Heterozygosity, heterosis, and fitness in outcrossing plants. In: Conservation Biology. Edited by M. E. SOUL. Sinauer, Sunderland. pp. 77–104 (1986). — MITTON, J. B., LINHART, Y. B., STRUGEN, K. B. and HAMRICK, J. L.: Allozyme polymorphisms detected in mature needle tissue of ponderosa pine. J. Hered. **70**, 86–89 (1979). — NEI, M.: Genetic distance between populations. Am. Nat. **106**, 283–292 (1972). — NEI, M.: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**, 3321–3323 (1973). — NEI, M.: *F*-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41**, 225–233 (1977). — NUMATA, M.: The flora and vegetation of Japan. Kodansha Ltd., Tokyo (in Japanese) (1974). — ROHLF, F. J.: Numerical taxonomy and multivariate analysis system. Exeter Publ., Setauket (1988). — SATO, T., TANOUCHI, H. and TAKESHITA, K.: Initial regenerative processes of *Distylium racemosum* and *Persea thunbergii* in an evergreen broad-leaved forest. J. Plant Res. **107**, 331–337 (1994). — SLATKIN, M.: Rare alleles as indicators of gene flow. Evolution **39**, 53–65 (1985). — SOLTIS, D. E., HAUFLER, C. H., DARROW, D. C. and GASTONY, G. J.: Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Am. Fern J. **73**, 9–27 (1983). — TANOUCHI, H., SATO, T. and TAKESHITA, K.: Comparative studies on acorn and seedling dynamics of four *Quercus* species in an evergreen broad-leaved forest. J. Plant Res. **107**, 153–159 (1994). — WEEDEN, N. F. and WENDEL, J. F.: Genetics of plant isozymes. In: Isozymes in plant biology. Edited by D. E. SOLTIS and P. S. SOLTIS. Dioscorides, Portland. pp. 46–72 (1989). — WENDEL, J. F. and PARKS, C. R.: Genetic diversity and population structure in *Camellia japonica* L. (Theaceae). Amer. J. Bot. **72**, 52–65 (1985). — WENDEL, J. F. and WEEDEN, N. F.: Visualization and interpretation of plant isozymes. In: Isozymes in plant biology. Edited by D. E. SOLTIS and P. S. SOLTIS. Dioscorides, Portland. pp. 5–45 (1989). — WORKMAN, P. L. and NISWANDER, J. D.: Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Amer. J. Hum. Genet. **22**, 24–49 (1970). — WRIGHT, S.: The genetical structure of populations. Ann. Eugen. **15**, 313–354 (1951). — YAMAMOTO, S.: Gap characteristics and gap regeneration in primary evergreen broad-leaved forests of Western Japan. Bot. Mag. Tokyo **105**, 29–45 (1992). — YUMOTO, T.: Pollination systems in a warm temperature evergreen broad-leaved forest on Yaku Island. Ecol. Res. **2**, 133–145 (1987).

Genetic Variation of *Taxus cuspidata* SIEB. et ZUCC. in Korea

By S. W. LEE[1][3][4], W. Y. CHOI[1], W. W. KIM[1], and Z. S. KIM[2]

(Received 5th October 1999)

Abstract

Japanese yew (*Taxus cuspidata* SIEB. et ZUCC.) is a shade-tolerant, dioecious gymnosperm native to Korea, Japan, Manchuria and eastern Siberia. Five natural populations of *T. cuspidata* in Korea were investigated using starch-gel electrophoresis in an attempt to determine the extent and distribution of genetic variation. The level of genetic diversity ($A = 1.7$, $P_{95} = 45.7\%$, $P_{99} = 60\%$, $H_o = 0.172$, and $H_e = 0.168$) and the degree of genetic differentiation ($G_{ST} = 0.067$) were comparable to those of other conifers with similar life-histories and ecological traits. Five natural Japanese yew populations seemed to be in equilibrium with the expectations of HARDY-WEINBERG. Isolation by distance was detected. NEI's genetic distance, *D,* was positively correlated with geographic distance ($r = 0.794$, $p = 0.006$). *Nm,* the number of migrants per generation, was 3.48 and 2.87, depending on estimation procedure, and is similar to values in wind-pollinated conifers. Individual trees widely scattered around natural populations appeared to be critical to the maintenance of genetic variation in Japanese yew. Implications for the conservation of genetic diversity of *T. cuspidata* are discussed.

Key words: Taxus cuspidata, genetic diversity, differentiation, allozymes.

Introduction

In Korea, there are two species and two varieties of the genus *Taxus; Taxus cuspidata* SIEB. et ZUCC., *T. cuspidata* var. *latifolia* NAKAI, *T. cuspidata* var. *nana* HORT., and *T. caespitosa* NAKAI which was introduced from Japan. Of these, *Taxus cuspidata* SIEB. et ZUCC., the most abundant native yew species, grows on high mountainous regions throughout Korea, Japan and China (LEE, 1987). This species is a long-lived, shade-tolerant, dioecious gymnosperm. In Korea, it is generally found in mixed forest stands together with other conifer species, such as *Abies nephrolepis, A. koreana, A. holophylla, Pinus koraiensis* and *Picea jezoensis,* and alpine broad-leaved tree species like *Quercus* spp. and *Betula* spp. In recent time, the distribution of yew species has been severely reduced due to natural disturbances and/or human activity. Additionally, the demands for yew species has rapidly increased since it was identified as the primary source of the compound Taxol® (paclitaxel), a promising new anticancer drug (see WHEELER, 1995 and references therein). So, the conservation and sustainable management of this species has gained much attention.

Allozyme variation in species and within and among populations has been extensively studied in forest trees, especially in conifers (HAMRICK et al., 1992; LEDIG, 1986, 1998). Comparatively, only a few studies have been made on yew species. Population genetic studies using DNA markers are also very sparse in yews. Recently, EL-KASSABY and YANCHUK (1994) used allozyme markers to study genetic diversity and were able to determine the inheritance of 21 allozyme loci in Pacific yew (*T. brevifolia*). WHEELER et al. (1995) studied genetic diversity and structure of Pacific yew sampled from North America and Canada using 22 isozyme loci. In addition, GÖÇMEN et al. (1996) constructed a genetic linkage map for Pacific yew based on RAPD markers. LEWANDOWSKII et al. (1992) and HERTEL (1996) studied inheritance of some isozyme markers in English yew (*T. baccata*) and HERTEL and KOHLSTOCK (1996) studied genetic variation and geographic structure of English yew in north eastern Germany, using 7 isozyme loci. However, to the best of our knowledge, there is a complete lack of information

---

[1]) Tree Breeding Department, Forestry Research Institute, Suwon 441-350, Rep. of Korea

[2]) Department of Forest Resources, Korea University, Seoul 136-701, Rep. of Korea

[3]) Present address: Institute of Forest Genetics, 2480 Carson Road, Placerville, CA 95667, USA
Phone) (530) 622-1225; Fax) (530) 622-2633;
E-mail) swlee66@hotmail.com or slee02@fs.fed.us

[4]) To whom correspondence should be addressed

about genetic variation in Japanese yew using allozyme marker systems in the literature.

The objectives of this study were to identify and describe levels and patterns of genetic variation in *Taxus cuspidata* in Korea and to compare the results with previous reports for other conifers in general, and yews in particular.

Materials and Methods

In the fall of 1997, open-pollinated seeds were collected from 142 parent trees in 5 populations located throughout the native range of Japanese yew in Korea (*Fig. 1*). Individuals within populations were separated by a minimum of 25 m in order to decrease the risk of relatedness. The goal was to sample more than 30 trees for each population, but that was not attainable in some populations (Ohdae and Taebaek) because of the low frequency of trees with seeds. Collected seeds were transported to the laboratory and stored at –20 °C until needed. Six or more megagametophytes from each individual tree were extracted according to CONKLE et al. (1982) and utilized to identify tree genotypes.

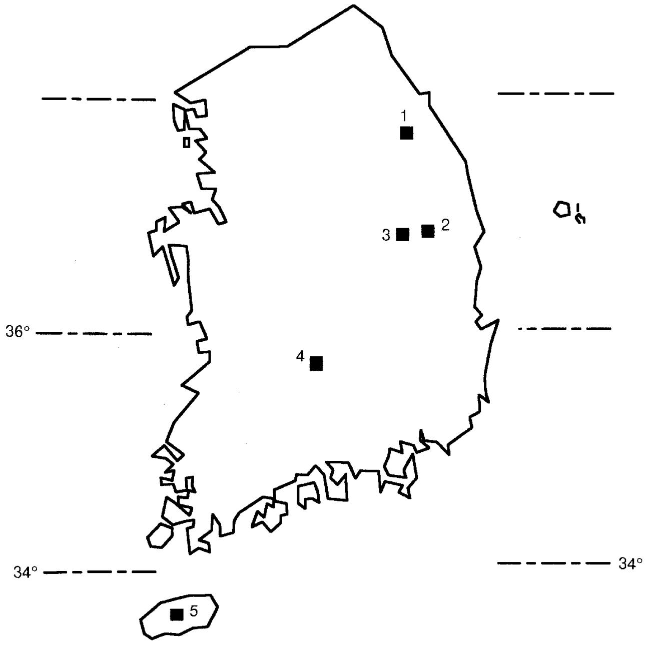

Fig. 1. – Locations of the five study populations of Japanese yew in Korea. 1, Ohdae; 2, Taebaek; 3, Sobaek; 4, Dukyoo; 5, Halla.

Samples were electrophoresed on horizontal starch gels (12%) using two gel electrode buffer systems (KIM et al., 1994). The gels were stained according to the methods of CONKLE et al. (1982) with some modifications in order to produce isozyme bands in 8 enzyme systems (abbreviations and EC number in brackets): aconitase (ACO, 4.2.1.3), glutamate-oxaloacetate transaminase (GOT, 2.6.1.1), isocitrate dehydrogenase (IDH, 1.1.1.42), malate dehydrogenase (MDH, 1.1.1.37), menadion reductase (MNR, 1.6.99.2), phosphoglucose isomerase (PGI, 5.3.1.9), phosphoglucomutase (PGM, 2.7.5.1), and shikimate dehydrogenase (SKDH, 1.1.1.25). We interpreted the number of loci and alleles by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species, as well as by comparing it to reports on the inheritance patterns of other yew species (LEWANDOWSKI, et al., 1992; HERTEL, 1996). Where several zones of activity were observed for a single enzyme, hyphenated numerals following the enzyme abbreviation were used for identification. Fourteen presumptive loci were consistently scored and used in the statistical analysis.

We used BIOSYS-1 (SWOFFORD and SELANDER, 1987) to estimate genetic diversity (number of alleles per locus, proportion of polymorphic loci, observed and expected heterozygosities), WRIGHT's (1965) *F*-statistics (F_{IS}, and F_{IT}), genetic distance of NEI (1978), and UPGMA-derived dendrogram. Deviations of genotypic distributions from the HARDY-WEINBERG expectations were tested by exact tests (ROUSSET and RAYMOND, 1995). The exact *P*-values were estimated by the Markov chain method (RAYMOND and ROUSSET, 1995a). To test whether the allelic distribution was independent across populations for each locus, an unbiased estimate of *P*-value of probability test was performed as described by RAYMOND and ROUSSET (1995a). In addition, an unbiased estimate of the *P*-value of an F_{ST}-based exact test was conducted to test genotypic differentiation among populations for each locus (RAYMOND and ROUSSET, 1995a). In this case, F_{ST} was estimated by the method of WEIR and COCKERHAM (1984). These exact tests were carried out by the GENEPOP software (RAYMOND and ROUSSET, 1995b). The amount of gene flow (*Nm*) based on the method of SLATKIN (1985) was estimated using the GENEPOP program as well. We also calculated *Nm* by the method of WRIGHT (1951), modified by substituting G_{ST} for F_{ST}. Finally, gene diversity (H_T, H_S, and G_{ST}) of NEI (1973) was obtained using the GST program (LEE and KIM, 1993).

Results

Of the 14 loci, 4 (ACO, GOT-3, MDH-1, and MDH-4) were invariant in every population. One locus (IDH-2) was polymorphic in two populations. Only one population had a private allele (i.e., alleles found in only one population) at one locus (Ohdae population at locus SKDH-1). In most polymorphic loci, a single allele was the most common in all populations, although alternative alleles were more common in at least one population at three different loci (*Table 1*). At the locus SKDH-2, allele frequencies were nearly intermediate in all populations. At the locus SKDH-1, allele *c* was most common in Halla, while allele *b* was most common in the other populations with a frequency of more than 0.800 (*Table 1*). Additionally, in the case of locus MNR-1, the frequency of allele *c* was relatively high in most populations (0.232 to 0.521), while that in Halla was very low (0.014). Actually, only one of 37 trees had the allele *c*. Thus, we could find that the distribution pattern of alleles in Halla seems to be distinct from that of the other populations. On the other hand, allele frequencies at polymorphic loci tended toward a U-shaped distribution (*Fig. 2*), which is in good agreement with the distribution in most conifers (e.g., GURIES and LEDIG, 1982). Allele frequency distributions are generally U-shaped with many alleles at low (<0.05) or high (>0.95) frequency (CHAKRABORTY et al., 1980).

Esimates of polymorphisms and heterozygosity are given in *table 2*. Within populations we detected an average of 1.7 alleles per locus, 60% (45.7% at the 95% (P_{95}) criterion for frequency of the most common allele) of polymorphic loci, 0.172 observed heterozygosity and 0.168 expected heterozygosity. In 4 of 5 populations, observed heterozygosity was slightly higher than expected heterozygosity (in one population, observed heterozygosity was lower than expected heterozygosity), but there were no statistically significant differences in either direction based on comparisons with 95% confidence levels. The heterozygote excess is reflected in a mean of WRIGHT's F_{IS} of –0.049. F_{IS} is a measure of the deviation of the genotypic proportions from HARDY-WEINBERG equilibrium at the population level. Negative values suggest the excess of observed heterozygotes relative to the HARDY-WEINBERG ratio. Conversely, the value of WRIGHT's F_{IT} was positive, but small (0.050). This means a minor heterozygote deficiency at the

Table 1. – Allele frequencies for 10 polymorphic loci in 5 populations of Japanese yew.

Locus/allele	Ohdae (24.2)[1]	Taebaek (19.0)	Sobaek (28.9)	Dukyoo (29.9)	Halla (35.5)
GOT-2/a	.875	.816	.862	.983	.909
b	.125	.184	.138	.017	.091
IDH-1/a	.040	.342	.224	.233	.270
b	.960	.658	.776	.767	.730
IDH-2/a	.000	.026	.000	.033	.000
b	1.000	.974	1.000	.967	1.000
MDH-2/a	.020	.000	.052	.000	.013
b	.980	1.000	.948	1.000	.987
MDH-3/a	.080	.132	.328	.267	.405
b	.920	.789	.672	.717	.595
c	.000	.079	.000	.017	.000
MNR-1/a	.354	.553	.482	.483	.986
b	.125	.211	.286	.033	.000
c	.521	.237	.232	.483	.014
PGI-2/a	.000	.000	.017	.000	.026
b	.980	.947	.948	1.000	.882
c	.020	.053	.034	.000	.092
PGM/a	.020	.053	.000	.000	.000
b	.980	.947	.983	1.000	.947
c	.000	.000	.017	.000	.053
SKDH-1/a	.040	.000	.000	.000	.000
b	.900	.816	.828	1.000	.429
c	.060	.184	.172	.000	.571
SKDH-2/a	.400	.579	.569	.518	.500
b	.600	.421	.431	.482	.500

[1]) Mean number of trees per locus

whole species level. Regardless, these small values for F_{IS} and F_{IT} show that the Japanese yew populations studied here are generally in good agreement with the HARDY-WEINBERG expectations. In fact, genotype frequencies observed for most loci in most populations conformed to the HARDY-WEINBERG expectations. Four of 34 exact tests for polymorphic loci indicated a significant deviation from the HARDY-WEINBERG expectations. In three of four significant tests, populations had a deficiency of heterozygotes (Ohdae at the GOT-2, Taebaek at the PGI-2, and Halla at the PGI-2), in contrast to population Sobaek which had an excess of heterozygotes at SKDH-2. A multi-locus exact test for the HARDY-WEINBERG proportions (ROUSSET and RAYMOND, 1995) in each population showed two significant deviations. Population Sobaek had a excess of heterozygotes, whereas Taebaek had a deficiency of heterozygotes. However, a global test across all loci as well as all populations showed no significant deviations.

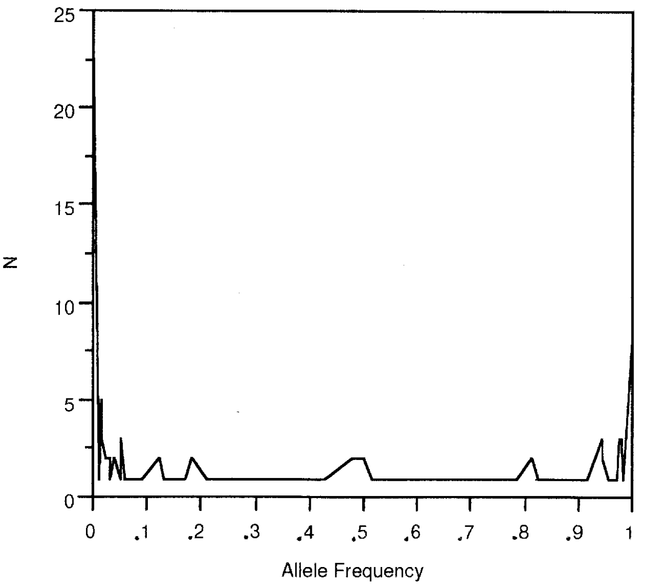

Fig. 2. – The distributions of allele frequencies in Japanese yew.

Total genetic diversity (H_T) in the species, averaged over 10 polymorphic loci, was 0.254, and H_S, the genetic diversity within populations, was 0.230 (*Table 3*). G_{ST} was 0.067, a moderate value for conifers (HAMRICK et al., 1992; LEDIG, 1998). This can be interpreted to mean that 93.3% of the total genetic variation is within populations and 6.7% among populations. Exact tests for genic differentiation and genotypic differentiation indicated significant population differentiation at 6 and 4 polymorphic loci, respectively. The degree of population differentiation was especially high at MDH-3, MNR-1, and SKDH-1 both at allele and genotype levels. This differentiation might be caused by distinctive allelic and genotypic frequency distributions in the Halla population (see *Table 1*).

Estimates of genetic distance (NEI, 1978) among populations also provides an indication of genetic structure of Japanese yew (*Table 4*). Values ranged from 0.000 between Taebaek and Sobaek to 0.062 between Ohdae and Halla. The average was

Table 2. – Genetic diversity estimates for 5 Japanese yew populations. Standard errors appear in parentheses.

Population	A	P_{95}	P_{99}	H_o	H_e
Ohdae	1.8	35.7	64.3	0.137	0.132
	(0.2)			(0.062)	(0.051)
Taebaek	1.8	57.1	64.3	0.180	0.201
	(0.2)			(0.055)	(0.058)
Sobaek	1.8	57.1	64.3	0.226	0.194
	(0.2)			(0.074)	(0.059)
Dukyoo	1.6	28.6	42.9	0.143	0.138
	(0.2)			(0.061)	(0.057)
Halla	1.7	50.0	64.3	0.175	0.174
	(0.2)			(0.059)	(0.056)
Mean	1.7	45.7	60.0	0.172	0.168
Species level	2.1 (0.2)	50.0	71.4	0.172 (0.054)	0.181 (0.054)

A, average number of alleles per locus including monomorphic loci; P_{95}, proportion of polymorphic loci at 95% level (A locus is considered if the frequency of most common allele does not exceed 0.95); P_{99}, proportion of polymorphic loci at 99% level; H_o, observed heterozygosity; H_e, expected heterozygosity (unbiased estimate).

Table 3. – Nei's (1973) genetic diversity for 10 polymorphic loci of the 5 Japanese yew populations.

Locus	H_T	H_S	G_{ST}
GOT-2	0.197	0.191	0.031
IDH-1	0.345	0.325	0.058
IDH-2	0.023	0.023	0.018
MDH-2	0.033	0.032	0.022
MDH-3	0.395	0.367	0.070
MNR-1	0.568	0.475	0.164
PGI-2	0.093	0.090	0.029
PGM	0.056	0.055	0.023
SKDH-1	0.330	0.252	0.235
SKDH-2	0.500	0.491	0.016
Mean	0.254	0.230	0.067

H_T, total genetic diversity of the species; H_S, mean within-population genetic diversity; G_{ST}, the proportion of the total genetic diversity found among populations.

Table 4. – Nei's (1978) unbiased genetic distance estimates among 5 populations of Japanese yew.

Population	Ohdae	Tabaek	Sobaek	Dukyoo	Halla
Ohdae	–	0.013	0.013	0.007	0.062
Taebaek		–	0.000	0.007	0.029
Sobaek			–	0.006	0.029
Dukyoo				–	0.049
Halla					–

0.022. This is a modest value compared with that of other conifers. To better visulaize the results, a dendrogram produced by the UPGMA clustering technique (Sneath and Sokal, 1973) is presented in *figure 3*. This dendrogram shows some decisive geographic trends; the most closely located populations, Taebaek and Sobaek were the most closely related genetically, while the Halla population, the most distinctive population geographically (*Fig. 1*), was the most distinctive genetically. In fact, a positive correlation existed between genetic and geographic distances ($r = 0.794$, $p = 0.006$).

Nm, the number of migrants per generation, estimated from G_{ST} was 3.48. The measure of gene flow based on the number of private alleles after correction, as proposed by Barton and Slatkin (1986), gave an *Nm* estimate of 2.87, similar to the estimate of Wright's (1951). The number of private alleles was one and the average of population sample size, *n,* was 27.5.

Discussion

As compared to other gymnosperms and outcrossing, wind-pollinated woody plants, Japanese yew in Korea possesses similar allozyme variation (A, P, H_e, H_T, H_S; *Tables 2* and *3;* comparisons from Hamrick et al., 1992 and Ledig, 1998); and when the genetic variation maintained by Japanese yew was compared to that of other yew species, it was similar to or slightly higher than that of *T. brevifolia* (A = 1.7; P = 42.3%, H_e = 0.166, El-Kassaby and Yanchuk, 1994; A = 1.5, P = 41.6%, H_e = 0.124, Wheeler et al., 1995), but less than that of *T. baccata* (A = 2.7, H_e = 0.44 (polymorphic loci only); Hertel and Kohlstock, 1996). Japanese yew in Korea generally occurs in rather small, widely scattered populations with low densities. It is generally known that genetic diversity within populations is influenced mainly by the geographic distribution of the species, mating system, the method of seed dispersal, and the methods of reproduction (Hamrick et al., 1992). Among these factors, geographic range seems to be most highly correlated with genetic diversity (Hamrick et al., 1992). That is to say, species with restricted ranges and discontinuous distribution often have low genetic diversity relative to more widespread species with similar life history traits. So, from the distribution pattern of Japanese yew in Korea, we expected a low level of genetic diversity, as previously reported in other yew species with similar geographic distribution and biological traits (Wheeler et al., 1995). However, genetic variation of Japanese yew at the species level (A_s = 2.1, P_s = 71.4, H_{es} = 0.181) is similar to that of other gymnosperms (A_s = 2.38, P_s = 71.1, H_{es} = 0.169; Hamrick et al., 1992) in general, and slightly higher than that of woody plants with regional geographic range (A_s = 1.87, P_s = 55.7, H_{es} = 0.169; Hamrick et al., 1992).

One possible explanation for the moderate level of genetic variation of Japanese yew may be its breeding system; i.e., outcrossing by wind pollination. In addition, inbreeding via selfing cannot occur because Japanese yew is a dioecious species, and monoecious trees are rarely reported (Lee, 1987). Thus, mating

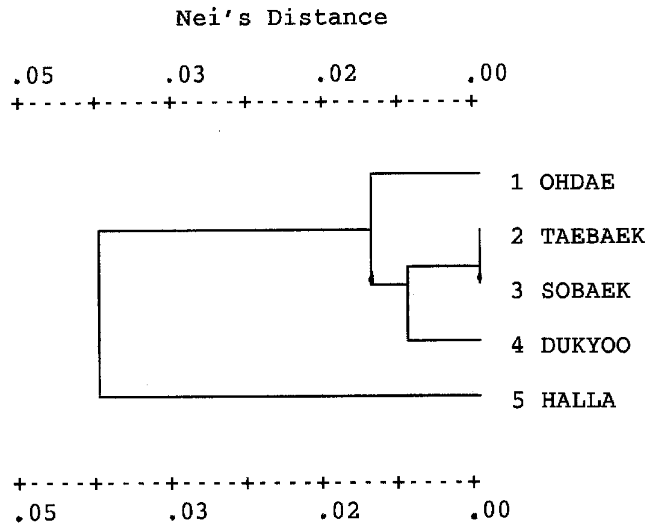

Fig. 3. – UPGMA-derived dendrogram showing the clustering of the five natural populations of Japanese yew based on the genetic distance of NEI (1978).

system might contribute to the maintenance of genetic variation in this species. It is generally known that the genetic diversity value for outcrossing wind-pollinated tree species is higher than that of mixed-mating species, which allow outcrossing as well as selfing (HAMRICK et al., 1992).

The fixation index can be used to estimate the outcrossing rate under the assumption that equilibrium has been reached (ALLARD et al., 1968):

$$t = (1 - F_e)/(1 + F_e),$$

where F_e is the equilibrium fixation index. Assuming F_{IS} represents the equilibrium, t is 1.10. This value suggests that Japanese yew is a completely outcrossing species (actually, outcrossing rates greater than 1.00 are not biologically possible, even though values of t greater than 1.00 are statistically possible). However, we should be very cautious when interpreting this result, because the t value is an indirect estimating (LEDIG et al., 1997). For a better understanding of this issue, we need more detailed specific studies such as the estimation of the outcrossing rates by isozyme analysis of the embryo alongside its megagametophyte. On the other hand, it is generally known that seeds of Japanese yew are dispersed by birds. These vectors could provide long-distance dispersal, and possible establishment of new populations by seeds from relatively few individuals (i.e., founder effect). In this case, inbreeding can occur through consanguineous mating. If inbreeding were significant, observed levels of heterozygosity should be lower than expected. However, in this study, observed levels of heterozygosity were higher than expected in 4 of 5 populations. Additionally, observed heterozygosity was higher than expected at the species level. From these results, we could deduce that inbreeding in Japanese yew is not effective. This is in good agreement with the estimate of t value.

Another possible explanation may be population structure. As already mentioned, Japanese yew occurs rather sporadically in its range in Korea. However, it sometimes occurs in pure stands and many individual trees are widely scattered around denser concentrations. If these scattered individual trees contribute to the effective population size by means of gene flow between individuals within populations and themselves, the level of genetic diversity of this species might be maintained at a level comparable to that of other conifers. Additionally, these scattered individual trees might have played an important role in the yew's genetic structure. In fact, genotype frequencies observed for most polymorphic loci in most populations conformed well to the HARDY-WEINBERG expectations.

WHEELER et al. (1995) reported the low level of genetic diversity in Pacific yew which has ecological characteristics and geographic distribution patterns similar to Japanese yew. They suggested that inbreeding and genetic drift (and/or founder effect) might be critical reasons for the lack of genetic diversity. However, in Japanese yew, we could find no evidence for inbreeding or genetic drift. For example, the distribution of allele frequencies of Japanese yew showed the typical U-shaped distribution (*Fig. 2*) which is commonly observed in conifers (CHAKRABORTY et al., 1980). When a species is influenced by genetic drift, the distribution of allele frequencies may approach a uniform distribution (LEDIG et al., 1997). Besides, populations that have experienced a recent reduction of their effective population size generally show a correlative reduction of allele numbers and gene diversity (H_e, or HARDY-WEINBERG heterozygosity) at polymorphic loci (CORNUET and LUIKART, 1996; LUIKART and CORNUET, 1998). In this case, the allele number is reduced faster than the gene diversity. Thus, in a recently reduced population and/or a recently expanded population after a reduction (i.e., bottlenecked population), the observed gene diversity (H_e) is higher than the expected equilibrium gene diversity (H_{eq}) which is computed from the observed number of alleles under the assumption of a constant-size (equilibrium) population (CORNUET and LUIKART, 1996; LUIKART and CORNUET, 1998). In the meantime, in a population at mutation-drift equilibrium (i.e., the effective size of which has remained constant in the recent past), there is approximately an equal probability that a locus shows a gene diversity excess or a gene diversity deficit. We conducted the WILCOXON sign-rank test to determine whether each population studied here shows a significant number of loci with gene diversity excess under three different models; Infinite Allele Model (IAM), Stepwise Mutation Model (SMM), and Two-phased Model of Mutation (TPM) (for more details, see LUIKART and CORNUET, 1998). For this test, we used the BOTTLENECK computer program (CORNUET and LUIKART, 1996). From this analysis, we could not find a significant excess of heterozygosity ($H_e > H_{eq}$) in all populations under all three models. Consequently, we could deduce that Japanese yew populations have not recently experienced either a severe reduction in population size (genetic drift) or a bottleneck effect, although we do not have records on the historical population sizes of Japanese yew. The lack of linkage disequilibrium (only six (3.9%) of 154 two-locus combination in disequilibrium) also suggests that genetic drift and/or inbreeding have not effectively affected the genetic structure of Japanese yew (LEDIG et al., 1999).

With Japanese yew in Korea, more than 93% of genetic variation resided within populations (G_{ST} = 0.067, see *Table 3*). Generally, the level of allozyme diversity is influenced by ecological and life-history characteristics of species, and significantly correlated with those traits (HAMRICK et al., 1992); namely, woody species with widespread distributions, outcrossing mating systems, and widely dispersed seeds (by means of wind and animal vectors) tend to have more genetic variation within populations and less variation among populations than species with other combinations of traits. The mean G_{ST} value for Japanese yew is comparable to that of woody plants with similar ecological traits as well as similar life history (G_{ST} = 0.073 for gymnosperm; G_{ST} = 0.065 for regional geographic range; G_{ST} = 0.077 for outcrossing-wind breeding system; G_{ST} = 0.051 for seed dispersal by animal ingestion, see HAMRICK et al., 1992). On the other hand, the G_{ST} value for Japanese yew is much lower than that of Pacific yew (G_{ST} = 0.104; Wheeler et al., 1995). As mentioned before, Japanese yew in Korea has wide-spread populations in its native range, even though the size of those populations is relatively small. In addition, it has numerous individual trees widely scattered around populations. These small, but widely distributed populations as well as individual trees may be a plausible explanation for the lower degree of genetic differentiation of Japanese yew compared to that of Pacific yew; namely, it is likely that those two factors have functioned as a bridge increasing gene flow among populations (i.e., corridor effect). Actually, when we excluded the Halla population, the most distinctive genetically as well as geographically (*Fig. 1* and *3*), from the analysis, the G_{ST} value (= 0.037) for Japanese yew was reduced by half. The moderate G_{ST} value resulted in a moderate estimate of gene flow (Nm = 3.48 based on Wright's (1951) method and Nm = 2.87 based on Slatkin's (1985) method) compared with that of wind-pollinated tree species (Govindaraju, 1988).

A positive correlation existed between genetic and geographic distance (r = 0.794, p = 0.006): the populations in close geographic proximity, tended to cluster into the same group. So, we tested "Isolation by Distance" according to the method of Slatkin (1993). For this test, we calculated $\hat{M}$ (Slatkin, 1993) based on θ (Weir and Cockerham, 1984) as well as G_{ST} (Nei, 1973) for each pair of populations. Then we estimated values of regression coefficients of log($\hat{M}$) against log(d), where d is the geographic distance ($\log_{10}(\hat{M}) = a + b\log_{10}(d)$, see Slatkin, 1993). A log-log graph of $\hat{M}$ against distance would be approximately linear in a population at equilibrium under restricted dispersal (Slatkin, 1993). *Figure* 4 shows plots of all pairwise $\hat{M}$ values against geographic distance. This shows that there is an apparent pattern of isolation by distance. The slopes of the regression of $\log_{10}(\hat{M})$ on $\log_{10}$(*distance*) are –0.782 when using θ and –0.702 when using G_{ST}, respectively, and the regression line for each case θ and G_{ST}) explains 43.5% and 66.6% of the variation in the data, respectively. The above results suggest that Japanese yew in Korea does reveal isolation by distance as if it were at equilibrium under dispersal and genetic drift. However, without direct observations of dispersal, we cannot draw a firm conclusion about the population structure of this species. Regardless, the above results suggest a possible hypothesis about population structure, namely that Japanese yew is at an approximate equilibrium under certain levels of gene flow that is geographically restricted to some extent. As a result, we can be reasonably sure that long distance dispersal is not sufficiently common to prevent isolation by distance. Actually, gene flow between Halla and other populations must have been very difficult due to the geographic distance (see *Fig. 1*), even though Japanese yew allows outcrossing by wind-pollination and long-distance seed dispersal by birds. We can also be reasonably certain that Japanese yew has not colonized its current habitat very recently, because it would not have had sufficient time for isolation by distance to become apparent (Slatkin, 1993).

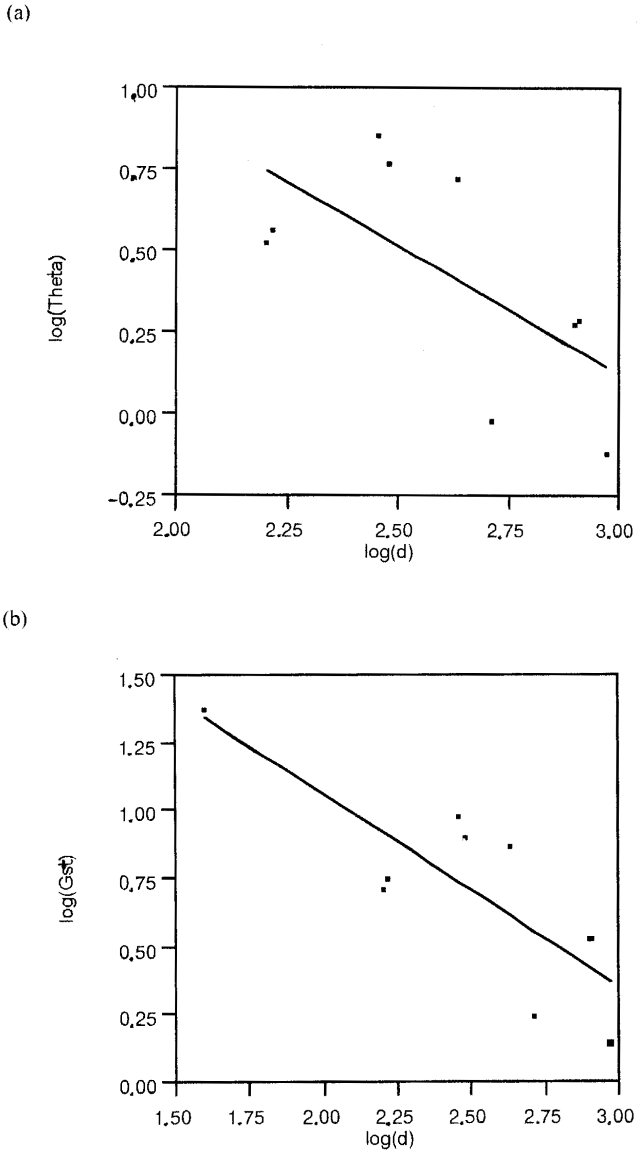

Fig. 4. – $\text{Log}_{10}(\hat{M})$ based on Weir and Cockerham's θ (a) and Nei's G_{ST} (b) plotted against $\log_{10}$(*distance*) for the Japanese yew. The lines were generated using a conventional regression: $log(\hat{M}) = 2.473 - 0.782\ log(d)$ with an R^2 value of 0.435 for θ and $log(\hat{M}) = 2.467 - 0.702\ log(d)$ with an R^2 value of 0.666 for G_{ST}.

Selection (even though isozymes are generally considered selectively neutral), drift by isolation, colonization and/or founder effect might be explanations for the distinctness of the Halla population. The Halla population is located on Cheju island, the most southern part of Korea. The climate of Cheju island is very different from the inland areas. So, environmental factors might function as a selection force. In addition, gene flow from other populations inland may be restricted. If that is the case, then the Halla population has a unique genetic structure. We can find evidence for this issue from the distribution pattern of alleles and genotypes. For instance, at the locus SKDH-1, allele c was most common in Halla, while allele b was most common in the other populations with a frequency of more than 0.800 (*Table 1*). And the frequency of genotype bc (0.571) at Halla was much higher than that of the other populations. Additionally, in the case of locus MNR-1, the frequency of allele c was relatively high in most populations (0.232 to 0.521), while at Halla it was very low (0.014); only one of 37 trees had the allele c as heterozygote ac, while the other 36 trees were homozygotes, aa. In addition, allele b of MNR-1 was not found at Halla, whereas the other populations had it with a frequency of 0.033 to 0.286.

In situ conservation efforts could be focused on a population such as Sobaek with high genetic diversity as measured by number of alleles per locus, percent polymorphic loci and expected heterozygosity. In fact, Sobaek has been preserved, designated as a natural protection forest. However, given the fact that genetic variation in Japanese yew is distributed more or less randomly among populations, sampling or preservation of any one population may not insure that all the variation in Japanese yew is captured. In other words, we should consider that several populations are probably worthy of conservation;

especially, the Halla population, because of its unique genetic structure. Finally, we can anticipate a restoration program for populations destroyed by natural and/or man-made disturbances. Some populations, such as Dukyoo in this study have been severely disturbed recently, by the construction of a ski resort and other activities. In this case, we can collect seeds, grow seedlings and transplant them back to the disturbed sites. If the seedlings survive and mature, this would help maintain an effective population size.

Acknowledgement

This study was funded by the Forestry Research Institute of the Republic of Korea. The authors are thankful for the helpful reviews of this manuscript by Dr. F. T. Ledig, and an anonymous reviewer. We also thank D. Johnson for English correction.

Literature

Allard, R. W., Jain, S. K. and Workman, P. L.: The genetics of inbreeding populations. Adv. Genet. **14**, 55–131 (1968). — Barton, N. H. and Slatkin, M.: A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. Heredity **56,** 409–415 (1986). — Chakraboraty, R., Fuerst, P. A. and Nei, M.: Statistical studies on protein polymorphism in natural populations. III. Distribution of allele frequencies and the number of alleles per locus. Genetics **94**, 1039–1063 (1980). — Conkle, M. T., Hodgskiss, P. D., Nunally, L. B. and Hunter, S. C.: Starch gel electrophoresis of pine seed: a laboratory manual. U.S. Forest Service General Technical Report PSW-64 (1982). — Cornuet, J. M. and Luikart, G.: Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics **144**, 2001–2014 (1996). — El-Kassaby, Y. A. and Yanchuk, A. D.: Genetic diversity, differentiation and inbreeding in Pacific yew from British Columbia. J. Hered. **85**, 112–117 (1994). — Govindaraju, D. R.: Relationship between dispersal ability and levels of gene flow in plants. Oikos **52**, 31–35 (1988). — Guries, R. P. and Ledig, F. T.: Genetic diversity and poulation structure in pitch pine (*Pinus rigida* Mill.). Evolution **36**, 387–402 (1982). — Göçmen, B., Jermstad, K. D., Neale, D. B. and Kaya, Z.: Development of random amplified polymorphic DNA markers for genetic mapping in Pacific yew (*Taxus brevifolia*). Can. J. For. Res. **26**, 497–503 (1996). — Hamrick, J. L., Godt, M. J. W. and Sherman-Broyles, S. L.: Factors influencing levels of genetic diversity in woody plant species. New Forests **6**, 95–124 (1992). — Hertel, H.: Vererbung von Isoenzymmarkern bei Eibe (*Taxus baccata* L.). Silvae Genetica **45**, 284–290 (1996). — Hertel, H. and Kohlstock, N.: Genetische Variation und geographische Struktur von Eibenvorkommen (*Taxus baccata* L.) in Mecklenburg–Vorpommern. Silvae Genetica **45**, 290–294 (1996). — Kim, Z. S., Lee, S. W., Lim, J. H., Hwang, J. W. and Kwon, K. W.: Genetic diversity and structure of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Korea. Forest Genetics **1**, 41–49 (1994). — Ledig, F. T.: Heterozygosity, heterosis, and fitness in outbreeding plants. In: Soule, M. E. (Ed.): Conservation Biology: The Science of Scarcity and Diversity. Sinauer, Sunderland, MA. pp. 77–104 (1986). — Ledig, F. T.: Genetic variation in *Pinus,*. In: Richardson, D.M. (Ed.): Ecology and Biogeography of *Pinus.* Cambridge University, pp. 251–280 (1998). — Ledig, F. T., Conkle, M. T., Bermejo-Velázquez, B., Eguiluz–Piedra, T., Hodgskiss, P. D., Johnson, D. R. and Dvorak, W. S.: Evidence for an extreme bottleneck in a rare Mexican pinyon: genetic diversity, disequilibrium, and the mating system in *Pinus maximartinezii.* Evolution **53**, 91–99 (1999). — Ledig, F. T., Jacob-Cervantes, V., Hodgskiss, P. D. and Eguiluz-Piedra, T.: Recent evolution and divergence among populations of a rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. Evolution **51**, 1815–1827 (1997). — Lee, S. W. and Kim, Z. S.: Computer program for hierarchical analysis of gene diversity. Korean J. Genet. **15**, 379–384 (1993). — Lee, T. B.: Dendrology. Hyang Moon Sa, Seoul. 331 pp. (in Korean) (1987). —Lewandowski, A., Burczyk, J. and Mejnartowicz, L.: Genetic structure of English yew (*Taxus baccata* L.) in the Wierzchlas Reserve: implications for genetic conservation. Forest Ecology and Management **73**, 221–227 (1995). — Luikart, G. and Cornuet, J. M.: Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology **12**, 228–237 (1998). — Nei, M.: Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA **70**, 3321–3323 (1973). — Nei, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics **89**, 583–590 (1978). —Raymond, M. and Rousset, F.: An exact test for population differentiation. Evolution **49**, 1280–1283 (1995a). — Raymond, M. and Rousset, F.: GENEPOP (ver. 1.2) : A population genetics software for exact test and ecumenicism. J. Hered. **86**, 248–249 (1995b). — Rousset, F. and Raymond, M.: Testing heterozygote excess and dificiency. Genetics **140**, 1413–1419 (1995). — Slatkin, M.: Rare alleles as indicators of gene flow. Evolution **39**, 53–65 (1985). — Slatkin, M.: Isolation by distance in equilibrium and non–equilibium populations. Evolution **47**, 264–279 (1993). — Sneath, P. H. A. and Sokal, R. R.: Numerical Taxonomy. Freeman, San Francisco, CA. 573 pp. (1973). — Swofford, P. L. and Selander, R. B.: BIOSYS–1: A computer program for the analysis of allelic variation in population genetic and biochemical systematics. Release 1.7. User's Manual. Illinois Natural History Survey, IL. USA (1989). — Weir, B. S. and Cockerham, C. C.: Estimating *F*-statistics for the analysis of population structure. Evolution **38**, 1358–1370 (1984). — Wheeler, N. C., Jech, K. S., Masters, S. A., O'Brien, C. J., Timmons, D. W., Stonecypher, R. W. and Lupkes, A.: Genetic variation and parameter estimates in *Taxus brevifolia* (Pacific yew). Can. J. For. Res. **25**, 1913–1927 (1995). — Wright, S.: The genetical structure of populations. Ann. Eugen. **15**, 323–354 (1951). — Wright, S.: The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution **19**, 395–420 (1965).

Analysis of Half-diallel Mating Design with Missing Crosses: Theory and SAS Program for Testing and Estimating GCA and SCA Fixed Effects

By H. X. Wu and A. C. Matheson[1])

(Received 17th January 2000)

Abstract

The half-diallel mating design, particularly a series of disconnected half-diallels has been widely adopted as a mating design for estimating genetic parameters and for future selection in many commercially important tree species. Standard commercial statistical packages do not allow direct specification of the linear model associated with the half-diallel design and therefore are not capable of analysing diallel mating designs, even for balanced diallel matings (no missing crosses). Published special computer programs for diallel analyses do not provide an adequate solution for GCA and SCA fixed effects in diallels with missing crosses. This paper presents the least squares theory for analysing half-diallel mating designs with missing crosses, and a SAS computer program (DIAFIXED.SAS), developed to test the significance of GCA and SCA effects and estimate the GCA and SCA fixed effects. The program is flexible enough to accommodate different number of parents, multiple environments and missing individual trees as well as missing whole plots. The DIAFIXED.SAS output includes (1) hypothesis testing for GCA and

---

[1]) CSIRO, Division of Forestry and Forest Products, PO Box E4008, Kingston, ACT 2604, Australia