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Genetic Diversity and Phylogenetic Relationships in Alder, *Alnus firma*, Revealed by AFLP

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Molecular markers for alder, *Alnus firma* Sieb. et Zucc., have not been studied extensively. Here, we used amplified fragment length polymorphism (AFLP) to investigate genetic relationships among 15 natural populations. EcoRI-ACG + MseI-CTG combinations revealed the highest polymorphism (62.2%). A total of 171 DNA fragments were identified. On average, 58.1% of the AFLP markers that were generated using four primer pairs were polymorphic. Diversity was insignificant among the populations. The combination of a wind-pollinated, outcrossing breeding system along with large population sizes, and the ability to regenerate by stump sprouting may explain the high level of genetic diversity within this species. The majority (98%) of the genetic variance resided within populations. The average number of individuals that were exchanged between populations per generation was very high (N_em = 12.3). Gene dispersal in alder is apparently by seed dispersalvia water and human activity as well as through pollen. Five individuals per population were claded in the same cluster.

Keywords: AFLP, alder, *Alnus firma*, genetic diversity

Alders (*Alnus* sp.) are early-successional, monoecious species that fix nitrogen symbiotically with the actinomycete *Frankia* (Normand and Lalonde, 1986). According to early fossil records, the genus may have originated on the Asian landmass, around the Cretaceous period (Furlow, 1979). Alder is distributed in the subtropical and tropical New World, north to the southern United States, and also into Southeast Asia and the East Indies (Woodland, 1991). Because speckled alder and green alder also occur in central Quebec in North America (Bousquet et al., 1988), one could assume that those two species preceded the spread of the rest of the genus to the Northern Hemisphere, but this event has not been documented precisely in time (Furlow, 1979). Bousquet et al. (1988) suggested that Asia and the East Indies were probably the centers of origin for alder. The Asian regions of China, Korea, Japan, and Siberia are well known for their various alder species. In fact, the genus *Alnus* in Korea comprises 15 species.

Molecular markers have not been studied extensively in *Alnus firma* Sieb. et Zucc., despite this species' economic importance (e.g., for furniture, forestation, firewood, and windbreak forest) and its transcontinental distribution. *A. firma* was introduced into Korea from Japan, and has been widely distributed at low elevations, especially on public forests. Therefore, our main goal in this study was to determine whether the establishment of plantations has eroded the level of genetic variation in these artificial populations, as has been shown in many recombination or artificial populations.

DNA markers are powerful tools for assessing the genetic relationships both within and among plant populations. These methods allow the assay of large numbers of DNA fragments that are distributed throughout a plant genome (An and An, 2000). DNA markers can reveal an immense numbers of genetic loci, are phenotypically neutral, and are not subject to environmental effects (Kim and An, 1996). Therefore, they are especially informative, providing results that are superior to those revealed by traditional methods that use morphological traits and allozymes to resolve genetic differences. In addition, the debate is still ongoing about the selective neutrality of allozyme polymorphism. Molecular maker techniques may offset these limitations. Amplified fragment length polymorphism (AFLP) uses PCR-based genetic markers to rapidly screen for genetic diversity. AFLP is effective for analyzing genetic variation below the species level, particularly when investigating population structure and differentiation of subpopulations (Maughan et al., 1996; Paul et al., 1997).

The objective of this study was to use AFLP markers to assess the amount and structure of genetic diversity

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within a natural population and to more finely discriminate all the tested genotypes rather than just with the allozymes. In addition, we compared the extent of genetic relatedness between Korean (man-made plantation) populations and the natural Japanese populations.

MATERIALS AND METHODS

Plant Materials

A. firma was gathered from five natural populations in Japan and ten artificial populations in Korea (Fig. 1). One young leaf per mature tree was collected, with five plants randomly sampled in each population. On average, each population covered approximately 1200 m^2. The distance between the selected individuals was about 20 m to avoid including individuals with a common lineage. In addition, one population of another species, *Alnus hirsuta*, was used for the outgroup samples and to compare phylogenetic relationships.

DNA Extraction and AFLP Analysis

DNA was extracted using the plant DNA Zol Reagent (Life Technologies Inc., Grand Island, NE, U.S.A.), following the manufacturer's protocol. AFLP analysis was carried out according to the method of Vos et al. (1995), with some minor modifications. We digested 125 ng rather than 500 ng of genomic DNA,

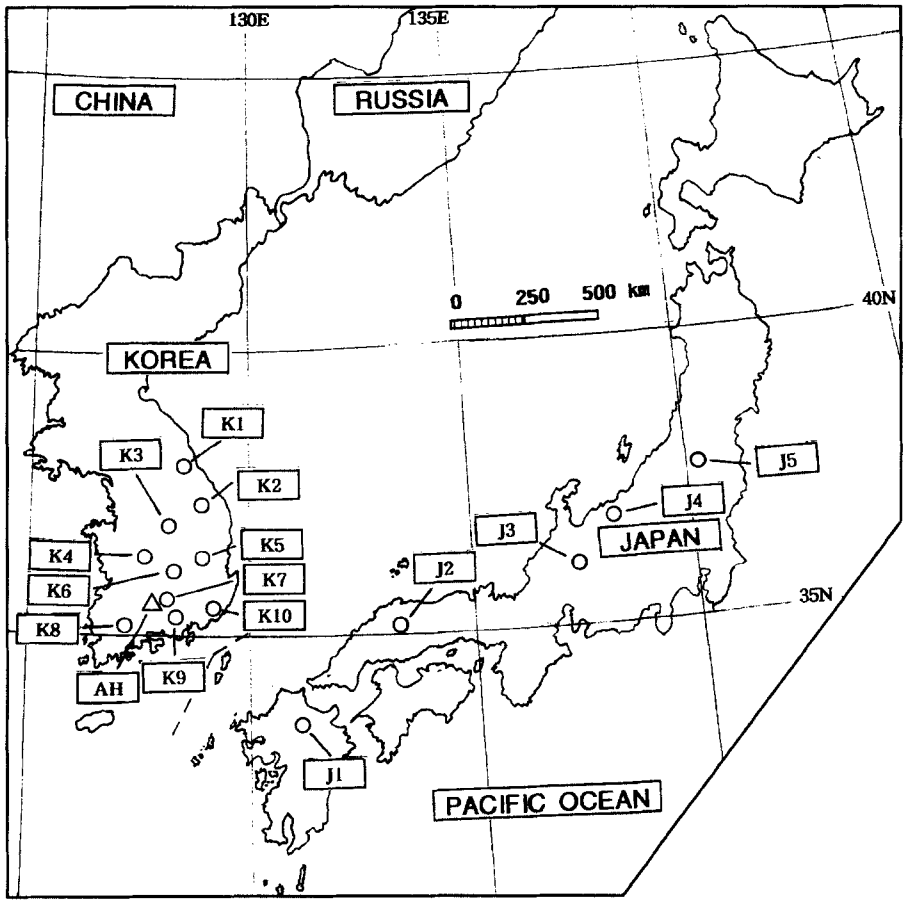

Figure 1. Location of the population studies. **K1,** Henbuck-myen, Yangyang-gun, Kangwon-do; **K2,** Buck-myen, Cheongseon-gun, Kangwon-do; **K3,** Kyerae-myen, Wonju-ci, Kangwon-do; **K4,** Soi-myen, Umseong-gun, Chungchengbuk-do; **K5,** Namseon-myen, Andong-ci, Gyeongsangbuk-do; **K6,** Sangcheon-myen, Youngdong-gun, Chungchengbuk-do; **K7,** Samchang-myen, Sancheong-gun, Gyeongsangnam-do; **K8,** Kymjeong-myen, Youngam-gun, Chonllanam-do; **K9,** Yehang-myen, Haman-gun, Gyeongsangnam-do; **K10,** Banye-dong, Haeundae-gu, Pusan-ci; **AH,** Samchang-myen, Sancheong-gun, Gyeongsangnam-do; **J1,** Nakatsu, Fukuoka Pref., Kyushu; **J2,** Okayama-ci, Okayama Pref., Honshu; **J3,** Ontake, Gifu Pref., Honshu; **J4,** Nagaoka, Niigata Pref., Honshu; **J5,** Nandai, Fukushima Pref., Honshu.

Table 1. Adapters and primers used for preamplification and selective amplification.

Names	Sequence (5′–3′)
EcoRI adapter	CTCGTAGCTGCGTACCCTGACGCATGGTTAA
MseI adapter	GACGATGAGTCCTGAGTACTCAGGACTCAT
Primer	
E-AAC	GACTGCGTACCAATTCAAC
E-ACC	GACTGCGTACCAATTCACC
E-ACG	GACTGCGTACCAATTCACG
M-CAT	GATGAGTCCTGAGTAACAT
M-CTC	GATGAGTCCTGAGTAACTC
M-CTG	GATGAGTCCTGAGTAACTG

E and M refer to primers EcoRI and MseI, respectively.

and omitted the selection of biotinylated fragments after ligation.

The genomic DNA was digested with EcoRI and MseI (AFLP core Reagent Kit; GibcoBRL Inc., Grand Island, NE, U.S.A.) in a final volume of 12.5 µL (Table 1). After inactivation at 70°C for 15 min, ligation on the adapters was performed; i.e., 12 µL of adapter ligation solution and 0.5 µL of T_4 ligase (AFLP core Kit) were added to the digested DNA. The resulting reaction mixture was incubated at 20°C for 2 h. This was followed by a preamplification step using primers that were complementary to the adapters, with one additional selective 3′ nucleotide. This step was performed in a total volume of 25.5 µL that contained 2.5 µL of ligation mixture (diluted 10 times in TE). PCR was performed under the following temperature conditions: 20 cycles of denaturation at 94°C for 30 s, followed by annealing at 56°C for 60 s, ending with extension at 72°C for 60 s.

Selective amplification was performed in a 20-µL final volume containing 5 µL of preamplification products (diluted 50 x in TE), with primers having additional nucleotides (i.e., 4-bp EcoRI primers and

Table 2. Informativeness of four combinations of selective primers used to detect AFLP between alder genotypes.

Pop./Primer	EcoRI	ACG	ACG	AAC	ACC	Total (above)
	MseI	CTC	CTG	CTG	CAT	Mean (below)
J1	No. Band	57.0	37.0	38.0	38.0	170.0
	Pp[a]	56.1	62.2	55.3	57.9	57.9
J2	No. Band	58.0	37.0	38.0	37.0	170.0
	Pp	56.9	62.2	55.3	56.8	57.8
J3	No. Band	58.0	35.0	38.0	36.0	167.0
	Pp	56.9	60.0	55.3	55.6	56.9
J4	No. Band	57.0	34.0	37.0	36.0	164.0
	Pp	56.1	58.8	54.1	55.6	56.1
J5	No. Band	56.0	31.0	36.0	35.0	158.0
	Pp	55.4	54.8	52.8	54.3	54.3
K1	No. Band	51.0	32.0	34.0	35.0	152.0
	Pp	51.0	56.3	50.0	54.3	52.9
K2	No. Band	51.0	29.0	36.0	32.0	148.0
	Pp	51.0	51.7	52.8	50.0	51.4
K3	No. Band	52.0	30.0	37.0	35.0	154.0
	Pp	51.9	53.3	54.1	54.3	53.4
K4	No. Band	53.0	33.0	37.0	35.0	158.0
	Pp	52.8	57.6	54.1	54.3	54.7
K5	No. Band	53.0	31.0	36.0	34.0	154.0
	Pp	52.8	54.8	52.8	52.9	53.3
K6	No. Band	52.0	35.0	35.0	32.0	154.0
	Pp	51.9	60.0	51.4	50.0	53.3
K7	No. Band	50.0	33.0	37.0	34.0	154.0
	Pp	50.0	57.6	54.1	52.9	53.6
K8	No. Band	50.0	34.0	36.0	33.0	153.0
	Pp	50.0	58.8	52.8	51.5	53.3
K9	No. Band	49.0	31.0	36.0	35.0	151.0
	Pp	49.0	54.8	52.8	54.3	52.7
K10	No. Band	49.0	32.0	37.0	31.0	149.0
	Pp	49.0	56.3	54.1	48.4	51.9
Total	No. Band	58.0	37.0	38.0	38.0	171.0
	Pp	56.9	62.2	55.3	57.8	58.1

Pp[a]: Percentage of polymorphism (including monomorphic band).

3-bp MseI primers). Amplification followed a temperature regimen of 1 cycle denaturation at 94°C for 60 s, followed by annealing at 65°C for 60 s, ending with extension at 72°C for 90 s. This was then followed by 10 cycles under the conditions listed above (except with a 1°C lower annealing temperature each cycle), and finally 23 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. Amplification was performed with four primer combinations (Table 2).

Gel electrophoresis followed the protocol for the AFLP™ Analysis System I (GibcoBRL). The staining procedure included incubation with a silver nitrate solution, development with a sodium carbonate solution, and fixing with acetic acid, according to the Silver Sequence DNA Sequencing™ System's protocol (Promega Co., Madison, WI, U.S.A.).

Statistical Analyses

All monomorphic and polymorphic AFLP bands visible by eye were scored; only unambiguously scored bands were used in the analyses. Each polymorphic AFLP band was given a score of either 1 (present) or 0 (absent). The degree of polymorphism was quantified using Shannon's index of phenotypic diversity (Bowman et al., 1971):

$$Ho = -\sum p_i \log p_i,$$

where p_i is the frequency of a particular phenotype i (King and Schaal, 1989). H_O can be calculated and compared for different populations (Paul et al., 1997). Let

$$H_{POP} = 1/n \sum H_O$$

be the average diversity over 'n' different populations and let

$$H_{SP} = -\sum p \log p$$

be the diversity calculated from the phenotypic frequencies p in all the populations considered together (Paul et al., 1997). Then the proportion of diversity present within populations, H_{POP}/H_{SP}, can be compared with that between populations, $(H_{SP} - H_{POP})/H_{SP}$.

The sum of the effective number of alleles (A_E) was calculated by determining the effective number of alleles for each locus (Powell et al., 1996):

$$A_E = \sum \{(1/\sum pi^2) - 1\}.$$

The estimation of genetic similarity (GS) between genotypes was based on the probability that an amplified fragment from one individual will also be present in another (Nei and Li, 1979). GS = 2 x Number of shared fragments between A and B/(Number of fragments in A + Number of fragments in B). GS was converted to genetic distance, i.e., 1-GS (Le Thierry et al., 2000). Homogeneity of variance among populations was tested by Bartlett's statistics. Genetic differentiation measured by G_{ST} among populations was also calculated. Finally, gene flow between population pairs was calculated from G_{ST} values by $N_em = 1/4(1/G_{ST} - 1)$ (Wright, 1951).

Cluster Analyses

A phylogenetic tree was constructed according to the neighborjoining (NJ) method (Saitou and Nei, 1987), using the NEIGHBOR program in PHYLIP version 3.57 (Felsenstein, 1993). The correlation between geographical and genetic distances was evaluated using a modified Mantel's test (Smouse et al., 1986).

RESULTS

Genetic Diversity

AFLP fingerprinting of alder with four primer combinations revealed a total of 171 unambiguous amplified DNA fragments (Table 2). On average, 39.5 bands were scored per primer combination. The EcoRI-ACG + MseI-CTC combinations showed the highest polymorphism (62.2%). Although primers vary in their capacity to detect polymorphism, those used in this experiment were not significantly different. Banding patterns were distinct and reproducible in the size interval of 100-900 bp, so the fragments from that interval were included in further analyses. Our analysis permitted unique identification of each selected individual, thereby ensuring that the collection did not contain genetic duplicates. This facilitated analysis of the distribution of genetic diversity among individuals as populations.

The phenotypic frequencies detected with the four primer combinations were calculated and used in estimating genetic diversity (H_O) within populations (Table 3). The average H_O was 3.607 across populations, ranging from 3.550 to 3.675. Notably, the natural Japanese populations of *A. firma* showed higher population variability than did the artificial Korean populations, in terms of the mean percentage of polymorphism, the mean number of bands, and Ho). In particular, the sum of the effective number of alleles (A_E) differed significantly between the Korean and

Table 3. Estimates of genetic diversity (H_O) within populations of alder.

	J1	J2	J3	J4	J5	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
ACG-CTC	3.985	4.016	4.010	4.013	3.987	3.911	3.893	3.927	3.932	3.930	3.929	3.898	3.892	3.868	3.883
ACG-CTG	3.558	3.555	3.499	3.473	3.399	3.444	3.329	3.373	3.449	3.416	3.463	3.462	3.491	3.420	3.444
AAC-CTG	3.579	3.580	3.582	3.572	3.523	3.501	3.548	3.561	3.549	3.543	3.495	3.56	3.535	3.529	3.565
ACC-CAT	3.579	3.549	3.533	3.535	3.507	3.517	3.431	3.506	3.499	3.474	3.357	3.489	3.462	3.509	3.393
Mean	3.675	3.675	3.656	3.648	3.604	3.593	3.55	3.592	3.607	3.591	3.561	3.602	3.595	3.582	3.572

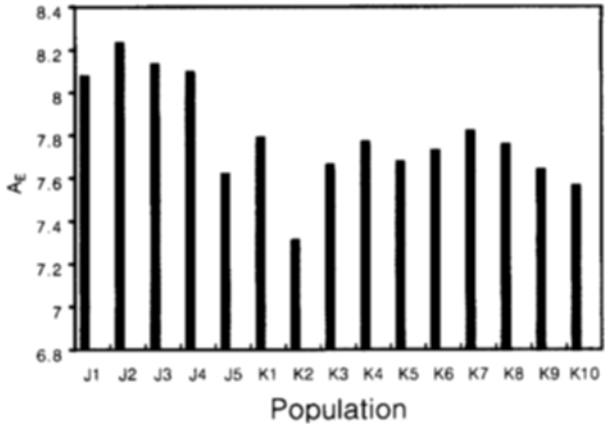

Figure 2. Sum of effective number of alleles (A_E) among 15 populations of alder for four primer combinations.

Japanese groups (F = 14.66, df = 1; Fig. 2). However, these two groups did not show significant differences in Bartlett's test for homogeneity of variance ($0.50<P<0.75$). Although the Korean and Japanese populations were isolated and sporadically distributed, they maintained a high level of genetic diversity. The same was observed for the average diversity (H_{SP}), where the mean was 3.680 (Table 4).

Table 4. Partitioning of the genetic diversity into 'within' and 'among' populations of alder for four primer combinations.

	H_{SP}	H_{POP}	H_{POP}/H_{SP}	$(H_{SP}-H_{POP})/H_{SP}$
ACG-CTC	4.002	3.938	0.016	0.984
ACG-CTG	3.549	3.452	0.027	0.973
AAC-CTG	3.588	3.548	0.011	0.989
ACC-CAT	3.580	3.489	0.025	0.975
Mean	3.680	3.607	0.020	0.980

Population Structure

Shannon's index of phenotypic diversity was used to partition the diversity into within- and among-population components. On a per primer-combination basis, the proportion of total genetic variation due to differences among populations (G_{ST}) ranged from 0.011 for AAC-CTG to 0.025 for ACC-CAT, with a mean of 0.020. Thus, the majority (98.0%) of the genetic variation resided within populations (Table 4). Likewise, this proportion of diversity within populations, H_{POP}/H_{SP}, meant that only 2.0% of the total genetic diversity was among populations. The average number of individuals exchanged between populations per generation was very high for our 15 alder populations (N_em = 12.3).

A similarity matrix based on the proportion of

Table 5. Similarity matrix (below diagonal) of 15 populations based on AFLPs using Nei and Li (1979) and genetic distances (above diagonal) according to Le Thierry et al. (2000).

Pop.	J1	J2	J3	J4	J5	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10
J1	–	0.012	0.023	0.041	0.064	0.105	0.135	0.164	0.135	0.117	0.146	0.152	0.158	0.170	0.187
J2	0.988	–	0.029	0.058	0.099	0.129	0.158	0.140	0.117	0.146	0.152	0.158	0.170	0.187	0.199
J3	0.977	0.971	–	0.035	0.076	0.117	0.146	0.117	0.105	0.129	0.135	0.140	0.152	0.170	0.187
J4	0.959	0.942	0.965	–	0.058	0.105	0.070	0.099	0.082	0.111	0.123	0.129	0.140	0.152	0.170
J5	0.936	0.901	0.924	0.942	–	0.088	0.117	0.088	0.070	0.099	0.105	0.111	0.123	0.140	0.158
K1	0.895	0.871	0.883	0.895	0.912	–	0.058	0.029	0.012	0.041	0.047	0.053	0.064	0.082	0.099
K2	0.865	0.842	0.854	0.930	0.883	0.942	–	0.029	0.047	0.018	0.018	0.023	0.035	0.041	0.064
K3	0.836	0.860	0.883	0.901	0.912	0.971	0.971	–	0.029	0.035	0.047	0.053	0.064	0.070	0.082
K4	0.865	0.883	0.895	0.918	0.930	0.988	0.953	0.971	–	0.023	0.029	0.035	0.047	0.053	0.058
K5	0.883	0.854	0.871	0.889	0.901	0.959	0.982	0.965	0.977	–	0.006	0.018	0.018	0.029	0.035
K6	0.854	0.848	0.865	0.877	0.895	0.953	0.982	0.953	0.971	0.994	–	0.012	0.018	0.029	0.035
K7	0.848	0.842	0.860	0.871	0.889	0.947	0.977	0.947	0.965	0.982	0.988	–	0.012	0.023	0.041
K8	0.842	0.830	0.848	0.860	0.877	0.936	0.965	0.936	0.953	0.982	0.982	0.988	–	0.018	0.029
K9	0.830	0.813	0.830	0.848	0.860	0.918	0.959	0.930	0.947	0.971	0.971	0.977	0.982	–	0.035
K10	0.813	0.801	0.813	0.830	0.842	0.901	0.936	0.918	0.942	0.965	0.965	0.959	0.971	0.965	–

shared fragments (GS) was used to establish the relationships between the populations (Table 5). The similarity of GS ranged from 0.801 between Populations 2 and 15 to 0.994 between Populations 10 and 11, with a mean of 0.949.

The genetic relationships among the populations can be seen in the NJ phylogenetic tree (Fig. 3). Five individuals per population were claded in the same cluster. The single outgroup population (from *A. hirsuta*) clustered separately from the all populations of *A. firma*. Genetic distance and geographic distance were positively correlated ($r = 0.65$, $P < 0.05$).

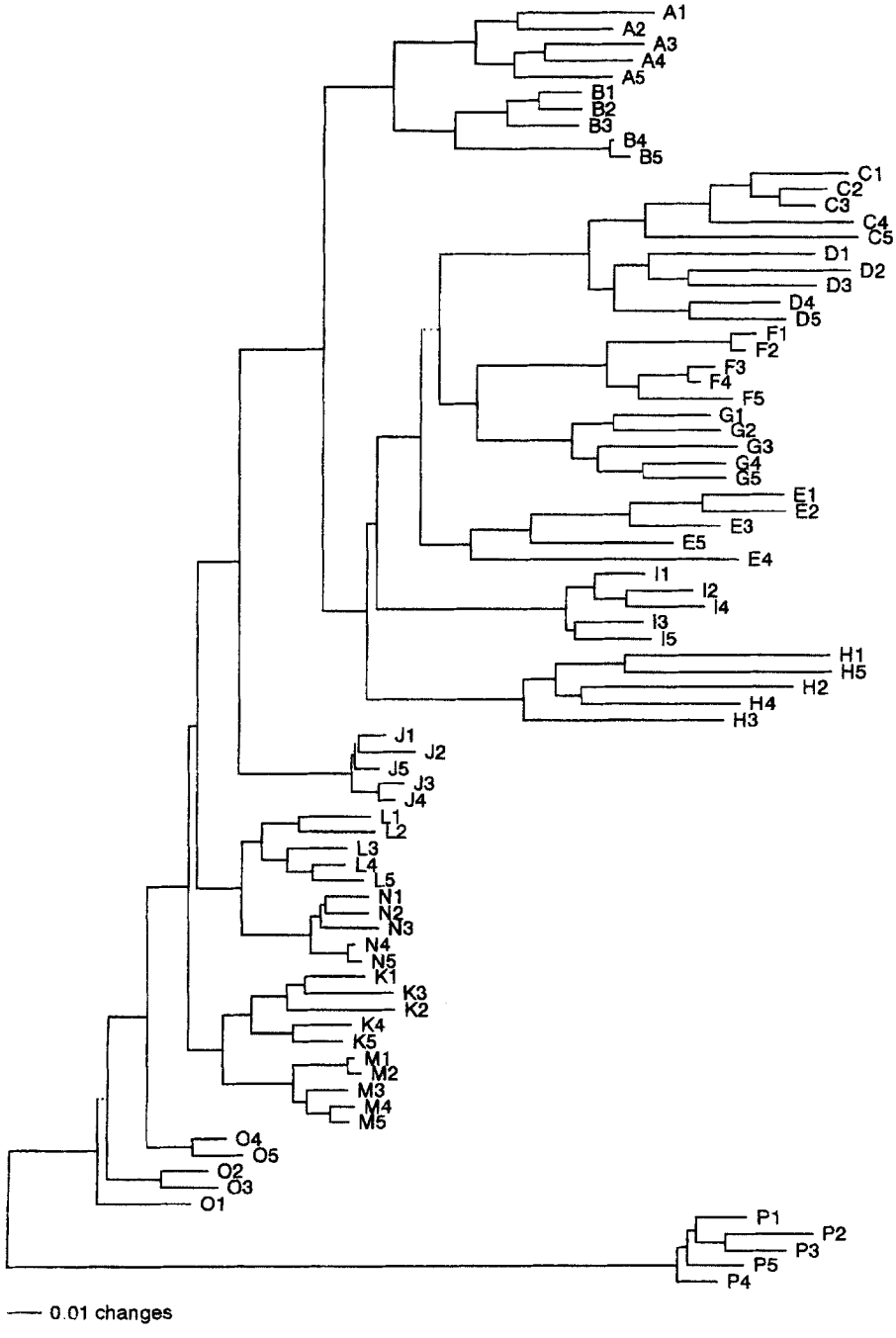

Figure 3. A neighbor-joining tree for alder based on AFLP analysis. A1~A5, K1; B1~B5, K2; C1~C5, K3; D1~D5, K4; E1~E5, K5; F1~F5, K6; G1~G5, K7; H1~H5, K8; I1~I5, K9; J1~J5, K10; K1~K5, J1; L1~L5, J2; M1~M5, J3; N1~N5, J4; O~O5, J5; P1~P5, AH (outgroup: *A. hirsuta*).

DISCUSSION

Genetic Diversity

One advantage of AFLP-based DNA fingerprinting is its potential for exposing large genetic polymorphism, thereby providing nearly complete coverage of the whole genome (Aggarwal et al., 1999). On average, 58.1% of the AFLP markers were polymorphic (Table 2). This level of polymorphism is lower than that reported in *Azadirachta indica* (Singh et al., 1999), but is higher than for sunflower (48%; Hongtrackul et al., 1997), soybean (36%; Maughan et al., 1996), rice (22%; Maheswaran et al., 1997), and coconut (27%; Teulat et al., 2000).

A. hirsuta and *A. firma* in Korea also maintained higher levels of allozyme variation (Huh, 1999; Huh and Huh, 1999). For example, the mean percentage of polymorphic loci was 62.8%, while the mean genetic diversity within populations was 0.207. These values were higher than for two Canadian alder species [*Alnus rugosa* (Du Roi) Spreng. and *Alnus crispa* (Ait.) Parsh.]. Because the species and methodology differed in the current study, our results may preclude meaningful direct comparisons (Karron, 1987). The dispersal process might possibly erode the levels of genetic diversity in derived species, as has been shown by Purdy et al. (1994). Those allozyme and AFLP marker results are consistent with the hypothesis that East Asia is the primary center of diversity for alder species (Furlow, 1979; Bousquet et al., 1988).

The erosion of genetic diversity (*Ho*) occurred in Korean populations through geographical and sexual isolations from its supposed distribution center, which resulted in reduced genetic diversity. The combination of a wind-pollinated, outcrossing breeding system, along with large population sizes, and the ability to regenerate by stump sprouting may explain the high level of genetic diversity within populations (Huh 1999; Huh and Huh, 1999).

Population Structure

The estimated gene flow based on G_{ST} was high (N_em = 12.3). Gene dispersal of alder seems to be via seed dispersal (due to water and human activity) as well as through pollen and seed dispersal (Huenneke, 1985, 1987).

A striking feature of this study is the lack of intrapopulation variation. We found that 2.0% of the AFLP variation was among populations and 98.0% within populations (Table 4). Using G_{ST} to estimate the proportion of isozyme diversity among populations, Hamrick et al. (1992) reported average values of 8.4% for long-lived perennial woods and 7.9% for wind-pollinated outcrossers, versus 12.2% for selfers. In comparison, the alder populations in Korea and Japan are less differentiated than are other wind-pollinated outcrosses. The latter are outcrossing, monoecious, or dioecious, wind-pollinated species. Alders are monoecious, and their female flowers have pistils with two united carpels. This genus obligates outcrossing by wind-pollination (Bousquet et al., 1987a, 1987b). Common life-history traits, such as allogamy wind dispersal or animal dispersal of both pollen grains and seeds, high reproductive capability, and similar longevity and successional behavior, could more readily account for most of the homology in the population genetics of these shrubby species, and most likely are responsible for the low differentiation observed at the intraspecific level. These factors reduce the effect of geographic isolation on breeding and the chance for genetic divergence.

Phylogenetic Relationship

The allelic composition of the Korean populations was a subset of that for the Japanese populations. This suggests that the Korean populations of *A. firma* may constitute a gene pool with reduced diversity that has resulted from founder effect and local adaptation. It also gives a clue to the introduction of this species. Clearly, the *A. firma* in Korea has been introduced from Japan and is widely distributed in forests. Our results revealed relatively close similarity among populations. In particular, the logical explanation for the high similarity in the Korean populations is that they were collected in the wild from the same population or region.

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LITERATURE CITED

Aggarwal RK, Brar DS, Nandi S, Huang N, Khush GS (1999) Phylogenetic relationships among *Oriza* species revealed by AFLP markers. Theor Appl Genet 98: 1320-1328

An K, An G (2000) Overriding Photoperiod Sensitivity of Flowering Time by Constitutive Expression of a MADS Box Gene. J Plant Biol 43: 28-32

Bousquet J, Cheliak WM, Lalonde M (1987a) Allozyme variability in natural populations of green alder (*Alnus crispa*) in Quebec. Genome 29: 345-352

Bousquet J, Cheliak WM, Lalonde M (1987b) Genetic dif-

ferentiation among 22 mature populations of green alder (*Alnus crispa*) in Central Quebec. Can J For Res 17: 219-227

Bousquet J, Cheliak WM, Lalonde M (1988) Allozyme variation within and among mature populations of speckled alder (*Alnus rugosa*) and relationships with green alder (*A. crispa*). Am J Bot 75: 1678-1686

Bowman KD, Hutcheson K, Odum EP, Shenton LR (1971) Comments on the distribution of indices of diversity. Stat Ecol 3: 315-359

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5s. Distributed by the author. Department of Genetics, Univ Washington, Seattle

Furlow JJ (1979) The systematics of the American species of *Alnus* (Betulaceae). Rhodora 81: 1-121

Hamrick JL, Godt MJW, Sherman-Broyles SL (1992) Factors influencing levels of genetic diversity in woody plant species. New Forests 6: 95-124

Hongtrackul V, Huestis GS, Knapp SJ (1997) Amplified fragment length polymorphisms as a tool for DNA fingerprint of sunflower germplasm: genetic diversity among oilseed inbred lines. Theor Appl Genet 95: 400-407

Huenneke LR (1985) Spatial distribution of genetic individuals in thickets of *Alnus incana* ssp. *rugosa*, a clonal shrub. Am J Bot 72: 152-158

Huenneke LR (1987) Demography of a clonal shrub, *Alnus incana* ssp. *rugosa* (Betulaceae). Am Midl Nat 117: 43-55

Huh MK (1999) Genetic diversity and population structure of Korean Alder (*Alnus japonica* : Betulaceae). Can J For Res 29: 1311-1316

Huh MK, Huh HW (1999) Genetic diversity and population structure of *Alnus hirsuta* (Betulaceae). J Plant Res 112: 437-442

Karron JD (1987) A comparison of levels of genetic polymorphism in geographically restricted and widespread plant congenera. Evol Ecol 1: 47-58

Kim S-R, An G (1996) Characterization of an Easter Lily Calmodulin cDNA Clone. J Plant Biol 39: 9-13

King LM, Schaal BA (1989) Ribosomal DNA variation and distribution of *Rudbeckia missouriensis*. Evolution 42: 1117-1119

Le Thierry díEnneequin M, Poupance B, Starr A (2000) Assessment of genetic relationships between *Setaria italica* and its wild relative *S. viridis* using AFLP markers. Theor Appl Genet 100: 1061-1066

Maheswaran M, Subudhi PK, Nandi S, Xu JC, Parco A, Yang DC, Huang N (1997) Polymorphism, distribution, and segregation of AFLP markers in a doubled-haploid rice population. Theor Appl Genet 94: 39-45

Maughan PJ, Maroof MAS, Buss GR, Huestis GM (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor Appl Genet 93: 392-401

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 74: 5267-5273

Normand P, Lalonde M (1986) The genetics of *Frankia*: a review. Plant Soil 90: 429-453

Paul SP, Wachira FN, Powell W, Waugh R (1997) Diversity and genetic differentiation among populations of Indian and Kenyan tea (*Camellia sinensis* (L.) O. Kuntze) revealed by AFLP markers. Theor Appl Genet 94: 255-263

Powell W, Michele M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2: 225-238

Purdy BG, Bayer RL, Macdonald SR (1994) Genetic variation, breeding system evolution, and conservation of the narrow sand dune endemic *Stellaria arenicola* and the widespread *S. longipes* (Caryophyllaceae). Am J Bot 81: 904-911

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425

Singh A, Negi MS, Rajagopal J, Bhatia S, Tomar UK, Srivastava PS, Lakshmikumaran M (1999) Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theor Appl Genet 99: 272-279

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool 35: 627-632

Teulat B, Aldam C, Trehin R, Lebrun P, Barker JHA, Arnold GM, Karp A, Baudouin L (2000) An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. Theor Appl Genet 100: 764-771

Vos P, Hogers R, Bleeker M, Reijans M, van Lee T, Hornes M, Freijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res 23: 4407-4414

Woodland DW (1991) Contemporary Plant Systematics. Prentice-Hall, Inc, Englewood Cliffs, NJ, pp 222

Wright S (1951) The genetic structure of populations. Ann Eugen 15: 313-354