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Genetic Diversity and Structure of Hinoki (*Chamaecyparis obtusa*) in Chichibu District

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Introduction

The genetic diversity of forests can provide the best assurance for maintenance and enhancement of adaptability to the changing environment, and the opportunity to select and breed new varieties suitable for a range of environments and increasing usage. In general, genetic diversity is higher in natural forests than in plantations, as it is reduced by seed collection from a scarce number of mother trees or tree-improvement practices such as plus-tree selection (EL-KASSABY, 1995; EL-KASSABY and NAMKOONG, 1995). The maintenance of genetic diversity in plantations of forest trees therefore becomes more important, and genetic diversity should be monitored for optimal management in plantations.

Hinoki (*Chamaecyparis obtusa* Endl.) is widely distributed throughout Japan and is one of the most commonly planted tree species. Several natural forests of Hinoki are preserved in Chichibu district, Saitama Pref., Japan, and Hinoki plantations are widely spread throughout the district. It is necessary to obtain basic information on genetic aspects in order to maintain a high genetic diversity within plantations, and also to conserve the natural forests as a gene pool. In the present study, we have surveyed genetic variation in several natural stands and plantations of Hinoki in Chichibu district, and compared the differences in genetic diversity and in genetic structure between them by using allozyme markers.

Materials and Methods

Collection of sample

Tissues of current-year needles were collected from trees in 5 natural stands and in 5 plantation stands in Chichibu district during winter. Data on the sampling stands are presented in Table 1 and their locations are shown in Fig. 1. Samples were stored in plastic bags at −80℃ until electrophoresis was conducted. About 50 individuals in each stand were randomly sampled as test materials.

Electrophoresis

Polyacrylamide gel electrophoresis was run on vertical slab gels, according to the procedures described by TSUMURA *et al.* (1990). Eight enzyme systems coding 10 loci were stained in this study. They were Shikimate dehydrogenase (ShD, E.C. 1.1.1.25), 6-Phosphogluconate dehydrogenase (6PGD, E.C. 1.1.1.44), Glucose-6-Phosphate dehydrogenase (G6PD, E.C. 1.1.1.49), Diaphorase (DIA, E.C. 1.6.4.3), Peroxidase (POD, E.C. 1.11.1.7), Glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), Glucokinase (GK, E.C. 2.7.5.1) and Phosphoglucomutase (PGM, E.C. 2.7.5.1). These systems have been identified as allozyme marker genes by the segregation data of selfed and crossed families obtained from isozyme

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Table 1. Location, sample size, area, elevation and population code of *C. obtusa* included in the study

Forest type	Population	No. of compartment	Sample number	Area (ha)	Elevation (m)	Age (years)	Thinning (%)	Seed suorce	Population code
Natural stand	Tohbakuzawa I*1	17-Ha-6	52	5.2	1250	up to 100	No	—	Tohbaku I
	Tohbakuzawa II*1	17-Ha-2	52	0.8	1350	up to 100	No	—	Tohbaku II
	Hidanazawa I*1	22-1	49	1.2	1300	up to 100	No	—	Hidana I
	Hidanazawa II*1	22-2	43	1.3	1300	up to 100	No	—	Hidana II
	Mameyakizawa*1	17-Ri-2	42	2.0	1300	up to 100	No	—	Mameyaki
Plantation	Ogano*2	44-So	52	4.1	450	14	Below 5%	*3	Ogano
	Yoshida*2	49III-E	52	0.5	250	14	No	*3	Yoshida
	Urayama*2	26-Ne	52	1.9	580	13	No	*3	Urayama
	Tohbakuzawa*1	17-I-2	52	1.1	1300	26	No	*4	Tohbaku
	Yohkurazawa*1	1-I-11	38	1.5	700	85	65.5%	*4	Yohkura

*1: University Forests in Chichibu, The University of Tokyo.
*2: Saitama Pref. Forestry Agency.
*3: Saitama Pref. plus-tree seed-orchard.
*4: Natural stands in Chichibu Univ. Forests.

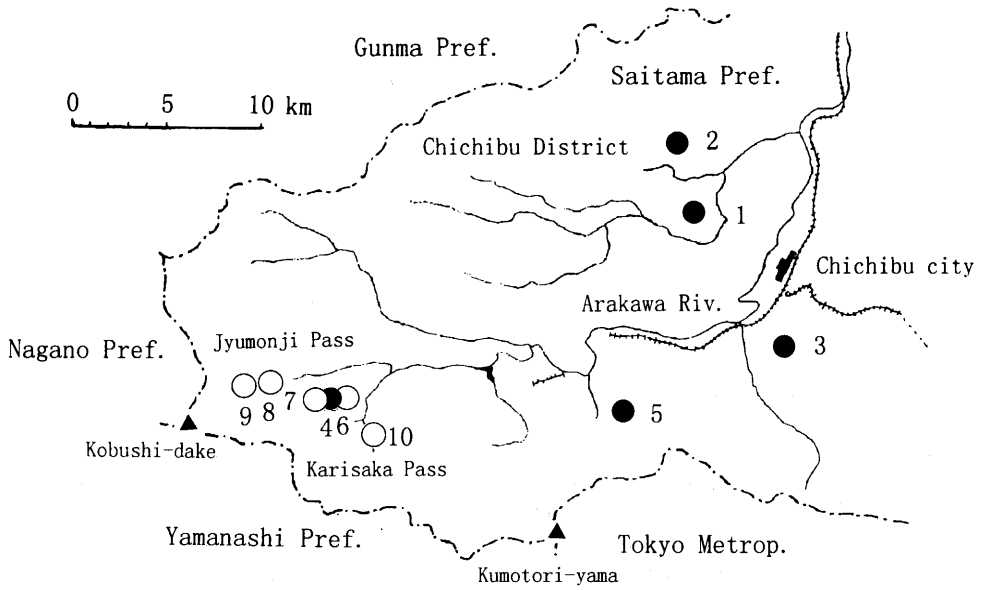

Fig. 1. Location of plantations and natural stands.
Plantations: 1. Ogano, 2. Yoshida, 3. Urayama, 4. Tohbakuzawa, 5. Yohkurazawa
Natural stands: 6. Tohbakuzawa I, 7. Tohbakuzawa II, 8. Hidanazawa I, 9. Hidanazawa II, 10. Mameyakizawa

analyses (UCHIDA *et al.*, 1991).

Statistical analysis

The allelic frequencies of the different loci were calculated directly from the bands of

corresponding loci, $x_i = X_{ii} + \Sigma X_{ij}/2$, where x_i is allelic frequency in i-allele, and X_{ii} and X_{ij} are the genotype frequencies of homozygote and heterozygote in the i-allele respectively. The extent of differentiation can be quantified by analysis of genetic diversity represented as heterozygosity (H). The heterozygosity of the total population (H_T) is partitioned into heterozygosity within (H_S) and between (D_{ST}) populations (NEI, 1973). The heterozygosity of the total population (H_T) was calculated from the mean allele frequencies at 10 loci of all populations, whereas the heterozygosity within the populations, that is the mean value of the heterozygosity in each population, was presented as the average observed heterozygosity (H_o) and the average expected heterozygosity (H_e) from the allele frequencies of 10 loci respectively (NEI and ROYCHOUDHURY, 1974; NEI, 1987). The relative magnitude of genetic differentiation between stands (G_{ST}) is measured as the proportion of D_{ST} to H_T, that is, $G_{ST} = D_{ST}/H_T$ (NEI, 1973). Genetic distance coefficients were also calculated from allelic frequencies over all loci inferred according to the procedure of NEI (1987). On this basis, the results are visualized in a dendrogram by the unweighted pair-group method using arithmetic averages (UPGMA) (SNEATH and SOKAL, 1973). To investigate the deviation of the observed genotype frequencies from those expected under panmixia, the multilocus heterozygote deficit (F_{IS}) is calculated according to the formula $F_{IS} = 1 - H/2pq(1 + 1/(2N - 1))$, where H is the number of heterozygotes observed and $2pq(1 + 1/(2N - 1))$ is the number of heterozygotes expected (KIRBY, 1975). The significance of genetic parameters between plantations and natural stands, and the deviation of F_{IS} at each loci from the expectations under the panmixia were tested using t-test.

Results and Discussion

The allelic frequencies at 10 loci in natural stands and plantations are shown in Table 2. Note the presence of three alleles highlighted in bold type. In natural stands, Mameyakizawa had all these three alleles. This stand was characterized by a low frequency of $G6pd^{-a}$ (less than 0.16), which was above 0.40 in the other stands, absence of Gk^{-b}, and a high frequency of Pod^{-b}. Hidanazawa I and Hidanazawa II, even though the distance between is less than 5 kilometers, were discriminated by the absence of Gk^{-b} and the highest frequency of Pod^{-b}, respectively. In plantations, with the exception of a low frequency of Gk^{-b} in both Tohbakuzawa and Yohkurazawa, the other allele frequencies were homogeneous among the five stands. Hence, it appears from this preliminary survey, that heterogeneity exists among the natural stands, and homogeneity in genetic composition exists among the plantations. The statistical significance of means of allelic frequencies at each locus was also tested between the natural stands and plantations (Table 2). Of all the ten loci investigated, only in *Shd* did the difference reach a significant level ($p = 0.042$). A little difference could also be found in *G6pd* ($p = 0.078$), *Gk* ($p = 0.055$) and *Pgm* ($p = 0.078$). Considering the lack of data from three natural stands for *Shd*-2 and *Dia*-1, plantations in Chichibu district seem to have a similar genetic composition to natural stands.

Intrastand genetic structure was estimated by observed heterozygosities (H_o) and expected heterozygosities (H_e) at all loci (Table 3). Among the natural stands, the H_e were 0.210, 0.245, 0.198, 0.286 and 0.235 in Tohbakuzawa I, Tohbakuzawa II, Hidanazawa I, Hidanazawa II and Mameyakizawa, respectively. Mean of estimates was 0.235. Among the plantations, the H_e were 0.281, 0.282, 0.260, 0.246 and 0.253 in Ogano, Yoshida, Urayamazawa and Yohkurazawa, respectively, averaging 0.265. There was no significant difference between natural forests and plantations in average expected heterozygosity ($p = 0.76$), implying plantations could conserve as large an intrastand genetic diversity as natural stands. The same results were reported between seed-orchard original seedling plantations and natural forests of Douglas-fir (ADAMS, 1981), and between seed-orchard and seed zone

Table 2. Allelic frequencies in plantations and natural stands in Chichibu district. Statistically significant *t*-values between plantations and natural stands are also shown. Alleles of special interest are in bold characters. Means-specific interest between plantations and natural stands are underlined

Locus	Allele	Allele frequencies												Heterogeneity	
		Plantations						Natural stands							
		Ogano	Yoshida	Urayama	Tohbaku	Yohkura	Mean±s.e.	Tohbaku I	Tohbaku II	Hidana I	Hidana II	Mameyaki	Mean±s.e.	*t* [4]	*p*
6*Pgd*-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000±.000	1.000	1.000	1.000	1.000	1.000	1.000±.000		
6*Pgd*-2	*a*	0.529	0.519	0.577	0.540	0.661	0.565±.026	0.645	0.645	0.602	0.500	0.655	0.609±.029	1.34	0.126
	b	0.471	0.481	0.423	0.460	0.339	0.435±.026	0.355	0.355	0.398	0.500	0.345	0.391±.029		
G6pd	*a*	0.538	0.548	0.587	0.519	0.569	0.552±.012	0.529	0.559	0.449	0.407	**0.159**	0.421±.071	1.75	0.078
	b	0.462	0.452	0.413	0.481	0.431	0.448±.012	0.471	0.441	0.551	0.593	0.841	0.579±.071		
Got	*a*	0.221	0.183	0.154	0.192	0.197	0.189±.011	0.184	0.333	0.071	0.163	0.378	0.226±.057	0.68	0.268
	b	0.779	0.817	0.846	0.808	0.803	0.801±.011	0.816	0.667	0.929	0.837	0.622	0.774±.057		
Gk	*a*	0.875	0.788	0.885	0.962	0.981	0.898±.034	0.961	0.947	1.000	0.872	1.000	0.956±.023	1.33	0.127
	b	0.125	0.212	0.115	**0.038**	**0.019**	0.102±.034	0.039	0.053	**0.000**	0.128	**0.000**	0.044±.023		
Shd-2	*a*	0.365	0.413	0.442	0.394	0.308	0.384±.023	0.039	0.192	—	—	—	0.116±.076	3.29*	0.042
	b	0.635	0.587	0.558	0.606	0.692	0.616±.023	0.961	0.808	—	—	—	0.885±.076		
Pod	*a*	0.885	0.902	0.932	0.933	0.926	0.916±.009	0.947	0.949	0.967	0.791	0.882	0.907±.032	0.22	0.418
	b	0.115	0.098	0.068	0.067	0.074	0.084±.009	0.053	0.051	0.033	**0.209**	**0.118**	0.093±.032		
Dia-1	*a*	0.212	0.269	0.183	0.106	0.231	0.200±.027	0.263	0.276	—	—	—	0.270±.007	2.25	0.055
	b	0.788	0.731	0.817	0.894	0.769	0.800±.027	0.737	0.724	—	—	—	0.731±.007		
Dia-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000±.000	1.000	1.000	—	—	—	1.000±.000		
Pgm	*a*	0.853	0.923	0.856	0.709	0.800	0.828±.036	0.882	0.921	0.878	0.893	0.854	0.886±.011	1.75	0.078
	b	0.147	0.077	0.144	0.204	0.200	0.172±.036	0.118	0.079	0.122	0.107	0.146	0.114±.011		

* Significant at 5% level.

Table 3. Intrastand genetic measures

Plantation	H_o		H_e	
	Mean	S.E.	Mean	S.E.
Ogano	0.313	0.066	0.281	0.058
Yoshida	0.296	0.071	0.282	0.061
Urayama	0.269	0.064	0.260	0.059
Tohbaku	0.304	0.080	0.246	0.063
Yohkura	0.316	0.084	0.253	0.061
Means	0.300	0.073	0.265	0.060
Natural stand				
Tohbaku I	0.242	0.074	0.210	0.060
Tohbaku II	0.280	0.084	0.245	0.062
Hidana I	0.188	0.079	0.198	0.080
Hidana II	0.306	0.074	0.286	0.066
Mameyaki	0.222	0.065	0.235	0.072
Means	0.248	0.075	0.235	0.068

H_o: observed heterozygosity
H_e: expected heterozygosity

Table 4. F statistics (F_{IS}) and genetic diversity interpopulations in plantations and natural stands

Locus	Plantations				Natural stands			
	F_{IS}	H_T	H_S	G_{ST}	F_{IS}	H_T	H_S	G_{ST}
6Pgd-1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6Pgd-2	−0.205*	0.491	0.485	0.014	−0.182	0.476	0.468	0.017
G6pd	−0.268*	0.495	0.493	0.003	−0.092	0.487	0.437	0.103
Got	−0.238*	0.307	0.306	0.004	−0.070	0.350	0.317	0.092
Gk	0.225*	0.183	0.171	0.065	0.011	0.084	0.079	0.066
Shd-2	0.143	0.473	0.468	0.011	0.195	0.204	0.181	0.115
Pod	−0.058	0.155	0.154	0.006	0.031	0.168	0.158	0.062
Dia-1	−0.273*	0.320	0.313	0.023	0.005	0.394	0.394	0.000
Dia-2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm	−0.237*	0.285	0.272	0.045	−0.024	0.203	0.201	0.006
Means	−0.091	0.271	0.266	0.017	−0.013	0.237	0.223	0.046
S.E.	0.057	0.059	0.058	0.007	0.031	0.058	0.055	0.015

* Significant at 5% level.

original seedling plantations and natural forests of both *Pinus banksiana* and *Picea mariana* (KNOWLES, 1985). Overall, general afforestation practices seem to have little effect on genetic diversity of forest trees.

The summary of interstand statistics is given in Table 4. For the estimate of F_{IS}, regardless of the positive or negative variation, eight of the ten loci investigated were close to zero, and averaged −0.013 in natural stands. This indicates that F_{IS} fits the expected under panmixia in those natural stands investigated. However, in plantations, values of F_{IS} were negative in six out of ten loci, and five deviated significantly from the expected under

Table 5. Pair-wise genetic distance measurements between natural stands

Stand	6	7	8	9	10	11
6. Tohbakuzawa I	—					
7. Tohbakuzawa II	0.0060	—				
8. Hidanazawa I	0.0194	0.0180	—			
9. Hidanazawa II	0.0282	0.0225	0.0112	—		
10. Mameyakizawa	0.0499	0.0356	0.0348	0.0304	—	
11. plantations	0.0175	0.0108	0.0091	0.0117	0.0429	—

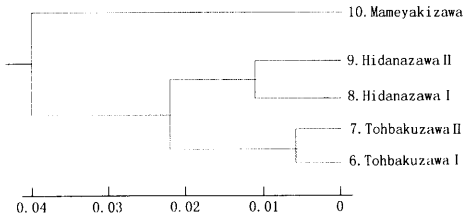

Fig. 2. A dendrogram of natural stands of Hinoki in Chichibu district, based on NEI's genetic distances.

panmixia. The mean of F_{IS} for all the loci was −0.091, more than six-fold the value from natural stands ($p=0.001$). This indicates an excess of heterozygous types over the expected value under panmixia in plantations. Genetic structural composition in these plantations is therefore characterized by the favoring of more heterozygotes than in natural populations.

Several mechanisms can produce an excess of heterozygous genotypes over expected values (BROWN, 1979). In general, the F_{IS} under panmixia is zero, and will be increased by inbreeding and decreased by selection favoring heterozygotes. It is reported that some self-fertilization occurs in the seed-orchard of *Chamaecyparis obtusa*, and the rate of self-fertilization may reach 9.8–16% (TAJIMA, 1979; SEIDO, 1990). In natural stands, the effects of selfing are small, since inbred individuals are eliminated early under the harsh conditions. Accordingly, the natural stands could be retained under panmixia. However, during afforestation, selective practices associated with genetic improvement, such as handling of seeds, culling in the nursery, thinning during growth and so forth, may bring about an elimination of homozygotes. The selective removal of inbreeds decreased mostly homozygosity during the early developmental phases or, stated alternatively, by "heterosis for outcross" (BROWN, 1979). Similar results to ours were reported in *Pinus sylvestris* between a seed-tree stand and young trees in naturally regenerated understorey (YAZDANI *et al.*, 1985), and natural and nursery-grown seedlings from collected seeds (MUONA *et al.*, 1987).

Genetic diversity analysis indicated that the total genetic diversity (H_T) in natural stands averaged 0.237, whereas the genetic diversity within each stand (H_S) was 0.223 for all the loci. Of the total genetic variation, 4.6% reflected the differences between the investigated stands (G_{ST}), and 95.4% accounted for that within stands. The range of G_{ST} values for each locus was from 0.05% (*Pgm*) to 11.5% (*Shd*-2) (Table 4). The unbiased genetic distances were computed in Table 5. The genetic distances between each of Tohbakuzawa I, Tohbakuzawa II, Hidanazawa I and Hidanazawa II were small (0.006–0.028). However, Mameyakizawa had rather large genetic distances compared to all other stands (0.030–0.050). This showed that Mameyakizawa appeared to be the most divergent from the other four stands in terms of genetic distance. The dendrogram constructed with genetic distance coefficients (Fig. 2) illustrates the same results. The first divergence dividing Mameyakizawa and the other four stands into two clusters reached 0.04. Tohbakuzawa and Hidanazawa could be clustered at 0.022, only half of the first divergence. This

indicated that Mameyakizawa is very important as a gene resource.

Many studies have been contributed on intra- and interpopulation genetic variation of *C. obtusa* natural forests (SHIRAISHI *et al.*, 1987; UCHIDA *et al.*, 1991). Our study reaffirmed the results of previous studies in that there was much less divergent genetic diversity between populations than within populations. Considering the component alleles and genetic distances in the 10 loci, these populations in Chichibu district are the same as northern populations in Shizuoka Prefecture (UCHIDA *et al.*, 1991), and belong to the central population of Hinoki classified by SHIRAISHI *et al.* (1987). However, the value of G_{ST} was 0.046 in our study, which was much larger than that in Shizuoka Prefecture (G_{ST}=0.0146, UCHIDA *et al.*, 1991) based on the mean of 10 common loci. Furthermore, the divergence dividing Mameyakizawa and the other four stands into two clusters reached 0.04. This value could gather Tanakamiyama, Kiso, Imaichi, and Akaidake populations into a group (SHIRAISHI *et al.*, 1987). Overall, the Chichibu population possesses great genetic variation, even though it is confined in geographical range, and is one of most important sources for gene pool preservation.

The average total genetic diversity (H_T) and intrastand genetic diversty (H_S) were 0.271 and 0.266 in plantations, respectively. Notably, 98.3% of genetic diversity (H_S/H_T) was distributed within stands, and only 1.7% of the genetic diversity (G_{ST}) was distributed between the stands (Table 4). The genetic relationship between stands could also be demonstrated by the genetic distance between stands (Table 6). Except for the values between Yoshida and Tohbakuzawa, and Yoshida and Yohkurazawa (0.0124 and 0.0116, respectively), the values between each of the remaining stands did not exceed 0.006.

Compared with natural stands, some conspicuous interstand genetic characteristics could be observed in plantations. Firstly, in contrast to high interstand variation (G_{ST}= 0.046, Table 4) and heterogenous genetic distance (D=0.026, Table 5) in natural stands, plantations have a much lesser G_{ST} and a uniform genetic distance. This implies that plantation stands are genetically very similar to each other. Secondly, the genetic distances between the plantations and each of the natural stands (Mameyakuzawa excluded) did not exceed 0.018, and averaged 0.012 (Table 5), suggesting there was little genetic difference between plantations and natural stands. In this study, the age of the plantation stands investigated ranged from 14 to 100 years. The similarity in allelic compositions between plantations and natural forests (Table 2) and uniformity in genetic distances between plantations (Table 6) showed that the seeds for afforestation of these plantations might have been collected mainly from Chichibu local areas. In fact, as shown in Table 1, Ogano, Yoshida and Urayama were from seed-orchard which was composed of plus-trees from outside Saitama Prefecture. This resulted in the differences in frequency of Gk^{-b} (Table 2). However, the values of Gk^{-b} were too small to alter the significance of difference in multiloci frequencies between natural stands and plantations, and in genetic distances between plantations. The differences of partition in genetic variation between plantations and natural forests, reconfirmed the importance of natural forests as a gene pool as

Table 6. Pair-wise genetic distance mearsurements between plantations

Stand	1	2	3	4
1. Ogano	—			
2. Yoshida	0.0028	—		
3. Urayama	0.0053	0.0038	—	
4. Tohbakuzawa	0.0048	0.0124	0.0046	—
5. Yohkurazawa	0.0051	0.0116	0.0056	0.0060

emphasized by SHIRAISHI *et al.* (1987).

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Summary

The genetic diversity and structure of hinoki were investigated in natural forests and plantations in Chichibu district by using 10 allozyme loci in 8 enzyme systems as marker genes. The analysis of gene composition and genetic structure, both intrastand and interstand, showed that in natural forests, 95.4% of the total genetic variation was maintained intrastand. It is shown that Chichibu natural forests possess great genetic variability. The analysis of estimated average heterozygosities revealed that plantations possess as large an intrastand genetic diversity as natural forests. But only 1.7% of genetic variation (G_{ST}) was attributable to genetic differences interstand, much less than the 4.6% in natural forests. Notably, plantations possessed less genetic variation interstand than the natural forests. The estimated average multilocus heterozygote deficit (F_{IS}) reached -0.091, which deviated significantly from the expected value under panmixia that was fitted in natural forests. This reconfirmed that some procedures during afforestation gave rise to a reduction of homozygotes and resulted in an excess of heterozygotes.

Key words: Allozyme, *Chamaecyparis obtusa*, Genetic diversity, Genetic structure

References

ADAMS, W. T., 1981. Population genetics and gene conservation in Pacific Northwest conifers. Evol. Today, Proc. Int. Congr. Syst. Evol. Biol. 2nd, 1980. pp. 401–415.

BROWN, A. H. D., 1979. Enzyme polymorphism in plant populations. Theor. Pop. Biol., **15**, 1–42.

EL-KASSABY, Y. A., 1995. Evaluation of the tree-improvement delivery system: Factors affecting genetic potential. Tree Physiol., **15**, 545–550.

EL-KASSABY, Y. A. and G. NAMKOONG, 1995. Genetic diversity of forest tree plantations: consequences of domestication, In: Consequences of changes in biodiversity. IUFRO World Congress, Tampere, Finland, Vol. 2, pp. 218–228.

KIRBY, G. C., 1975. Heterozygote frequencies in small subpopulations. Theor. Pop. Biol., **8**, 31–48.

KNOWLES, P., 1985. Comparison of isozyme variation among natural stands and plantation: jack pine and black spruce. Can. J. For. Res., **15**, 902–908.

MUONA, O., R. YAZDANI and D. RUDIN, 1987. Genetic change between life stages in *Pinus sylvestris*: allozyme variation in seeds and planted seedlings. Silvae Genet., **36**, 39–42.

NEI, M., 1973. Analysis of gene diversity of subdivided populations. Proc. Natl. Acad. Sci. USA, **70**, 3321–3323.

NEI, M., 1987. Molecular Evolutionary Genetics. pp. 512, Columbia University Press, New York.

NEI, M. and A. K. ROYCHOUDHURY, 1974. Sampling variances of heterozygosity and genetic distance. Genetics, **76**, 379–390.

SEIDO, K., 1990. The isozyme variation and estimation of self-fertilization rate in a seed orchard of *Chamaecyparis obtusa*. Trans. Jpn. For. Soc., **101**, 301–302. (in Japanese)*

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.

SKROPPA, T., 1994. Impacts of tree improvement on genetic structure and diversity of planted forests. Silva Fennica, **28**, 265–274.

SNEATH, P. H. A. and R. R. SOKAL, 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.
TAJIMA, M., 1979. Genetic analysis for self-fertilization in *Chamaecyparis obtusa*, The Reports of the Kyushu University Forests, **51**, 39–124. (in Japanese)
TSUMURA, Y., N. TOMARU, Y. SUYAMA, M. NAITEM and K. OHBA, 1990. Laboratory manual of isozyme analysis. Bull. Tsukuba University Forests, **6**, 63–95. (in Japanese).
UCHIDA, K., Y. TSUMURA and K. OHBA, 1991. Inheritance of isozyme variants in leaf tissue of hinoki, *Chamaecyparis obtusa*, and allozyme diversity of two natural forests. Japan J. Breed., **41**, 11–24.
YAZDANI, R., O. MUONA, D. RUDIN and A. E. SZMIDT, 1985. Genetic structure of *Pinus sylvestris* L. seed tree stand and naturally regenerated understorey. For. Sci., **31**, 430–436.

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秩父地域におけるヒノキ林のアイソザイム変異

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要　　旨

ヒノキ林遺伝子組成と遺伝構造解明するために，秩父地域の5か所の天然林（トウバク沢I，トウバク沢II，豆焼沢，ヒダナ沢I，ヒダナ沢II）と5か所の人工林（小鹿野，吉田，浦山，トウバク沢，要倉沢）を調査した。分析は8酵素種10遺伝子座のアイソザイム遺伝子について，平板ポリアクリルアミドゲル垂直電気泳動法を行った。その結果，天然林では全遺伝変異の95.4%が集団内に存在しており，集団間の遺伝的隔りは小さかった。その中で，豆焼沢集団は他の四集団と多少異なる遺伝子組成を有し，隔離された小集団の遺伝的特徴を示したことから，ヒノキ天然林の遺伝的多様性を保持する上で重要な遺伝子プールであると考えられた。また，5か所の人工林では林分間の遺伝変異は認められず，また林分内の遺伝的多様性（平均ヘテロ性接合体率の期待値）は天然林の集団内とほとんど違わなかった。またこれらの人工林には天然林と余り変わらない遺伝的多様性が保有されていることが示唆された。さらに，人工林の遺伝的構成は天然林と異なり，ヘテロ接合体過剰であることが示された。これは，人工林造成過程において何らかの選抜などにより，自殖に起因するホモ個体が消滅してきたことによるものと推察される。

キーワード：アイソザイム，ヒノキ (*Chamaecyparis obtusa*)，遺伝的多様性，遺伝構造

Abstract

Genetic Diversity and Structure of Hinoki (*Chamaecyparis obtusa*) in Chichibu District

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The genetic diversity and structure of hinoki were investigated in natural forests and plantations in Chichibu district by using 10 allozyme loci in 8 enzyme systems as marker genes. The analysis of gene composition and genetic structure, both intrastand and interstand, showed that in natural forests, 95.4% of the total genetic variation was maintained intrastand. It is shown that Chichibu natural forests possess great genetic variability. The analysis of estimated average heterozygosities revealed that plantations possess as large an intrastand genetic diversity as natural forest. But only 1.7% of genetic variation (G_{ST}) was attributable to genetic differences interstand, much less than the 4.6% in natural forests. Notably, plantations possessed less genetic variation interstand than the natural forests. The estimated average multilocus heterozygote deficit reached -0.091, which deviated significantly from the expected value under panmixia that was fitted in natural forests. This reconfirmed that some procedures during afforestation gave rise to a reduction of homozygotes and resulted in an excess of heterozygotes.

Formulation of the Shear Stress/Shear Strain Relationship Using the Torsion Testing Data

Hiroshi YOSHIHARA, Masamitsu OHTA and Kazuhiro ORIGUCHI

In this paper, we tried to formulate the shear stress/shear strain relationship of wood by approximating the torsional moment-shear strain relationship with Ludwik's n-power function.

Sitka spruce (*Picea sitchensis* Carr.) and konara (Japanere oak, *Quercus serrata* Murray) were used for the specimens. Specimens were cut so as to have various angles between the grain and the geometrical axes. These specimens were twisted around the radial axis, and the torsional moment-shear strain relationships were obtained. This relationship was approximated to Ludwik's n-power function, and this relationship was transformed into the shear stress-shear strain relationship which was formulated by a similar power function.