chamaecyparis thyoides* (L.)B.S.P.

this swamp is relatively genetically depauperate and has probably been through a bottleneck; it may have been founded by one or a very few trees. Allele 3 of G6PDH (the only locus having more than two alleles) was found in only one of 40 trees, and could have blown in on pollen carried by a south or east wind from the main coastal range. With High Point's expected heterozygosity at only 0.03, one might wonder whether any inbreeding depression could be present. We did not find any in seed viability or germination in an earlier experiment; it was one of the more vigorous populations in those respects in our 1992 tests (Boyle and Kuser, 1994). It may or may not be coincidental that some of the most vigorous stands of cedar in New Jersey are in Lebanon State Forest, where we found the populations with highest heterozygosity levels.

We think that the lack of correlation between geographic and genetic distances among populations means that cedar did not advance northward up the Atlantic coast swamp-by-swamp after the last glaciation. The wind-dispersed seeds may be carried as far as 1.6 km from the top of a 21-m tree by a 48 km/h wind (Korstian and Brush, 1931). If cedar had moved north this maximum distance once each generation (70 yr), it would have taken nearly 25000 yr to travel from Gates County, North Carolina to High Point, New Jersey and ~50000 yr to reach its current northern limit in Maine, or if it had started from Brunswick County, 50% longer. It is more likely that cedar possessed some means of long-distance dispersal such as a bird species, which may have used swamps and fed on the small cones or seeds. Water or snowpack dispersal may be additional methods at the local level in New England (Eckert, 1997), and farther south water dispersal is considered likely (R. C. Kellison, personal communication, North Carolina State University).

Perhaps cedar did not have to travel from a Carolina glacial refugium to occupy the northern part of its present range. The existence of highest heterozygosity levels in New Jersey, rather than North Carolina, implies that cedar's refugium might have been offshore of New Jersey on the wide continental shelf now covered by a shallow sea (USDC, 1972), or perhaps under present-day Chesapeake Bay. At sea levels 100–125 m lower than today's there could have been room for extensive cedar swamps. On the other hand, North Carolina (Cape Hatteras) lies

close to the edge of the continental shelf, and a 100–125 m drop in sea level might have drained and dissected the present swampy areas in the eastern part of that state without creating new swamps on the steeper continental slope. Reconstructions of full-glacial climate have estimated summer water temperatures along the Atlantic coast to have been 8°C colder than now. This would have made the ocean off Delaware Bay about as cool then as that off the Maine coast today, where white-cedar reaches the northern end of its present range (CLIMAP 1981). An alternate, warmer refugium might have been the continental shelf from south of Cape Hatteras to offshore of present-day South Carolina and Georgia.

Our results suggest that the genetic stock of *C. thyoides* to be used for cedar swamp restoration on the New Jersey coastal plain should come from a source such as Lebanon State Forest, which is centrally located in the area and has the highest heterozygosity of the six stands sampled. For North Carolina, either Gates or Brunswick County stock could be used for local planting. Because North Carolina's original cedar swamp area was three to four times as large as New Jersey's (Fig. 1), more thorough sampling should be done there (as well as the rest of the southern range). More detailed recommendations await results of provenance tests underway in both states.

#### LITERATURE CITED

- BELLING, A. J. 1977. Postglacial migration of *Chamaecyparis thyoides* (L.) B.S.P. (southern white cedar) in the northeastern United States. Ph.D. dissertation, New York University, New York, NY.
- BOYLE, E. D., AND J. E. KUSER. 1994. Propagation of Atlantic white-cedar by seed and cuttings in New Jersey. *Tree Planters' Notes* 45: 104–111.
- CLIMAP. 1981. CLIMAP Project Members, 1981. Seasonal Reconstructions of the Earth's Surface at the Last Glacial Maximum. Geological Society of America Map Chart Series MC-36. Geological Society of America, Boulder, CO.
- CONKLE, M. T. 1992. Genetic diversity—seeing the forest through the trees. *New Forests* 6: 5–22.
- ECKERT, R. T. 1997. Population genetic analysis of *Chamaecyparis thyoides* in New Hampshire and Maine. In A. Laderman [ed.], *Coastally restricted forests*. Oxford University Press, New York, NY. In press.
- HAMRICK, J. L., AND M. J. GODT. 1990. Allozyme diversity in plant species. In A. H. D. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir. [eds.] *Plant population genetics, breeding, and genetic resources*. Sinauer, Sunderland, MA.
- KORSTIAN, C. F., AND W. D. BRUSH. 1931. Southern white cedar. Technical Bulletin 251, United States Forest Service, Washington, DC.
- KUSER, J. E. 1996. Artificial regeneration of cedar swamps. Atlantic White-cedar Restoration Conference, June 25, 1996, Stockton State College, Pomona, NJ. NJ Water Resources Research Institute, Rutgers University, Piscataway, NJ.
- , AND G. ZIMMERMAN. 1995. Restoring Atlantic White-cedar swamps: a review of techniques for propagation and establishment. *Tree Planters' Notes* 46: 78–85.
- LADERMAN, A. D. 1989. The ecology of Atlantic white cedar wetlands: a community profile. U.S.D.I. Fish and Wildlife Service R & D, National Wetlands Research Center, Washington, DC.
- LI, H. 1962. A new species of *Chamaecyparis*. *Bulletin of the Morris Arboretum* 13: 43–46.
- LITTLE, E. L. 1966. Varietal transfers in *Cupressus* and *Chamaecyparis*. *Madroño* 18: 161–167.
- . 1971. Atlas of United States Trees, vol. 1. Conifers and Important Hardwoods. Miscellaneous Publication No. 1146 United States Forest Service, Washington, DC.
- . 1979. Checklist of United States trees (native and naturalized). Handbook, no. 541, United States Forest Service, Washington, DC.
- MILLAR, C. I., AND K. A. MARSHALL. 1991. Allozyme variation of Port-Orford-Cedar (*Chamaecyparis lawsoniana*): Implications for genetic conservation. *Forest Science* 37: 1060–1077.
- MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.
- NATIONAL RESEARCH COUNCIL. 1991. Managing global genetic resources: forest trees. National Academy Press, Washington, DC.
- PERRY, D. J., P. KNOWLES, AND F. C. YEH. 1990. Allozyme variation of *Thuja occidentalis* L. in northwestern Ontario. *Biochemical Systematics and Ecology* 18: 111–115.
- SCHNABLE, A., J. L. HAMRICK, AND P. V. WELLS. 1993. Influence of quaternary history on the population genetic structure of Douglas fir (*Pseudotsuga menziesii*) in the great basin. *Canadian Journal of Forest Research* 23: 1900–1906.
- SHEELY, D. L., AND T. R. MEAGHER. 1996. Genetic diversity in Micronesian island populations of the tropical tree *Campnosperma brevipetiolata* (Anacardiaceae). *American Journal of Botany* 83: 1571–1579.
- SLATKIN, M. 1985. Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16: 393–430.
- . 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* 47: 264–279.
- SUMMERVILLE, K. O. 1995. Atlantic white-cedar provenance study in North Carolina. Proceedings of Current Developments with White-cedar Management, Aug. 1–3, 1995, Washington, NC. North Carolina Division of Forest Resources, Raleigh, NC.
- USDC. 1972. United States Department of Commerce, National Oceanic and Atmospheric Administration, National Oceanic Service, Coast and Geodetic Survey, Washington, DC.
- WATTS, W. A. 1979. Late quaternary vegetation of central Appalachia and the New Jersey coastal plain. *Ecological Monograph* 49: 427–469.
- WEIR, B. S. 1990. Genetic data analysis. Sinauer, Sunderland, MA.
- WENDEL, J. F., AND N. F. WEEDEN. 1989. Visualization and interpretation of plant isozymes. In D.E. Soltis and P.S. Soltis [eds.], *Isozymes in plant biology*, 5–45. Dioscorides Press, Portland, OR.
- WRIGHT, S. 1978. Evolution and the genetics of populations: variability within and among natural populations, vol. 4. University of Chicago Press, Chicago, IL.
- YEH, F. C. 1988. Isozyme variation of *Thuja plicata* (Cupressaceae) in British Columbia. *Biochemical Systematics and Ecology* 16: 373–377.