

Genetic Variation of *Juglans sinensis* in Korea

By S. W. LEE¹⁾ and M. H. LEE

Forest Genetics Research Institute, Suwon 441-350, Rep. of Korea

(Received 12th November 1996)

Abstract

The genetic diversity and structure of 8 Korean populations of *Juglans sinensis* (D.C.) DODE, an introduced and a semi-domesticated crop plant, was examined. Korean *J. sinensis* populations had notably less allozyme diversity than most other tree species with similar ecological characteristics, however, showed similar level of genetic diversity in Italian populations of *J. regia*. For example 33.7% of the 13 loci examined were polymorphic, the mean number of alleles per locus was 1.40, and mean expected heterozygosity was 0.088. A considerable high level of heterozygote deficiency was observed in Korean populations of *J. sinensis* (mean F_{IS} = 0.156). About 12% of the total genetic variation was found among populations (mean F_{ST} = 0.122). Indirect estimated of the number of migrants per generation (N_m = 1.80) indicated that gene flow among populations was relatively low. There was little relationship between geographic and genetic distance between pairs of populations. Factors contributing to the low level of genetic diversity and moderate level of genetic differentiation found in Korean *J. sinensis* populations include founder effect, some level of inbreeding, the narrow and discontinuous distribution and probably man's impacts.

Key words: allozyme, *Juglans sinensis*, genetic diversity, genetic structure.

FDC: 165.3; 165.5; 176.1 *Juglans sinensis*; (519.5).

Introduction

The genus *Juglans*, a long-lived angiosperm, is an economically important tree in Northern hemisphere because of its fruit and wood production. It is generally considered that it includes 15 species from South Europe to East Asia as well as North and South America (KRÜSSMAN, 1986). Of these, *Juglans regia*, the Persian or English walnut and its varieties have been widely cultivated. In Korea, *J. sinensis* (D.C.) Dode known as the natural hybrid between *J. mandshurica* and *J. regia* (SLATE, 1981) has been cultivated since long. According to the information by word of mouth and some records, it was introduced from China around 700 to 800 years ago and then spread over the country. However, the exact introduction time is unknown. Traditionally in Korea, *J. sinensis* has been planted as a single tree or small groups of trees near farms or on the borders of drainages and roads without structural order. However, as farmers have interest in walnut cultivation due to its dual function of wood and fruit production as well as its usefulness for reforestation and/or reconversion of wasted agricultural land, it has been widely and systematically planted all over the country since the beginning of 1980's and consequently the plantation area reaches 13,000 ha at the present. Additionally, the Forest Genetics Research Institute (FGRI) has developed breeding programs for the production of better quality fruits since 1975.

An assessment of genetic variability in *J. sinensis* is needed to verify the availability of genetic diversity, which is required

for continued crop improvement. However, little genetic information using isozymes and/or DNA markers is available for the *Juglans* tree species (ARULSEKAR and PARFITT, 1986; ARULSEKAR et al., 1985, 1986; MCGRANAHAN et al. 1986; ALETÀ et al., 1989, 1993; GERMAIN et al., 1993; MALVOLTI et al., 1993; SOLAR et al., 1993; FJELLSTROM et al., 1994). The purpose of this study was to examine genetic variation in *J. sinensis* throughout its range in Korea employing isozyme marker. Specific objectives were (1) to describe how genetic variation is distributed within and among populations and (2) to compare the results for *J. sinensis* with previous reports for other woody species including *Juglans* species.

Materials and Methods

A total of 235 individuals from 8 populations were selected from the geographical range of *J. sinensis* in Korea (Fig. 1). The individuals collected were ca. 8 m to 20 m high and 30 to 70 years old depending on the population. On average, each area that was defined as a population covered about 3 ha. Early spring in 1996, we collected dormant vegetative buds individually, with more than 30 m between sampled trees, to avoid erroneous sampling of half-sib and/or identical ramet individuals. Samples were placed on ice immediately, returned

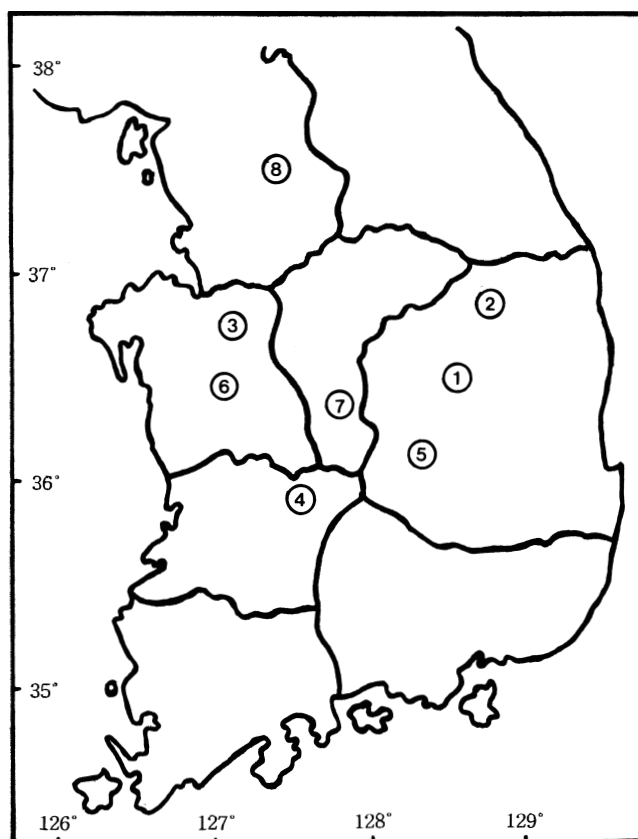


Fig. 1. - Distribution of the *J. sinensis* populations examined in Korea (numerical codes as in Table 1).

¹⁾ To whom correspondence should be addressed.

to the laboratory at the FGRI within 4 days and stored at 5 °C until enzyme extraction.

Enzyme extraction from buds was performed according to LEE et al. (1995). Isozyme analysis was carried out by a similar procedure described by KIM et al. (1993) and CONKLE et al. (1982). Horizontal starch gel electrophoresis was used for analysis of 6 enzyme systems: aconitase (*Aco*: EC 4.2.1.3); malate dehydrogenase (*Mdh-1*, *Mdh-2*, and *Mdh-3*: EC 1.1.1.37); menadien reductase (*Mnr-1*, *Mnr-2*, and *Mnr-3*: EC 1.6.99.2); phosphoglucose isomerase (*Pgi-1* and *Pgi-2*: EC 5.3.1.9); 6-phosphogluconate dehydrogenase (*6Pg-1* and *6Pg-2* EC 1.1.1.44); and shikimate dehydrogenase (*Skdh-1* and *Skdh-2*: EC 1.1.1.25). It was not possible to confirm patterns of inheritance for these enzymes owing to the lack of controlled-bred of half-sib progenies. Genetic control of polymorphic enzymes was inferred, however, from observation of functional subunit numbers in heteromultimer types of putative heterozygotes, and published reports of work with these enzymes in other walnuts and angiosperms. Loci and alleles were numbered according to the mobility of the protein they encoded from the fastest to the slowest one. Thirteen putative loci with a total of 21 alleles were inferred for these 6 enzyme systems.

Calculation of parameters of intrapopulation genetic diversity (mean number of alleles per locus, A , percentage of polymorphic loci, P , expected and observed heterozygosity, H_e and H_o , respectively), WRIGHT's F -statistics (F_{IS} , F_{IT} and F_{ST} by WRIGHT, 1978) and genetic distances, clustering and construction of dendrograms of walnut populations were carried out using the computer program BIOSYS-1 (SWOFFORD and SELANDER, 1981). Mean effective number of alleles per locus or gene pool diversity (A_e or v , GREGORIUS, 1987), hypothetical gametic multilocus diversity (v_{gam} , GREGORIUS et al., 1986), GREGORIUS' distance (d_o , GREGORIUS, 1974), and subpopulation genetic differentiation of the gene pool (D_j and δ , GREGORIUS, 1984; GREGORIUS and ROBERDS, 1986) were computed using the GSED program (GILLET, 1994).

With our data we estimated different measures of genetic distance and used several methods of clustering, but almost all dendrograms resulted in nearly the same topology. Thus, we will present the most commonly used measures: NEI's (1978) genetic distance D , and GREGORIUS' distance d_o , and the UPGMA-dendrogram based on the D matrix (SNEATH and SOKAL, 1973).

Departures of observed heterozygote frequencies from HARDY-WEINBERG expectations for each polymorphic locus in each population were estimated with WRIGHT's fixation index, $F=1-H_o/H_e$ (WRIGHT, 1965). These values were tested for significance with X^2 analysis, $X^2=NF^2(a-1)$ with $df=a(a-1)/2$, where N is the total sample size and a is the number of alleles at a locus (LI and HORVITZ, 1953).

Null hypotheses ($F_{IS}=0$, $F_{IT}=0$ and $F_{ST}=0$) were tested using: $X^2=NF_{IS}^2$, $df=1$; $t=|F_{IT}|\sqrt{N}$, $df=infinity$; $X^2=2NF_{ST}$, $df=no. of populations-1$, where N is total number of individuals (LEE, 1994).

Gene flow (N_m) was calculated indirectly by the method of WRIGHT (1951): $N_m=(1-F_{ST})/4F_{ST}$, where N is the effective population size, m is the proportion of the population replaced by migrants every generation, N_m is the number of migrants per generation.

Results

Six of the 13 loci studied were monomorphic in all populations (*Aco*, *Mdh-3*, *Mnr-2*, *Mnr-3*, *Pgi-1*, and *Pgi-2*). The existence of 2 or 3 alleles was recognized at all polymorphic loci

Table 1. – Allele frequencies, WRIGHT's (1965) fixation index (F), test for deviation from HARDY-WEINBERG proportions based on LI and Horvitz's (1953) X^2 tests and sample size (N) for 7 polymorphic loci in 8 populations of *J. sinensis*.

Locus	Population							
	1	2	3	4	5	6	7	8
<i>Mdh-1</i>								
(N)	27	30	30	30	30	30	28	28
<i>a</i>	.000	.000	.000	.100	.033	.000	.054	.000
<i>b</i>	1.000	1.000	1.000	.900	.967	1.000	.946	1.000
F	-	-	-	-.111	-.034	-	-.057	-
<i>Mdh-2</i>								
(N)	27	30	30	29	30	30	30	28
<i>a</i>	.000	.000	.000	.034	.000	.000	.000	.000
<i>b</i>	1.000	1.000	1.000	.931	1.000	1.000	1.000	1.000
<i>c</i>	.000	.000	.000	.034	.000	.000	.000	.000
F	-	-	-	-.055	-	-	-	-
<i>Mnr-1</i>								
(N)	27	30	30	30	30	30	29	28
<i>a</i>	.037	.000	.033	.067	.133	.050	.190	.411
<i>b</i>	.963	1.000	.967	.933	.867	.950	.810	.589
F	-.038	-	-.034	-.071	-.154	-.053	.215	.484*
<i>6Pg-1</i>								
(N)	27	30	30	28	30	25	29	25
<i>a</i>	.093	.000	.033	.161	.100	.060	.086	.100
<i>b</i>	.907	1.000	.967	.839	.900	.940	.914	.900
F	.339	-	1.000*	.338	-.111	-.064	.343	.333
<i>6Pg-2</i>								
(N)	27	30	30	30	30	25	21	26
<i>a</i>	.204	.333	.500	.167	.333	.140	.619	.769
<i>b</i>	.796	.667	.500	.833	.667	.860	.381	.231
F	.429*	.100	.467*	.520*	-.050	.169	-.010	-.083
<i>Skdh-1</i>								
(N)	27	28	30	30	30	30	28	17
<i>a</i>	.056	.089	.050	.083	.000	.000	.000	.147
<i>b</i>	.944	.911	.950	.917	1.000	1.000	1.000	.853
F	.647*	-.098	-.053	.345	-	-	-	.297
<i>Skdh-2</i>								
(N)	27	28	30	30	30	28	21	23
<i>a</i>	.574	.768	.900	.883	.883	.875	.762	.891
<i>b</i>	.426	.232	.100	.117	.117	.125	.238	.109
F	.167	.299	-.111	.191	.191	-.143	-.050	-.122

1:Yecheon, 2:Ponghwa, 3:Cheonwon, 4:Muju, 5:Kimcheon, 6:Kongju, 7:Youngdong, 8:Kwangju, * $p < 0.05$

(Table 1). One locus (*Mdh-2*) exhibited polymorphism in one population only, based on so-called area specific allele (*Mdh-2^a*:0.034 and *Mdh-2^c*:0.034). In all loci a single allele was the most common in all populations, although alternate allele was more common in 2 populations at *6Pg-2*.

On average, 37.5% (P_{99}) of the loci were polymorphic within populations (33.7% if the 95% (P_{95}) criterion for frequency of the most common allele was used, Table 2). Across all loci, A ranged from 1.2 to 1.6. Population mean was 1.4. The mean effective number of alleles (A_e) or gene pool diversity (v) varied between 1.05 and 1.13 with a mean of 1.10 which was slightly less than A . Expected heterozygosities (H_e) ranged from 0.052 to 0.115 with a mean of 0.088, while observed heterozygosities (H_o) showed a range of 0.049 and 0.101 with a mean of 0.074. The hypothetical gametic multilocus diversities (v_{gam}) was found to range between 2.100 and 6.068 (mean value=4.100).

Slightly more than 48% of fixation indices were positive (19/39), and 6 of those departed significantly from zero ($p < 0.05$; see Table 1). On the contrary, of 20 negative fixation indices none was significantly different from zero.

WRIGHT's F coefficients showed that significant deficiencies of heterozygotes exist for 4 and 5 of the 7 polymorphic loci at the population level (mean F_{IS} =0.156) and the sample as a whole (mean F_{IT} =0.258), respectively (Table 3). The F_{ST} values ranged from 0.028 to 0.191 (Table 3), and overall, about 88% of the total variation in the species was common to all populations.

Table 2. – Genetic variation for 8 populations of *J. sinensis* (Standard errors in parentheses).

Population	<i>A</i>	<i>A_e</i>	<i>v_{gam}</i>	<i>P₉₉</i>	<i>P₉₅</i>	<i>H_o</i>	<i>H_e</i>	<i>D_j</i>
1	1.4 (.1)	1.10	4.189	38.5	30.8	0.063 (.033)	0.091 (.044)	0.048
2	1.2 (.1)	1.08	3.340	23.1	23.1	0.064 (.036)	0.075 (.043)	0.032
3	1.4 (.1)	1.08	3.079	38.5	23.1	0.049 (.025)	0.071 (.040)	0.032
4	1.6 (.2)	1.12	4.517	53.9	53.8	0.081 (.023)	0.105 (.031)	0.051
5	1.4 (.1)	1.10	3.844	38.5	30.8	0.090 (.041)	0.088 (.039)	0.019
6	1.3 (.1)	1.05	2.100	30.8	30.8	0.052 (.024)	0.052 (.025)	0.039
7	1.4 (.1)	1.12	5.665	38.5	38.5	0.101 (.045)	0.110 (.047)	0.041
8	1.4 (.1)	1.13	6.068	38.5	38.5	0.088 (.036)	0.115 (.047)	0.080
Mean	1.4	1.10	4.100	37.5	33.7	0.074	0.088	δ=0.042

A, the mean number of alleles; *A_e*, the effective number of alleles per locus; *v_{gam}*, hypothetical gametic multilocus diversity; *P₉₉*, percent of polymorphic loci at 99% criterion; *P₉₅*, percent of polymorphic loci at 95% criterion; *H_o*, the observed heterozygosity; *H_e*, the heterozygosity expected under HARDY-WEINBERG; *D_j* and *δ*, subpopulation genetic differentiation of the gene pool.

Table 3. – Heterogeneity *X*² values (with degrees of freedom) and WRIGHT's *F*-statistics.

Locus	<i>F_{IS}</i> ^a	<i>F_{IT}</i> ^b	<i>F_{ST}</i> ^a	<i>X</i> ² (df)
Mdh-1	-.081	-.024	.053**	24.632(7)**
Mdh-2	-.055	-.007	.046**	28.520(14)*
Mnr-1	.178**	.305**	.155**	71.245(7)**
6Pg-1	.264**	.285**	.028	13.228(7)
6Pg-2	.178**	.336**	.191**	79.745(7)**
Skdh-1	.227**	.265**	.048**	20.084(7)**
Skdh-2	.079	.147*	.074**	32.753(7)**
Mean	.156**	.258**	.122**	Total=270.206(56)**

F_{IS} and *F_{IT}*, deviations of genotype frequencies from HARDY-WEINBERG expectations within each population and over all populations, respectively; *F_{ST}*, proportion of the total genetic diversity partitioned among populations.

*) p < 0.05, **) p < 0.01

a) Significances of *X*² tests

b) Significances of *t*-test

At *6Pg-2*, *6Pg-2^b* was the most common allele in 6 populations, while in 2 populations, alternate allele (*6Pg-2^a*) was most common. This is why the *6Pg-2* has large F_{ST} value. Significant differences among populations in allele frequencies were evident for 6 of 7 polymorphic loci (Table 3). WRIGHT's (1951) estimate of the number of migrants per generation based on the mean F_{ST} gave N_m value of 1.80. As can be seen from the last column in table 2, D_j values differed from each other: the maximum value was evident for Kwangju population ($D_s=0.080$), the minimum value for Kimcheon population ($D_s=0.019$). This means that sample No. 8 is more differentiated than each of the remaining samples. Namely, it contains a smaller part of the common genetic information of the respective complement samples than any other sample. The opposite is true for sample No. 5. The gene pool differentiation in the whole investigated area (δ) was 0.042.

NEI's (1978) measure of genetic distance (D) among populations ranged from 0.001 to 0.047 (Table 4) with a mean of 0.013, which is well within the range of values expected for conspecific populations (CRAWFORD, 1989). On the other hand, GREGORIUS' genetic distance (d_o) averaged 0.054 (range: 0.028 to 0.104).

Table 4. – NEI's genetic distance estimates (D) among 8 populations of *J. sinensis* (below diagonal) and GREGORIUS' genetic distance (d_o ; above original).

Population	1	2	3	4	5	6	7	8
1 Yecheon	*****	.037	.053	.049	.049	.036	.067	.104
2 Ponghwa	.004	*****	.031	.053	.036	.038	.055	.087
3 Cheonwon	.015	.003	*****	.055	.033	.037	.044	.063
4 Muju	.008	.005	.010	*****	.039	.031	.075	.096
5 Keumreung	.009	.003	.002	.002	*****	.028	.038	.069
6 Kongju	.007	.004	.010	.001	.002	*****	.062	.092
7 Youngdong	.018	.009	.003	.020	.006	.020	*****	.055
8 Kwangju	.047	.031	.017	.042	.023	.045	.007	*****

In general, there was little relationship between geographic and genetic distance between pairs of populations (Fig. 2). The most distinctive populations were Youngdong (No. 7) and Kwangju (No. 8); the average distances (D) between these populations from all other populations were 0.012 and 0.030, respectively. UPGMA dendrogram based on GREGORIUS' genetic distance showed the same pattern. Also, populations in very close geographic proximity were often genetically relatively distinct. Most groupings simply did not exhibit predictable geographic trends.

Discussion

In the comparison of data, problems arise especially due to different sampling methods, population and site characteristics, choice of gene loci, inclusion of monomorphic gene loci and due to deviating concepts in the quantification of genetic variation. Additionally, *J. sinensis* in Korea is an introduced and a semi-domesticated crop plant rather than a wild species. Accordingly, direct comparison of genetic structure to that of natural populations may not be straightforward. However, the level of genetic variation of *J. sinensis* in Korea is likely to be very low as compared with that of similar life-history and ecological characteristics (A , A_e , P , H_e ; Table 2, comparisons from HAMRICK et al., 1992). In addition, the estimates of intra-population genetic diversity in Korean *J. sinensis* are at the

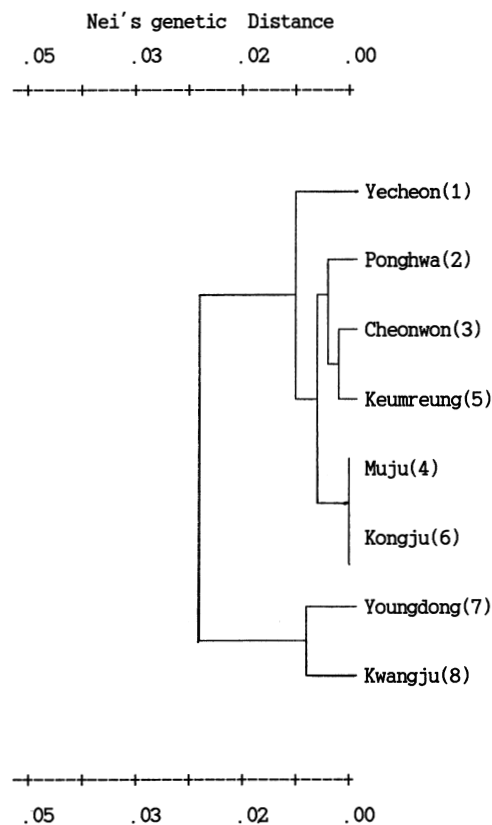


Fig. 2. – UPGMA dendrogram based on NEI's (1978) genetic distance among 8 populations of *J. sinensis*.

extreme low end of the range for values for other tree species summarized by LEDIG (1986). On the other side, the level of genetic diversity of Korean walnut populations is similar to that of Italian walnut (*J. regia*) populations ($A=1.37$; $A_e=1.163$; $P_{99}=31.3\%$; $H_o=0.141$; $H_e=0.142$; MALVOLTI et al., 1993).

As a possible explanation for the low level of genetic variation found in Korean *J. sinensis* populations, we could consider the introduction history. As described before, the Korean *J. sinensis* was introduced from China. At that time, if our naturalized populations originated from only a few introduced genotypes, the genetic diversity within a population might have been reduced due to founder effect. Secondly, we could consider the typical structure of walnut stands in Korea and the flowering habits. *J. sinensis* is a monoecious, dicogamous and wind-pollinated tree species. According to LUZA et al. (1988), *J. regia* is a self-compatible species. In addition, in the case of *J. nigra*, environmental conditions may cause a temporal overlap in maturation of male and female flowers, and consequently self-pollination can be successful. Furthermore, the frequency of cross-pollination depends only on the proportion of foreign pollen in the pollen cloud (RINK et al., 1989). If these are the case in *J. sinensis*, inbreeding via selfing presumably does occur, although it is unclear what level of inbreeding occurs through selfing. Besides, the spatial distribution of trees in open land and in the countryside can increase the rates of self-pollination with reduction of genetic diversity.

Most *J. sinensis* populations examined here showed a slight deficiency of heterozygotes. In 6 of 8 populations, observed heterozygosities were less than expected values; and for individual loci in each population, all significant deviations

from HARDY-WEINBERG expectations were deficiencies of heterozygotes. It is highly probable that inbreeding might occur within *J. sinensis* populations as described before. In addition, there might be limited gene flow due to the mode of seed (nut) dispersal. Nuts are dispersed by gravity and animals. These may have made a contribution to the low level of genetic diversity and a increase of homozygotes. On the other hand, genetic samples may have been collected from different origin groups (i.e., seed sources) within populations, each characterized by slightly different allele frequencies. In other words, we could suppose that local populations of *J. sinensis* in Korea arise from founder events with only rarely plants from different seed sources colonizing the same site where they may be crossed and ultimately create a new recombinant local population. This may favor the establishment of clusters of related individuals that could lead partial inbreeding and/or create the WAHLUND effect causing heterozygote deficiencies. If breeding systems and the WAHLUND effect affect the population genetic structure, all F_{IS} values for polymorphic loci should show similar patterns in a single population. However, this pattern was not observed in an analysis of fixation indices, calculated for all polymorphic loci in each population. This suggests that the acting evolutionary forces differ in their impact upon 7 polymorphic loci. Similar trends were observed in the plant allozyme literature (HOKANSON et al., 1993; ALVAREZ-BUYLLA and GARAY, 1994; LEE et al., 1995).

Most loci except those that were nearly monomorphic showed significant heterogeneity in allele frequencies among populations, which suggests some degree of population structuring. Namely, the mean F_{ST} value of 0.122 of *J. sinensis*, indicated that genetic differentiation among populations is moderate as compared with those of other tree species (HAMRICK et al., 1992). The moderate F_{ST} observed in this study resulted in a relatively low estimate of gene flow (1.80) compared with those of other woody species (GOVINDARAJU, 1988). These results may be explained in part by short distance of seed dispersal, the narrow and discontinuous distribution and probably founder effect. Finally, we could not rule out the man's impacts on the genetic structure of *J. sinensis*. Namely, random effects of man such as selection for desirable nut characters may have had an influence on the level and distribution of genetic variation of *J. sinensis*.

We did not find any correlation between genetic and geographic distances. This may be typical result for an introduced species such as walnut which is not native but could be considered naturalized (VILLANI et al., 1991). That is to say, we could consider that each local population might be built up by randomly chosen seed materials and stochastic events would have played a significant role in the establishment of *J. sinensis* populations in Korea.

When we concern the low level of genetic diversity, it is urgent to develop conservation program to retain appropriate levels of genetic diversity of *J. sinensis* in Korea. In addition, to cope with the need for breeding programs of *J. sinensis* we should broaden the genetic base through the way such as the introduction of more variable cultivars from other countries. Finally, further studies of *J. sinensis* populations in China which is supposed to be the origin of Korean populations would add more detailed information on the genetic variation and their spatial distribution.

Acknowledgements

The authors thank an anonymous reviewer for helpful comments on the manuscript. This study was funded by Forest Genetics Research Institute of the Republic of Korea.

Literature

- ALETÀ, N., OLARTE, C., TRUCO, M. J. and ARÚS, P.: Identification of walnut cultivars by isozyme analysis. *Acta Horticulturae* **284**, 91–96 (1989). — ALETÀ, N., ROVIRA, M., NINOT, A. and ARÚS, P.: Inheritance of four isozymes in walnut. *Acta Horticulturae* **311**, 62–67 (1993). — ALVAREZ-BUYLLA, E. R. and GARAY, A. A.: Population genetic structure of *Ceropia obtusifolia*, a tropical pioneer population. *Heredity* **48**, 437–453 (1994). — ARULSEKAR, S., MCGRANAHAN, G. H. and PARFITT, D. E.: Inheritance of phosphoglucosyltransferase and esterase isozymes in Persian walnut. *J. Hered.* **77**, 220–221 (1986). — ARULSEKAR, S. and PARFITT, D. E.: Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. *Hortscience* **241**, 928–933 (1986). — ARULSEKAR, S., PARFITT, D. E. and MCGRANAHAN, G. H.: Isozyme gene markers in *Juglans* species. *J. Hered.* **76**, 103–106 (1985). — BROWN, A. H. D.: Enzyme polymorphism in plant populations. *Theor. Pop. Biol.* **15**, 1–42 (1979). — CONKLE, M. T., HODGSKISS, P. D., NUNALLY, L. B. and HUNTER, S. C.: Starch-Gel Electrophoresis of Conifer Seeds: A Laboratory Manual. Pacific Southwest Forest and Range Experiment station, Berkeley, CA (1982). — CRAWFORD, D. J.: Enzyme electrophoresis and plant systematics. In: D. E. SOLTIS and P. S. SOLTIS (editors): *Isozymes in Plant Biology*. Dioscorides, Portland, Oreg. pp. 146–164 (1989). — FJELLSTROM, R. G., PARFITT, D. E. and MCGRANAHAN, G. H.: Genetic relationships and characterization of Persian walnut (*Juglans regia* L.) cultivars using restriction fragment length polymorphisms (RFLPs). *J. Amer. Soc. Hort. Sci.* **119**, 833–839 (1994). — GERMAIN, E., HANQUIER, I. and MONET, R.: Identification of eight *Juglans* spp. and their interspecific hybrids by isozymatic electrophoresis. *Acta Horticulturae* **311**, 73–85 (1993). — GILLET, E. M.: GSED: Genetic structures from electrophoretic data, ver. 1.0. Abteilung für Forstgenetik und Forstpflanzenzüchtung, Universität Göttingen (1994). — GOVINDARAJU, D. R.: Relationship between dispersal ability and levels of gene flow. *OIKOS* **52**, 31–35 (1988). — GREGORIUS, H.-R.: On the concept of genetic distance between populations based on gene frequencies. In: *Proc. IUFRO Joint Meeting of Working Parties on Popul. and Ecol. Genet., Breed Theory and Progeny Testing*. SO2.04.1-3. Stockholm. pp. 17–26 (1974). — GREGORIUS, H.-R.: Measurement of genetical differentiation in plant populations. In: H.-R. GREGORIUS (editor): *Population Genetics in Forestry*. Lecture Notes in Biomathematics. Vol. 60. Springer-Verlag, Berlin. pp. 276–285 (1984). — GREGORIUS, H.-R.: The relationship between the concepts of genetic diversity and differentiation. *Theor. Appl. Genet.* **74**, 397–401 (1987). — GREGORIUS, H.-R., KRAUHAUSEN, J. and MÜLLER-STARCK, G.: Spatial and temporal genetic differentiation among the seed in a stand of *Fagus sylvatica* L. *Heredity* **57**, 255–262 (1986). — GREGORIUS, H.-R. and ROBERDS, J. H.: Measurement of genetical differentiation among subpopulations. *Theor. Appl. Genet.* **71**, 826–834 (1986). — HAMRICK, J. L., GODT, M. J. and SHERMAN-BROYLES, S. L.: Factors influencing levels of genetic diversity in woody plant species. *New For.* **6**, 95–124 (1992). — HOKANSON, S. C., ISEBRANDS, J. G., JENSEN, R. J. and HANCOCK, J. F.: Isozyme variation in oaks of the Apostle Islands in Wisconsin: genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoides* (Fabaceae). *Am. J. Bot.* **80**, 1349–1357 (1993). — KIM, Z. S., LEE, S. W. and HYUN, J. O.: Allozyme variation in six native oak species in Korea. *Ann. Sci. For.* **50** (Suppl. 1), 253s–260s (1993). — KRÜSSMAN, G.: *Manual of Cultivated Broad-Leaved Trees and Shrubs*. Vol. 2. Timber Press, Portland, OR. (1986). — LEDIG, F. T.: Heterozygosity, heterosis and fitness in outbreeding plants. In: M. E. SOULÉ (editor): *Conservation Biology: The Science of Scarcity and Diversity*. Sinauer Associates, Sunderland, Mass. pp. 77–104 (1986). — LEE, S. W.: Genetic Diversity and Structure of Natural Populations of *Pinus thunbergii* in Korea. Ph. D. Thesis. Korea University (1994). — LEE, S. W., KIM, W. W., LEE, B. C., KIM, Y. Y. and KIM, S. C.: Genetic variation of acorn production stands in *Quercus acutissima* and *Q. variabilis*. *Korean J. Breeding* **27**: 345–358 (1995). — LI, C. C. and HORVITZ, D. G.: Some methods of estimating the inbreeding coefficient. *Am. J. Hum. Genet.* **5**, 107–117 (1953). — LUZA, J. G. and POLITO, V. S.: Cryoconservation of English walnut (*Juglans regia* L.) pollen. *Euphytica* **37**, 141–148 (1987). — MALVOLTI, M. E., PACIUCCI, M., CANNATA, F. and FINESCI, S.: Genetic variation in Italian populations of *Juglans regia* L. *Acta Horticulturae* **31**, 86–94 (1993). — MCGRANAHAN, G. H., TULECKE, X., ARULSEKAR, S. and HANSEN, J. J.: Intergeneric hybridization in the *Juglandaceae*: *Pterocarya* sp. x *Juglans regia*. *J. Amer. Soc. Hort. Sci.* **11**, 627–630 (1986). — NEI, M.: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583–590 (1978). — RINK, G., CARROL, E. R. and KUNG, F. H.: Estimation of *Juglans nigra* L. mating system parameters. *Forest Sci.* **35**, 623–627 (1989). — SLATE, G. L.: History of nut trees. In: R. A. JAYNES (editor): *The Culture in North America*. NNGA, Connecticut. pp. 9–11 (1981). — SNEATH, P. H. A. and SOKAL, R. R.: *Numerical Taxonomy*. W. H. Freeman, San Francisco. pp. 230–234 (1973). — SOLAR, A., SMOLE, J. and STAMPAR, F.: Identification of walnut cultivars by pollen isozymes. *Acta Horticulturae* **311**, 95–100 (1993). — SWOFFORD, D. L. and SELANDER, R. B.: BIOSYS-1: a FORTRAN program for the comprehensive analysis of

electrophoretic data in population genetics and systematics. *J. Hered.* **72**, 281–283 (1981). — VILLANI, F., PIGLIUCCI, M., BENEDETTI, S. and CHERUBINI, M.: Genetic differentiation among Turkish chestnut (*Castanea sativa* MILL) populations. *Heredity* **65**, 131–136 (1991). — 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). — WRIGHT, S.: *Evolution and the Genetics of Populations*. Vol 4. Variability within and among Natural Populations. University of Chicago Press Chicago (1978).

Genetic Parameters for Spiral Grain, Stem Form, Pilodyn and Growth in 13 Years Old Clones of Sitka Spruce (*Picea sitchensis* (BONG.) CARR.)

By J. K. HANSEN and H. ROULUND¹)

(Received 20th November 1996)

Summary

Spiral grain measured at ring number 6 or 8 from the pith on 13-years old ramets coming from 191 Sitka spruce clones tested at 4 sites showed a broad sense heritability on single tree level from 0.36 to 0.54. Standard deviations were in the interval from 1.61 to 1.97 degrees and the mean was on all sites about 5 degrees to the left. Predicted genetic gains equal to about 2 degrees reduction of spiral grain in the juvenile wood seems realistic even with moderate selection intensities. Genetic correlations with height, diameter, stem form and pilodyn were small or absent, and almost no genotype-environment interaction was present. Individual broad sense heritabilities for heights and diameters were moderate to low ranging from 0.08 to 0.31 and with coefficients of variation about 0.30. Moderate genotype-environment interaction was present for the 2 traits with genetic correlations across sites ranging from 0.57 to 0.85. Pilodyn had a moderate heritability about 0.32 and a coefficient of variation about 8%. The genetic correlation with diameter was 0.53 and with height 0.22 so the density must be taken in consideration when selecting for growth in Sitka spruce. The broad sense heritability for stem form using a scale from 1 to 9 was 0.37 and the coefficient of variation 0.22. Positive, but moderate genetic correlations with diameter and height was present, 0.27 and 0.21 respectively. Pilodyn and stem form were only measured in one trial.

Key words: Clonal trials, heritability, genetic correlations, genotype-environment interaction, spiral grain.

FDC: 165.3; 165.411; 232.11; 174.7 *Picea sitchensis*.

Introduction

Sitka spruce (*Picea sitchensis* (BONG.) CARR.) is an important tree species in the western parts of Denmark influenced by the Atlantic, where the salt impact makes it impossible to grow Norway spruce. It further plays a role in the eastern parts of Denmark on more swampy soils. It has therefore been subject for breeding since 1970 and the importance of the species has been growing in the eighties (ROULUND, 1990). The main disadvantage of Sitka spruce wood is that the sawn timber and boards are twisting considerably, which causes a considerable amount of wastage.

¹) Arboretum, Dept. of Botany, Dendrology and Forest Genetics, The Royal and Veterinary and Agricultural University Denmark, Hørsholm, Denmark.

The relationship between spiral grain and twist has theoretically and empirically been shown by STEVENS and JOHNSON (1960) and BALODIS (1972). Specifically for Sitka spruce, HARVALD (1988, 1989) and DANBORG (1994a) has shown significant correlations about 0.5 between twist and spiral grain with higher correlations in small dimensions and boards from the inner parts of the trees.

The spiral grain in the juvenile wood of Sitka spruce is of particular importance. Firstly because of the normally high level of spiral grain in the juvenile wood of Sitka spruce, (BRAZIER, 1967; PEDINI, 1990; JENSEN, 1994). Secondly because fast growth in the youth and a limited rotation length of about 50 to 60 years, means that the wood of the adult trees from Sitka spruce plantations in Denmark will normally have a considerable percentage of juvenile wood. Thirdly, because the boards from the inner parts of the stems, as mentioned before, are more susceptible to twist. Spiral grain in Sitka spruce has been found to be under considerably genetically control concerning the level of spiral grain, the radial pattern and the height pattern within same ring number (HANSEN and ROULUND, in preparation). However more broadly based genetic parameters such as heritability and genetic correlations with other traits still needs to be revealed. The high degree of genetic control is in agreement with findings in other tree species (see HARRIS, 1989, for a review).

Therefore spiral grain is being considered as an important selection criteria in the breeding programme (ROULUND, 1990). In the light of this, the objectives in this paper are primarily to describe the genetic variation of spiral grain in the juvenile wood and the correlations with other traits. The paper also looks at the genetic parameters for height, diameter, stem form and pilodyn 10 years after establishment. A comprehensive description of selection strategies in Sitka spruce will be discussed in a subsequent paper.

Material

The 4 trials designated A, B, C and D consist of 191 clones propagated in 1982 and planted in 1985. The clones were selected for height growth in a population of 4-years old seedlings, originating from a bulked seedlot of the provenance Havredal F. 379 of Washington origin. It was not possible to trace back the mother trees of the clones. The trials are