Allozyme Variation in Natural Populations of Hinoki, *Chamaecyparis obtusa* (SIEB. *et* ZUCC.) ENDL. and Its Comparison with the Plus-trees Selected from Artificial Stands

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Summary

Allozyme variation in 11 natural populations of hinoki, *Chamaecyparis obtusa* (SIEB. *et* ZUCC.) ENDL. was studied using 10 loci, and compared with that in 12 groups of hinoki plus-trees selected from artificial stands in the national project for plus-tree selection breeding. In the natural populations, it was evident that most of the genetic variation was retained within populations, and population differentiation was low, as observed in many other gymnosperms. However, there were genetic clines in allele frequencies at 4 loci, *G6p*, *Pod*, *Got-1* and *Pgm* along the geographic locations of the populations. The populations were mostly separated according to the locations using two clustering methods based on genetic distances among populations. Comparison of allozyme variation between the natural populations and plus-tree groups revealed that generally the variation in the natural populations was preserved even in the plus-tree groups. However, genetic differences were observed between the natural populations and the plus-tree groups in the presence or absence of rare alleles, the mean frequencies of alleles, and the distribution of allele frequencies across the natural populations or the plus-tree groups.

Key Words: Isozyme, *Chamaecyparis obtusa*, genetic variation, genetic differentiation, breeding material, genetic resources.

Introduction

Hinoki, *Chamaecyparis obtusa* (SIEB. *et* ZUCC.) ENDL., Cupressaceae which is native to Japan is one of the most important tree species in Japanese forestry. The current natural forests are scattered from Iwaki City in Fukushima Prefecture (37° 10' N) to Yaku Island (30° 15' N), mostly in areas with a small amount of snowfall and hinoki trees occur as small populations except for the forests in the Kiso area (Hayashi 1960). Hinoki wood is economically valuable due to its high quality for the production of housing timber such as pillars and beams. As the demand for timber production increased, the natural forests were rapidly depleted in particular after World War II. As a result, there are no natural

forests of hinoki on Kyushu Island at present.

Shiraishi *et al.* (1987) studied six natural populations and eleven artificial stands of hinoki with allozymes at two loci, *Pod* and *Got*. They reported that there was a genetic cline in allele frequencies at the *Got* locus in the natural populations, while no differentiation was observed among the artificial stands. Uchida *et al.* (1991) examined the genetic variation in natural populations of hinoki using 13 allozyme loci. However, the populations studied were limited and consisted of only three small populations in Shizuoka Prefecture. Hitherto no allozyme studies on natural populations across the entire distribution of the species had been carried out.

In the national project for plus-tree selection breeding in Japan, plus-trees of hinoki were selected from 4 basic breeding regions, which were further subdivided into several breeding districts. The plus-trees, being selected mainly from artificial stands, have been propagated by grafting to establish seed orchards in each breeding district. The seeds produced in the seed orchards are being used for practical reforestation. Meanwhile, plus-trees will be used as crossing parents for advanced breeding generations. For the promotion of advanced breeding programs, it is important to evaluate genetic differences among the plus-trees in different breeding districts. Therefore, genotypes for 11 allozyme loci were identified in the plus-trees selected from artificial stands, and genetic differences were examined among the 12 groups composed of the plus-trees from different breeding districts (Uchida *et al.* 1993). These groups were genetically homogeneous among the breeding districts.

In this report, using allozymes as genetic markers, we studied the genetic variation in natural populations of *C. obtusa* across most of its natural distribution and compared it with that in the plus-trees. Particularly, we examined whether the genetic variation preserved in the natural populations was retained in the plus-tree groups or not.

Materials and Methods

Samples

We studied eleven natural populations of hinoki, which were designated based on their location in this study: Iwaki, Imaichi, Chichibu, Hijiri, Ikawa, Kiso, Owase,

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Yamazaki, Yanase, Shiragayama and Yakushima (Fig. 1). Eight out of the 11 populations, Iwaki, Imaichi, Kiso, Owase, Yamazaki, Yanase, Shiragayama and Yakushima were sampled from national forests; other two populations, Chichibu and Ikawa were sampled from Tokyo and Tsukuba University Forests, respectively; and the last one, Hijiri was sampled from the forest belonging to Tokai Pulp Forest Co. Ltd. Kiso population was, however, generated by one-year-old seedlings grown from the seeds gathered in 564 seed traps in seven hinoki stands in the Kiso area, and in total 564 seedlings that were randomly selected (one per seed trap) were analyzed. The leaf samples were collected during the winter season and stored in plastic bags at -80°C prior to isozyme analysis. Sample size ranged from 34 for the Yakushima population to 564 for the Kiso population with a mean number of 109.2 trees (Table 1).

Electrophoresis

Enzyme extraction, electrophoresis and staining methods were the same as those described in the reports of Tsumura *et al.* (1990) and Uchida *et al.* (1991). All the samples were analyzed for 9 enzyme systems and scored for a total of 11 loci as follows: 6-Phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44), *6Pg-1* and *6Pg-2*; glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), *G6p*; glutamate dehydrogenase (GDH,

EC1.4.1.2), *Gdh*; peroxidase (POD, EC 1.11.1.7), *Pod*; glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1), *Got-1* and *Got-2*; glucokinase (GK, EC 2.7.1.2), *Gk*; phosphoglucomutase (PGM, EC 2.7.5.1), *Pgm*; esterase (EST, EC 3.1.1), *Est*; and leucine aminopeptidase (LAP, EC3.4.11.1), *Lap*. However, *Est* locus was excluded through all the following statistical analyses since a null allele was frequently observed which hampered the correct estimation of allele frequencies.

Statistical analysis

Allele frequencies in each natural population were estimated for the 10 loci encoding the 8 enzymes. The following parameters were used to quantify the amount of genetic variation within populations: 1) the proportion of polymorphic loci (95% criterion); 2) the effective number of alleles per locus (Kimura and Crow 1964); 3) the average heterozygosity observed; and 4) gene diversity (Nei 1973, 1978). Gene diversity was defined as follows: the probability that two randomly chosen alleles from a population are different.

Genetic variation between populations was estimated by the following parameters. 1) Gene diversity: $H_T = H_S + D_{ST}$; $G_{ST} = D_{ST}/H_T$ (Nei 1973), where H_T is the gene diversity in the total population, H_S and D_{ST} are the average gene diversities within and between populations, respectively, and G_{ST} is the relative extent of gene dif-

Fig. 1. Locations of 11 natural populations of *C. obtusa* studied.

ferentiation among populations. The statistical significance of the deviation of G_{ST} from zero was tested using χ^2 (Workman and Niswander 1970). 2) Nei's genetic identity and distance (Nei 1972, 1978). In the following, two dendrograms were constructed using two clustering methods, namely the UPGMA method (unweighted pair-group method with arithmetic mean; Sneath and Sokal 1973) and the NJ method (neighbor-joining method; Saitou and Nei 1987) based on the genetic distances. The UPGMA method is the most widely applied method to electrophoretic data. However, if there are bottleneck effects or inbreeding effects on genetic distances, the UPGMA method may lead to the formation of an erroneous dendrogram (Nei 1987). Nei (1987) recommended that in this case the NJ method should be selected. Furthermore, in order to identify relationships between genetic variation in populations and the latitudinal or longitudinal gradients, significance tests in linear regression (t -test) were performed for the regression coefficients of allele frequencies and H_S against latitude and longitude in the 11 populations (Table 1).

For a comparison of the genetic constitution between 11 natural populations in this study and 12 plus-tree groups of hinoki of which data were cited from a separate report (Uchida *et al.* 1993), we used heterogeneity tests in mean allele frequencies at polymorphic loci between natural populations and plus-tree groups, and principal component analysis based on a variance-covariance matrix of the most common alleles frequencies at each locus (SAS Institute 1985).

Results

Allele frequencies at four loci, *G6p*, *Pod*, *Got-1* and *Pgm*

were more heterogeneous among populations than those at the other loci. Seven alleles, *a* and *b* at *G6p*, *a* and *b* at *Got-1*, and *a*, *b* and *c* at *Pgm*, were significantly related to the latitude and longitude at least at $P < 0.05$ in the regression analysis. In addition, *a* and *b* at *Pod* were also significantly related only to the longitude at $P < 0.05$. Thus, there were apparently genetic clines in allele frequencies at the four loci along geographic locations for the 11 populations (Fig. 2).

Three loci, *6Pg-1*, *Gdh* and *Got-2* were monomorphic in all the populations (95% criterion). The other loci, *6Pg-2*, *G6p*, *Pod*, *Got-1*, *Gk*, *Pgm* and *Lap* were polymorphic in most of the populations. As an average across all populations, the proportion of polymorphic loci was 55.5%; the effective number of alleles per locus was 1.34; and the average gene diversity within population (H_S) was 0.202 (Table 1). The H_S values of each population tended to be lower in populations at more eastern locations; the tendency was significant at $P < 0.05$ when the regression coefficient of H_S against longitude was tested for 11 populations. The gene diversity within species (the total population; H_T) was 0.212, which was calculated for all the 10 loci. Both the mean G_{ST} values at all the 10 loci and only at seven polymorphic loci except for *6Pg-1*, *Gdh* and *Got-2* loci were 0.045 (Table 1), indicating that 4.5% of the total diversity maintained in the species was due to the genetic differences among populations and the remaining was maintained within populations. However, the deviation from zero for the mean value of G_{ST} at the polymorphic loci was significant at $P < 0.001$. Among the loci analyzed, *Got-1* and *Pgm* showed higher values of G_{ST} .

Analysis of the genetic identity and distance revealed the presence of small genetic differences among the 11 populations, as indicated by G_{ST} (Table 2). Based on the

Table 1. Comparison of genetic variation in natural populations with that in plus-tree groups of *C. obtusa*

	Latitude (N)	Longitude (E)	Sample size	Within-population				Within-species	Among-population
				Proportion of polymorphic loci	Effective no. of alleles per locus	Observed heterozygosity	Gene diversity (H_S)	Gene diversity (H_T)	G_{ST}
Natural population									
Iwaki	37°06′	140°50′	67	40.0	1.21	0.126	0.127		
Imaichi	36°44′	139°47′	70	50.0	1.34	0.214	0.201		
Chichibu	35°57′	138°47′	92	60.0	1.29	0.178	0.177		
Hijiri	36°25′	138°09′	108	60.0	1.31	0.172	0.190		
Ikawa	36°20′	138°14′	50	50.0	1.31	0.189	0.186		
Kiso	35°47′	137°38′	564	60.0	1.30	0.187	0.191	0.212	0.045
Owase	34°12′	136°08′	55	70.0	1.36	0.236	0.225		
Yamazaki	35°14′	134°29′	54	60.0	1.44	0.255	0.245		
Yanase	33°38′	134°05′	53	50.0	1.35	0.209	0.214		
Shiragayama	33°48′	133°36′	54	60.0	1.50	0.236	0.260		
Yakushima	30°18′	130°33′	34	50.0	1.39	0.231	0.207		
Mean			109.2 (1201) ¹⁾	55.5	1.34	0.203	0.202		
Plus-tree groups ²⁾									
			73.2 (878)	56.7	—	0.187	0.196	0.198	0.009

¹⁾ Total sample size in parentheses.

²⁾ Uchida *et al.* (1993).

Fig. 2 Allele frequencies at four allozyme loci in 11 natural populations and 12 plus-tree groups of *C. obtusa*. Histograms of each population and plus-tree group are roughly arranged in geographical location order from southwest to northeast.

Table 2. Genetic identity (above diagonal) and distance (below diagonal) among 11 natural populations of *C. obtusa*

Population	Iwaki	Imaichi	Chichibu	Hijiri	Ikawa	Kiso	Owase	Yamazaki	Yanase	Shiragayama	Yakushima
Iwaki	—	0.992	0.997	0.996	0.994	0.989	0.992	0.977	0.988	0.964	0.965
Imaichi	0.008	—	0.999	0.998	1.000	0.987	0.996	0.985	0.991	0.983	0.967
Chichibu	0.003	0.001	—	0.998	1.000	0.988	0.994	0.979	0.988	0.972	0.960
Hijiri	0.004	0.002	0.002	—	0.999	0.992	0.995	0.986	0.990	0.981	0.973
Ikawa	0.006	0.000	0.000	0.001	—	0.990	0.995	0.983	0.991	0.979	0.965
Kiso	0.011	0.013	0.012	0.008	0.010	—	0.993	0.992	0.992	0.981	0.983
Owase	0.008	0.004	0.006	0.005	0.005	0.007	—	0.993	1.000	0.987	0.979
Yamazaki	0.024	0.015	0.022	0.014	0.017	0.008	0.007	—	0.995	0.999	0.998
Yanase	0.012	0.009	0.012	0.010	0.009	0.008	0.000	0.000	—	0.988	0.982
Shiragayama	0.037	0.017	0.029	0.019	0.021	0.019	0.013	0.001	0.012	—	0.990
Yakushima	0.036	0.033	0.040	0.028	0.036	0.018	0.022	0.002	0.018	0.010	—

Fig. 3 UPGMA (above) and NJ (below) dendrograms using unbiased genetic distances among population of *C. obtusa*.

genetic distances, two dendrograms were constructed using the UPGMA and NJ methods (Fig. 3). The topology was roughly similar between the two dendrograms. In general, the populations were clustered according to the geographic locations.

Table 3 shows the mean allele frequencies at the 10 loci in the 11 natural populations and 12 groups of hinoki plus-trees (Uchida *et al.* 1993). We detected a total of 27 alleles in the natural populations, while in the plus-trees, 21 alleles were observed and seven alleles were absent. These seven alleles were detected as rare

alleles with frequencies less than 0.01 mostly in the Kiso population with the largest sample size of 564 individuals (Table 1). Heterogeneity tests in mean allele frequencies between natural populations and plus-tree groups showed that the frequencies at loci, *G6p*, *Got-1*, *Pgm* and *Lap* were significantly different between them (Table 3).

In the principal component analysis of the 11 natural populations and the 12 plus-tree groups using the frequencies of the most common alleles at the 9 loci except for *Gdh* since the locus was fixed, the first and second principal components accounted for 51.7% and 18.2% of the total variance, respectively. The values of eigen vectors of the alleles at the *Pgm*, *Got-1*, *G6p* and *Pod* loci in the first principal component were higher than those at the other loci. In addition, the values of eigen vectors at the *G6p*, *Got-1* and *6Pg-2* loci in the second principal component were higher than those at the other loci. In the loci except for *6Pg-2*, the alleles showed clinal variation in the natural populations (Fig. 2). In the scatter diagram (Fig. 4), the Kiso and Owase populations were plotted near the origin, and other plus-tree groups and Yanase population were plotted near the two populations of Kiso and Owase. Meanwhile, the other 8 natural populations were scattered peripherally around the plus-tree groups.

Discussion

Allozyme variation in natural populations

Despite the present natural distribution of hinoki in scattered small areas, hinoki displays relatively high levels of allozyme variation within species and within populations. For the averages in the 11 hinoki populations, the values of the effective number of alleles per locus, the average genetic diversity within populations (H_S) and within species (H_T) were higher than average values (1.20, 0.151 and 0.169, respectively) for many other gymnosperms (Hamrick *et al.* 1992). Hamrick *et al.* (1992) concluded that levels of within-population and within-species variation in long-lived tree species were partially explained by the geographic ranges. The values of these three parameters for hinoki were higher than

Table 3. Mean allele frequencies at 10 allozyme loci in 11 natural populations and 12 plus-tree groups of *C. obtusa*, and heterogeneity tests of frequencies between them

Locus	Allele	Mean (range) of frequency				P
		Natural population		Plus-tree group ¹⁾		
6Pg-1	a	1.000	(0.997–1.000)	1.000		
	b	<0.001	(0.000–0.003)	ND ²⁾		
6Pg-2	a	0.568	(0.456–0.676)	0.603	(0.500–0.700)	0.057
	b	0.432	(0.320–0.544)	0.397	(0.300–0.500)	
	c	<0.001	(0.000–0.004)	ND		
	d	<0.001	(0.000–0.001)	ND		
G6p	a	0.302	(0.074–0.460)	0.256	(0.158–0.349)	<0.01
	b	0.691	(0.540–0.912)	0.741	(0.643–0.906)	
	c	0.007	(0.000–0.022)	0.003	(0.000–0.015)	
	d	<0.001	(0.000–0.001)	ND		
Gdh	a	1.000		1.000		
Pod	a	0.818	(0.613–0.985)	0.836	(0.639–0.913)	0.230
	b	0.182	(0.015–0.387)	0.164	(0.087–0.361)	
Got-1	a	0.257	(0.090–0.485)	0.186	(0.149–0.230)	<0.001
	b	0.743	(0.515–0.910)	0.814	(0.770–0.851)	
Got-2	a	1.000		0.995	(0.983–1.000)	
	b	ND		0.005	(0.000–0.017)	
GK	a	0.958	(0.891–1.000)	0.953	(0.932–1.000)	0.562
	b	0.042	(0.000–0.109)	0.047	(0.000–0.068)	
Pgm	a	0.773	(0.594–0.932)	0.736	(0.613–0.790)	<0.001
	b	0.169	(0.068–0.292)	0.228	(0.170–0.355)	
	c	0.059	(0.000–0.172)	0.036	(0.000–0.074)	
Lap	a	0.970	(0.926–1.000)	0.986	(0.957–1.000)	<0.05
	b	0.005	(0.000–0.037)	0.001	(0.000–0.008)	
	c	0.022	(0.000–0.065)	0.013	(0.000–0.043)	
	d	0.001	(0.000–0.009)	ND		
	e	0.001	(0.000–0.010)	ND		
	o	0.001	(0.000–0.009)	ND		
Total unnumber of alleles	28		27		21	

¹⁾ Uchida *et al.* (1993).²⁾ Not detected.

average values for species with narrow-geographic ranges but almost similar to those of species with regional-geographic ranges (Hamrick *et al.* 1992). In addition, the respective values of these three parameters for other species in the genus *Chamaecyparis* and a Japanese conifer, *Cryptomeria japonica* were as follows. For the effective number of alleles per locus, *C. formosensis* and *C. taiwanensis* (Lin *et al.* 1994) had values of 1.10 and 1.07, respectively; *C. japonica* (Tomaru *et al.* 1994), 1.33. For H_S , *C. lawsoniana* (Millar and Marshall 1991) had a value of 0.15; *C. formosensis* and *C. taiwanensis*, 0.087 and 0.052; *C. japonica*, 0.189. For H_T , *C. japonica* had a value of 0.196. These values were also lower than those for hinoki in this study.

The average value of G_{ST} for other gymnosperms was 0.076, and the values for the species with narrow- and regional-geographic ranges were 0.124 and 0.065, re-

spectively (Hamrick *et al.* 1992). Comparison of the values of long-lived tree species with similar distributions to that of hinoki suggests that genetic differentiation among hinoki natural populations is low.

The fact that hinoki showed high genetic variation within populations and low population divergence despite the current small and scattered populations is obviously inconsistent with the generalization of genetic diversity for long-lived tree species (Hamrick *et al.* 1992). Then, it is postulated that the areas with hinoki forests were larger and more continuously distributed in the past, and that the past genetic diversity even now is maintained in the sporadically distributed populations. In large continuous populations, a high rate of gene flow may take place and prevent the occurrence of genetic drift. However, in this study, this hypothesis was not analyzed in detail. Paleogeographical data as

Fig. 4 Scatter plot resulting from principal component analysis of 11 natural populations and 12 plus-tree groups of *C. obtusa*.

●, natural populations; ○, plus-tree groups.

well as allozyme data from more populations of hinoki should be collected to test the hypothesis.

Similar genetic clines in allele frequencies along latitude and/or longitude, as observed in hinoki, have been reported for several tree species [e.g. *Picea abies* (Lagercrantz and Ryman 1990)]. Of the clines at the 4 loci in hinoki, that at *Got-1* had already been recognized by Shiraishi *et al.* (1987). Furthermore, allozyme variation within populations tended to be lower in populations at more eastern locations. Iwaki population near the northern limit of the distribution certainly showed the lowest variation. These clines have probably been generated by the events associated with genetic drift through the distributional shift from the past center of distribution, presumably in the Chugoku and Shikoku geographic districts and Kii Peninsula. It is also possible that closely linked loci with the allozyme loci may represent adaptive phenomena along environmental gradients.

Comparison of allozyme variation between natural populations and plus-tree groups

A comparison of the parameters describing genetic variation within populations and species between the natural populations in this study and the plus-tree groups (Uchida *et al.* 1993) is presented in Table 1. The values in the natural populations were approximately similar to those in the plus-tree groups. Therefore, in this study it is concluded that the allozyme variation in the natural populations was mostly present even in the plus-tree groups. However, small, but significant differences were observed between them as follows:

First, seven rare alleles found in the natural populations were absent in the plus-tree groups. This difference resulted probably from the large sample size of the

Kiso population. As the allozyme data of the plus-trees were derived from analyses of 878 clones, which accounted for 88.2% of the number (995) of registered plus-trees (Uchida *et al.* 1993), it is likely that the plus-tree group with a smaller finite size does not share the seven rare alleles found in the natural population with a greater population size. Similar results were reported for *Picea glauca* (Cheliak *et al.* 1988) and *C. japonica* (Tomaru *et al.* 1992). Tomaru *et al.* (1992) compared allozyme variation between artificial stands and a group of plus-trees of *C. japonica* within Ibaraki Prefecture. They reported that since the plus-tree group contained only 76% of the total number of alleles observed in the stands, the plus-tree group may not have all the genetic variation preserved in the artificial stands in the prefecture. This fact may be associated with the plus-tree selection practice where only one or a few superior phenotypes are selected within one stand, because alleles with low frequencies may not be included under such a strong selection pressure as in the practice (Cheliak *et al.* 1988, Tomaru *et al.* 1992).

Second, in the natural populations the profile of allele frequencies was characterized by the clinal variation of allele frequencies across the populations and the mean frequencies, in contrast to that in the plus-tree groups. Mean frequencies of alleles at the loci, *G6p*, *Got-1*, *Pgm* and *Lap* were significantly heterogeneous between the natural populations and the plus-tree groups. The ranges of allele frequencies in the natural populations were wider than those in the plus-tree groups; there was no clinal variation of allele frequencies across the plus-tree groups (Fig. 2); the population difference [$G_{ST} = 0.045 \pm 0.012$ (SE)] among the natural populations was five times that among the plus-tree groups (0.009 ± 0.004). Hinoki plus-tree groups were genetically

homogeneous among the groups (Uchida *et al.* 1993).

The discrepancies in the allele frequencies between the natural populations and the plus-tree groups were further illustrated by the principal component analysis. The results obtained also indicated that the allele frequencies in the plus-tree groups were close to each other (Uchida *et al.* 1993), and that the 8 natural populations except for the Kiso, Owase and Yanase populations showed different frequencies of alleles from those in the plus-tree groups. The homogeneous frequencies of alleles in the plus-tree groups possibly reflect the limited origin of artificial stands with ages approximately less than 80 years. Based on allozyme data for two loci of natural populations and artificial stands of hinoki, Shiraishi *et al.* (1987) reported that genetic variation in the artificial stands was very similar to one another, and thus that the seed sources for afforestation may have been limited in particular areas. Alternatively, genetic drift which may have occurred in marginal natural populations with limited population sizes could have possibly resulted in the wider distribution of the allele frequencies across the natural populations. Since advanced breeding generations should be included in the overall gene conservation program for predominantly outcrossing species, our results suggest that it is very important to establish in situ genetic resource conservation forests particularly for the 8 natural hinoki populations.

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Literature Cited

- Cheliak, W. M., G. Murray and J. A. Pitel (1988) Genetic effects of phenotypic selection in white spruce. *For. Ecol. Manage.* 24 : 139-149.
- Hamrick, J. L., M. J. W. Godt and S. L. Sherman-Broyles (1992) Factors influencing levels of genetic diversity in woody plant species. *New For.* 6 : 95-124.
- Hayashi, Y. (1960) Taxonomical and Phytogeographical Study of Japanese Conifers. Norin-Shuppan, Tokyo, Japan (In Japanese).
- Kimura, M. and J. F. Crow (1964) The number of alleles that can be maintained in a finite population. *Genetics* 49 : 725-738.
- Lagercrantz, U. and N. Ryman. (1990) Genetic structure of Norway spruce (*Picea abies*) : Concordance of morphological and allozymic variation. *Evolution* 44 : 38-53.
- Lin, T.-P., T.-Y. Lee, L.-F. Yang, Y.-L. Chung and J.-C. Yang. (1994) Comparison of the allozyme diversity in several populations of *Chamaecyparis formosensis* and *Chamaecyparis taiwanensis*. *Can. J. For. Res.* 24 : 2128-2134.
- Millar, C. I. and K. A. Marshall (1991) Allozyme variation of Port-Orford-Cedar (*Chamaecyparis lawsoniana*) : Implications for genetic conservation. *For. Sci.* 37 : 1060-1077.
- Nei, M. (1972) Genetic distance between populations. *Am. Nat.* 106 : 283-292.
- (1973) Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci. USA* 70 : 3321-3323.
- (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89 : 583-590.
- (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Saitou, N. and M. Nei. (1987) The neighbor-joining method : A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4 : 406-425.
- SAS institute (1985) *SAS User's Guide: Statistics*, Version 5th edition. SAS Institute, Cary, NC.
- Shiraishi, S., H. Kaminaka and N. Ohyama (1987) Genetic variation and differentiation recognized at two allozyme loci in hinoki (*Chamaecyparis obtusa*). *J. Jpn. For. Soc.* 69 : 88-93.
- Sneath, P. H. and R. R. Sokal (1973) *Numerical taxonomy*. W. H. Freeman, San Francisco.
- Tomaru, N., Y. Tsumura and K. Ohba (1992) Allozyme variation in artificial stands and a plus-tree group of sugi, *Cryptomeria japonica* in Ibaraki Prefecture. *J. Jpn. For. Soc.* 74 : 44-48.
- , — and — (1994) Genetic variation and population differentiation in natural populations of *Cryptomeria japonica*. *Plant Species Biol.* 9 : 191-199.
- Tsumura, Y., Tomaru, N., Suyama, Y., Na'iem, M. and Ohba, K. (1990) Laboratory manual of isozyme analysis. *Bull. Tsukuba Univ. For.* 6 : 63-95 (in Japanese).
- Uchida, K., Y. Tsumura and K. Ohba (1991) Inheritance of isozyme variants in leaf tissues of hinoki, *Chamaecyparis obtusa*, and allozyme diversity of two natural forests. *Japan. J. Breed.* 41 : 11-24.
- , N. Tomaru, Y. Tsumura, C. Takahashi and K. Ohba (1993) Allozyme variation in plus-tree of hinoki, *Chamaecyparis obtusa* selected from artificial stands. *Japan. J. Breed.* 43 : 485-494.
- Workman, P. L. and J. D. Niswander (1970) Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *Am. J. Hum. Genet.* 22 : 24-49.