Allozyme Variation of a Small Subshrub *Ardisia japonica* (Myrsinaceae) in North Eastern Asia

By C. P. CHEON, M. Y. CHUNG, S. G. CHUNG and M. G. CHUNG[1])

Department of Biology, Gyeongsang National University, Jinju 660-701, Republic of Korea

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Abstract

Ardisia japonica is a small subshrub native to coastal areas in northeastern Asia. The species is self-compatible, insect-pollinated but capable of clonal reproduction. We used allozymes as genetic markers to evaluate genetic and clonal diversity in 20 populations of *Ardisia japonica*, sampled from Korea and Japan. Within-population estimates of genetic diversity were low (%P_p of 19.1%, A_p of 1.19, and H_{ep} of 0.073). However, at the species level, *A. japonica* harbors high levels of allozyme diversity (%P_s of 66.7%, P_s of 2.11, and H_{es} of 0.185), because many alleles display large differences in frequencies among 20 populations (mean jackknifed F_{ST} = 0.629). A low degree of allozyme differentiation was observed between populations from Korea and Japan, despite lack of a land connection between the southern Korean peninsula and the southern Japanese archipelago since the middle Pleistocene. There was a very weak correspondence between genetic and geographic distances between populations. Multilocus genotypic diversity was low (D_G = 0.453). The abundance of multilocus genotypes which are homozygous at most loci and at several populations may be a result of inbreeding and genetic drift through a few founders coupled with limited pollen flow and extensive clonal reproduction. These factors are likely responsible for the low levels of allozyme diversity within populations and high genetic divergence between populations.

Key words: Ardisia japonica, Asian coastal forest, clonal reproduction, founder effect, genetic diversity, Myrsinaceae.

Introduction

Ardisia japonica (THUNB.) BLUME (Myrsinaceae), a small, evergreen shrub (10–30 cm high), grows on temperate and subtropical forests in northeastern Asia (China, Taiwan, southern Korea, and Japan). *Ardisia japonica* reproduces both sexually and extensively asexually by stolons. We have observed stolons up to 5.8 m long. During three-years of observations, seedlings were rarely found in populations of the species. Though many individuals flower, few individuals bear fruits. The terminal inflorescences contain 2–5 small, white, fragrant, bisexual flowers (6–8 mm long in diameter). Bees and flies visit flowers, and are considered to be pollinators (M. G. CHUNG, pers. obs.). Stamens of the species are clustered around a long style, and have introrse dehiscence through initially porous anthers, which then spilt longitudinally. *Ardisia* species are self-compatible and have a highly selfed or a mixed-mating system with intermediate levels of outcrossing (BAWA et al., 1985; PASCARELLA, 1997). Like other *Ardisia* species, *A. japonica* has showy drupes (ca. 5–6 mm long in diameter) that ripen from September through October and are dispersed by birds (PASCARELLA, 1997; M. G. CHUNG, pers. obs.).

Ardisia japonica is important members of coastal forest vegetation in Japan and Korea (NUMATA, 1974). It has been observed that the natural habitats of Korean *A. japonica* near the sea have been severely damaged by road construction. More recently, CHEON et al. (2000) examined allozyme diversity in 11 Korean populations of the species. Populations of the species in Korea maintain low levels of allozyme diversity (mean expected heterozygosity = 0.061). Since the distribution of genetic variation among populations is of primary importance to the conservation of genetic diversity of plant species in northeastern Asia (HAMRICK et al., 1991; S. Kawano of Kyoto University, pers. comm.), the previous study was conducted on only one edge of their distribution. The analysis of this local group of populations in Korea may provide little information that can be used to interpret the dynamics of variation over the range of the whole species. Although *A. japonica* is widely distributed in Japan (Honshu, Shikoku, and Kyushu), most Korean populations of the species occur on several islands near the southern coastal areas of the Korean peninsula. Thus, it is predicted that continuously distributed, mainland populations in Japan would harbor higher levels of genetic diversity than the marginal populations in Korea.

During the Ice Age (the glacial Würm), the Sea of Japan (East Sea) and Yellow Sea were about 100m lower than at present and a land connection existed between Korea and Japan (KIM and HONG, 1991; see *Fig. 1*). The degree of genetic differentiation among populations in plant species, which extend over Korea and the Islands of Japan, is not yet well understood (e.g., M.G. CHUNG and M.Y. CHUNG, 2000; M.Y. CHUNG and M.G. CHUNG, 2000).

This study is an extension of previous work (CHEON et al., 2000) to determine levels of clonal and allozyme diversity within populations in Japan and to assess the degree of geographical differentiation of allozymes among populations in this region.

Materials and Methods

A total of 861 leaf samples were collected from 20 populations of *A. japonica* in Korea (information on sample size in 11 populations is available in CHEON et al., [2000]) and Japan (236 from eight populations), including 58 samples from population 8 in Korea (*Fig. 1*). Because the species exhibits extensive clonal growth, samples were collected randomly from approximately 2,500 to 7,200-m^2 area at intervals of > 5m to avoid biased sampling toward certain clones. The collected leaves were wrapped with wet paper towels, placed in plastic bags and stored on ice to prevent protein denaturation prior to returning to the laboratory where they were stored at 4 °C until protein extraction.

Leaf samples were cut finely and crushed with a mortar and pestle. A phosphate-polyvinylpyrrolidone extraction buffer

[1]) Corresponding author and address: MYONG GI CHUNG, Department of Biology, Gyeongsang National University, Jinju 660-701, Republic of Korea
Telephone: +82-55-751-5953
Fax: +82-55-754-0086
Email: mgchung@nongae.gsnu.ac.kr

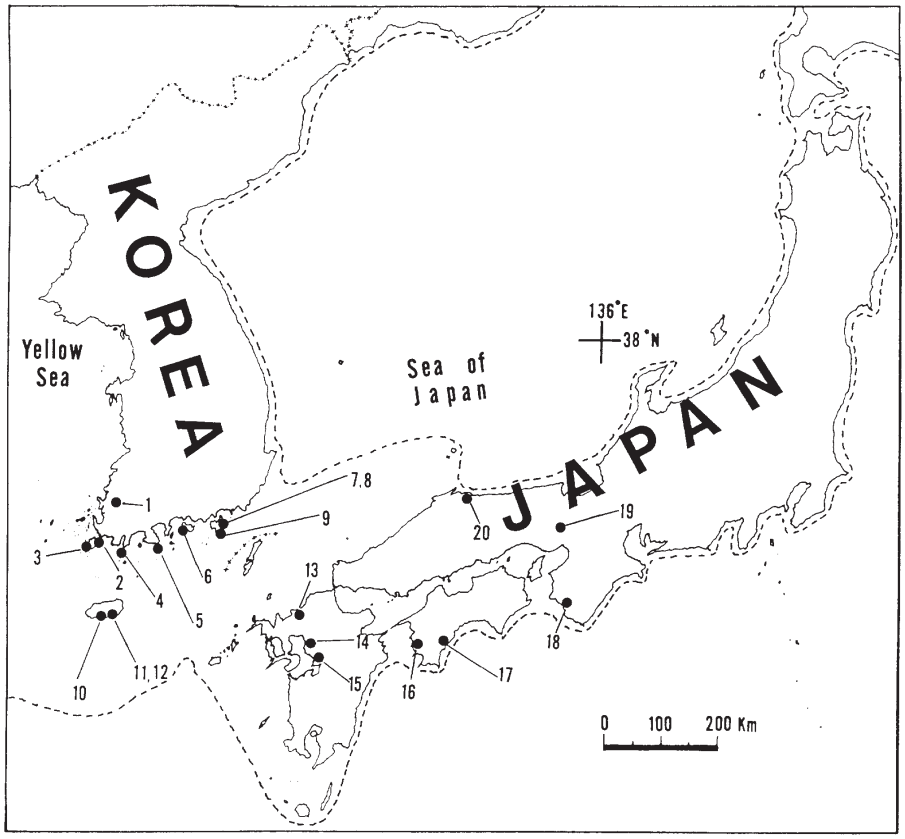

Figure 1. – Location map of the 20 populations of *Ardisia japonica* studied (Korea: 1-12 and Japan: 13-20). Broken line indicates a coastal line or land connection in the middle Pleistocene.

(MITTON et al., 1979) was added to the leaf samples to facilitate crushing and to aid enzyme stabilization. The crushed extract was absorbed onto 4-mm X 6-mm Whatman 3MM chromatography paper wicks which were stored at –70 °C until needed for analysis. Electrophoresis was performed using 11% starch gels. Eighteen putative loci for *A. japonica* from eight enzyme systems were resolved using a Poulik buffer system, a modification (HAUFLER, 1985) of SOLTIS et al.'s (1983) system 6. The eight enzyme systems were alcohol dehydrogenase (*Adh-1, Adh-2*; E.C.1.1.1.1), diaphorase (*Dia-1, Dia-2*; E.C.1.6.4.3), fluorescent esterase (*Fe-1, Fe-2, Fe-3*; E.C.3.1.1.1), leucine aminopeptidase (*Lap-1, Lap-2*; E.C.3.4.11.1), peroxidase (*Per-1, Per-2*; E.C.1.11.1.7), phosphoglucoisomerase (*Pgi-1, Pgi-2*; E.C.5.3.1.9), phosphoglucomutase (*Pgm-1, Pgm-2;* E.C.2.7.5.1), and triosephosphate isomerase (*Tpi-1, Tpi-2, Tpi-3*; E.C.5.3.1.1). Enzyme staining followed protocols of CHELIAK and PITEL (1984) for diaphorase and SOLTIS et al. (1983) for all others.

Putative loci were designated sequentially, with the most anodally migrating isozyme designated 1, the next 2, etc. Likewise, alleles were designated sequentially with the most anodally migrating alleles designated superscript a. Although the genetic bases of the loci were not documented by controlled crosses, the isozymes expressed phenotypes that were consistent in subunit structure and genetic interpretation with other isozyme studies in plants, as documented by WEEDEN and WENDEL (1989).

A locus was considered polymorphic if two or more alleles were detected, regardless of their frequencies. Five standard genetic parameters were estimated using the program POPGENE (YEH et al., 1999): percentage polymorphic loci (*%P*), mean number of alleles per locus (*A*), effective number of alleles per locus (A_e), observed heterozygosity (H_o), and expected heterozygosity or gene diversity (H_e) (BERG and HAMRICK, 1997). These parameters were estimated for species as a whole (subscript s) as well as within populations (p).

To assess the amount of clonal diversity within and between populations, three measures were estimated. First, the number of different multilocus genotypes (*G*) within populations was counted and then, diversity of *G* within populations (D_G) was calculated as a modification (PIELOU, 1969) of the SIMPSON index: $D_G = 1 - \Sigma\{[n_i(n_i\text{-}1)]/[N(N\text{-}1)]\}$, where n_i is the number of individuals of multilocus genotype i and N is the total number of individuals in the population. Second, the total probability (*PR*) that two identical multilocus genotypes could be produced by sexual reproduction was estimated, which is based on observed single-locus genotypic frequencies and the assumption of linkage equilibrium (BERG and HAMRICK, 1994). Third, for comparisons of genotype diversity among populations, the number of "widespread genotypes" (multilocus genotype occurring in more than 75% of the populations) and "local genotypes" (multilocus genotypes occurring in only one population) were counted (ELLSTRAND and ROOSE, 1987).

Estimates of levels of inbreeding within individuals in populations (F_{IS}) and the degree of genetic differentiation between populations (F_{ST}) were determined using WEIR and COCKERHAM's (1984) f and θ, because these estimators correct for unequal sample sizes. The estimates were calculated for all populations (subscript T) and populations in Korea (K) and Japan (J). The significance of F_{ST} per locus was tested based on 1,800 permutations. Means and standard errors were obtained by jackknifing over six loci. Bootstrap confidence intervals (95% CI) were constructed around jackknifed means of the F_{IS} and F_{ST} with 15,000 replicates; observed mean fixation indices

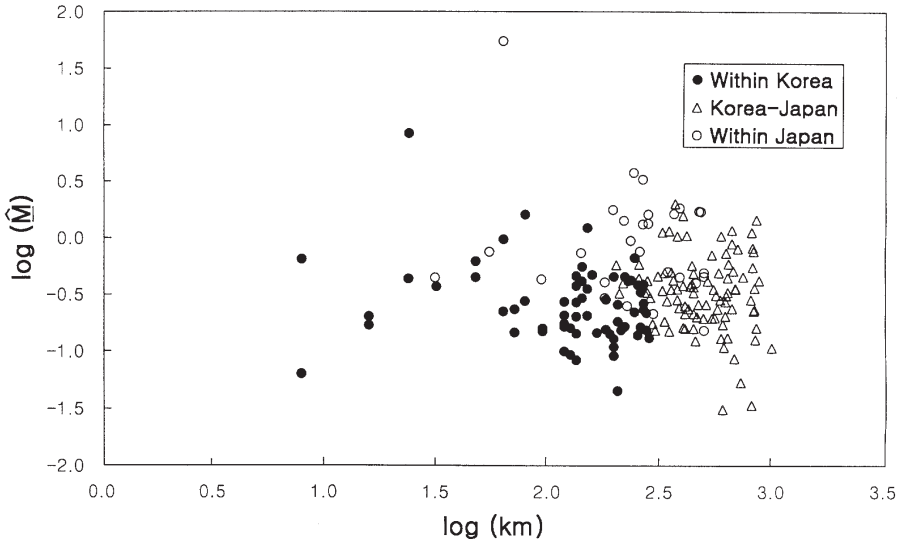

Figure 2. – Estimates of gene flow between population pairs as a function of genetic distance between populations. Log_{10} (M = [1-/F_{ST} - 1]/4) plotted against $\log_{10}$ (distance in kilometers between populations of each pair) for *Ardisia japonica.*

were considered significant when confidence intervals did not overlap zero. These calculations were made using the program FSTAT (version 2.9.1 by GOUDET [2000], see GOUDET, 1995).

In order to compare intra and inter regional differentiation (Korea vs. Japan), we used hierarchial F statistics (WRIGHT, 1978) to partition variation from expected heterozygosity among all populations into the effects of regions, populations within regions, and populations within the entire study area (F_{ST}).

An approach proposed by SLATKIN (1993) was used to estimate levels of gene flow and to study if there is a pattern of genetic isolation by distance in the study species (e.g., ALVUREZ-BUYALLA and GARAY, 1994; KUDOH and WHIGHAM, 1997; AGUIRRE-PLANTER et al., 2000; MATOLWENI et al., 2000; WU et al., 2001). SLATKIN has shown that for allozyme data, in populations with restricted dispersal and at a genetic equilibrium, there is a decreasing relationship between M [$M = (1/F_{ST} - 1)/4$] for pairs of populations and geographic distance separating population in each pair. The significance of association was determined by applying a MANTEL's permutation test (MANTEL 1967). Finally, genetic divergence among populations was also estimated by calculating NEI's (1972) genetic identity for all pairs of populations.

Results

Twelve of the 18 loci resolved were polymorphic in at least one population. *Fe-1, Fe-2, Lap-2, Per-1, Per-2,* and *Pgi-1* were monomorphic in all 20 populations. Three alleles were exclusively found in populations 2 (private allele, *Adh-2*[a], 0.120) and 14 (*Fe-3*[a], 0.029 and *Lap-1*[a], 0.029) at a low frequency. Six loci (*Adh-1, Dia-1, Pgi-2, Tpi-1, Tpi-2,* and *Tpi-3*) displayed common alleles with high frequencies that varied among populations. No allozyme variation was found in populations 8 and 19 (*Table 1*).

Populations of *A. japonica* maintained low levels of allozyme diversity: $\%P_p$ = 19.1%, A_p = 1.19, and A_{ep} = 1.09, and H_{ep} = 0.073 (*Table 1*). However, at the species level, *A. japonica* harbored considerably higher levels of allozyme diversity: $\%P_s$ = 66.7%, A_s = 2.11, and A_{es} = 1.39, and H_{es} = 0.185. The higher levels of genetic diversity were due to the occurrence of several alleles that displayed high frequencies in different populations (data not shown). There was a significant difference between populations in Korea and Japan for H_{ep} and $\%P_p$ (Wilcoxon signed rank coefficients, z = –2.240, P = 0.013;

Table 1. – Allozyme diversity within 20 populations of *Ardisia japonica*[a)] Population codes to those given in *Fig. 1.*

[a)] Abbreviations: N, sample size; $\%P_p$, percentage polymorphic loci; A_p, mean number of alleles per locus; A_{eP}, effective number of alleles per locus; H_{oP}, observed heterozygosity; SD, standard deviations; H_{eP}, HARDY-WEINBERG expected heterozygosity; G, number of multilocus genotypes; and D_G, multilocus genotypic diversity.

[b)] Mean of 11 Korean populations (567 individuals) from CHEON et al. (2000) and population 8 in Korea.

[c)] Mean of all 20 populations.

Pop	N	$\%P_p$	A_p	A_{eP}	H_{oP} (SD)	H_{eP} (SD)	G	D_G
Japanese populations								
13	21	27.78	1.39	1.30	0.214 (0.382)	0.143 (0.238)	8	0.786
14	34	38.89	1.50	1.29	0.199 (0.359)	0.146 (0.229)	10	0.838
15	46	33.33	1.39	1.18	0.167 (0.383)	0.091 (0.190)	4	0.128
16	29	16.67	1.17	1.17	0.167 (0.383)	0.083 (0.192)	1	0.000
17	33	16.67	1.17	1.17	0.167 (0.383)	0.083 (0.192)	1	0.000
18	18	22.22	1.33	1.22	0.179 (0.352)	0.111 (0.212)	6	0.784
19	29	0.00	1.00	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
20	26	27.78	1.26	1.26	0.216 (0.416)	0.133 (0.221)	3	0.443
Mean								
	29.5	22.86	1.27	1.20	0.164 (0.069)	0.099 (0.048)	4.3	0.372
Korean populations								
8	58	0.00	1.00	1.00	0.000 (0.000)	0.000 (0.000)	1	0.000
Mean[b)]								
	52.1	16.67	1.23	1.11	0.046 (0.042)	0.056 (0.041)	8.2	0.506
Mean (population level[c)])								
	43.1	19.14	1.19	1.09	0.093 (0.079)	0.073 (0.048)	6.6	0.453
Overall mean (species level)								
	861	67.67	2.11	1.39	0.083 (0.142)	0.185 (0.240)		

z = –0.169, P = 0.045, respectively), but *APp* (z = –0.676, P = 0.249), A_p (z = –0.169, P = 0.433), and A_{ep} (z = –1.260, P = 0.104) were not significantly different.

The number of multilocus genotypes (G) in all populations ranged from 1 to 43 (population 2: CHEON et al., 2000) with a mean of 6.6, indicating that all except for populations 8, 16, 17, and 19 were composed of "local" multiple genotypes (*Table 1*). Genotype diversity indices (D_G) ranged from 0.000 to 0.943 (population 2) with a mean of 0.453 (*Table 1*). The likelihood that two individuals could have identical multilocus genotypes when both are produced by sexual reproduction was less than 0.05 for populations 2 and 3 (PR = 0.006 and 0.048), suggesting that in these two populations identical multilocus genotypes would be clones.

Although populations 12, 16, and 17 had the same H_{ep} value (0.083), the number of multilocus genotypes (G) was different (G = 10 for population 12; G = 1 for populations 16 and 17). This value was caused by presence of one multilocus genotypes that was heterozygous at three out of 12 polymorphic loci for populations 16 and 17 (data not shown). Populations 2 (H_{ep} = 0.143), 13 (0.143), and 14 (0.146) had similar and high expected heterozygosities, and population 2 had the highest G (43), but the latter two populations had eight and 10, respectively, which also indicates that multilocus genotypes found in populations 13 and 14 are heterozygous at four loci. For these reasons, there is a significant, but weak correlation between H_{ep} and G (SPEARMAN's rank- correlation coefficient r_s = 0.588, P = 0.006).

Significant excess of heterozygotes (jackknifed $F_{IS\text{-}J}$ estimates = –0.695 ± 0.167 [means ± SE] with a 95% bootstrap CI ranged from –0.887 to –0.150) was detected in Japanese populations (data not shown). However, the 95% CI for populations in Korea overlaps zero (jackknifed $F_{IS\text{-}K}$ estimates = 0.101 ± 0.208 [means ± SE] with a 95% bootstrap CI ranged from –0.145 to 0.623).

Genotypic diversity among populations was large: all multilocus genotypes were "local genotypes", suggesting that the present populations might have been founded from sexually produced seed rather than by asexual fragmentation and dispersal of preexisting clones.

Significant differences in allele frequencies among 20 populations of *A. japonica* were found for 10 of 12 polymorphic loci (P < 0.001 in each case). The mean $F_{ST\text{-}T}$ value indicated that overall, about 37% of the total variation in *A. japonica* is common to all populations (*Table 2*). A higher F_{ST} value was detected among populations in Korea than in Japan (0.665 vs. 0.455). However, most of the genetic variance occurred in populations within regions (intra regional differentiation, 92.5%), with little differentiation between Korea and Japan (inter regional differentiation, 7.5%) (*Table 3*). NEI's genetic identity values were highly variable, ranging from 0.735 (populations 1 vs. 15) to 0.999 (populations 13 vs. 14), with a mean of 0.883 _ 0.057 (SD) which was below the range of values expected for conspecific populations (CRAWFORD, 1989). The high F_{ST} and low genetic identity values were due to the fact that alleles with high frequencies at several polymorphic loci varied among populations, regardless of their geographical distributions. For example, *Adh-1*^{b} was fixed in populations 1 and 4, whereas *Adh-1*^{a} was found in all other populations. In addition, although three populations are closely located in Kojae Island (populations 7 and 8 are separated by 1.3 km and populations 8 and 9 by 2.5 km), populations 8 and 9 shared allele *Pgi-2*^{b}, but *Pgi-2*^{d} was fixed in population 7. Again, populations 7 and 8 were fixed at *Tpi-1*^{c}, whereas the population 9 was not fixed (data not shown).

For total populations, the logs of all pairwise M values plotted against the geographic distances between populations in each pair showed no linear relationship ($\log_{10}$ (M) = –0.357 – 0.043 $\log_{10}$ (km) with R^2 value of nearly 0), expected from isolation by distance (r_T = 0.005, P = 0.575) (*Fig. 2*). A higher but insignificant association was found within populations in Korea (r_K = –0.208, P = 0.094). A similar pattern was found between population pairs in Japan (r_J = –0.199, P = 0.310).

Table 2. – Estimates of the degree of genetic differentiation between population (F_{ST}) following the method of WEIR and COCKERHAM's (1984) θ based on polymorphic loci of *Ardisia japonica.* SE, standard error; CI, confidence interval.

Dataset	# polymorphic loci	F_{ST} (θ) Mean ± 1 SE (95% CI)	Significance
All populations	12	0.629 ± 0.058 (0.517, 0.739)	10 loci P < 0.001
Korean populations	9	0.665 ± 0.068 (0.518, 0.776)	9 loci P < 0.001
Japanese populations	8	0.455 ± 0.125 (0.211, 0.702)	6 loci P < 0.001

Table 3. – Variance components for populations in Korea and Japan and F statistics combined across loci.

Source of variance	Variance component	% variation	F statistics
Total populations	1.86184	100	0.583
Populations within regions	1.72308	92.5	0.564
Between regions	0.13877	7.5	0.043

Discussion

Ardisia japonica maintains levels of allozyme diversity comparable to the reported mean values of long-lived woody perennials (N = 191, H_{es} = 0.177; HAMRICK et al., 1992), though, at the population level, allozyme diversity was low (N = 196, H_{ep} = 0.148; HAMRICK et al., 1992). As expected, the marginal island populations in Korea maintain lower levels of allozyme diversity than the continuously distributed mainland populations in Japan. Evidence from allozyme studies has shown that marginal populations are less variable (e.g., YEH and LAYTON, 1979; SCHNABEL and HAMRICK, 1990; SUN, 1997; CHUNG et al., 2001). The average multilocus genotypic diversity within populations of *A. japonica* is lower (D_G = 0.453) than the average (0.62) reported by ELLSTRAND and ROOSE (1987) in their review of genotypic diversity in clonal plants, which reflects the extensive clonal reproduction of the species. Previous studies have demonstrated that vegetative reproduction and spread have a marked effect on genetic diversity within plant populations (e.g., MURAWSKI and HAMRICK, 1990; PARKER and HAMRICK, 1992; PARKS and WERTH, 1993; MAYERS et al., 1998; BURKE et al., 2000; CHUNG et al., 2000a; WOLFE et al., 2000). *Ardisia japonica* is also a case in point because, in general, many multilocus genotypes which are homozygous at most loci and populations are detected in *A. japonica* (data not shown). This is in part responsible for the low levels of allozyme diversity within populations of *A. japonica*. Similar results were found in other clonal plant species in Korea (e.g., *Vitex rotundifolia:* YEEH et al., 1996; *Chimaphila japonica* and *Pyrola japonica:* CHUNG and KANG, 1996; four *Cimicifuga* species: LEE et al., 2000).

Populations of selfing species and animal-pollinated species with mixed mating systems (i.e., partially selfed, partially outcrossed) have lower levels of genetic diversity than obligately outcrossing species (HAMRICK and GODT, 1989). *Ardisia japonica* is self-compatible and highly-selfing, resulting from pollinator-mediated self-pollination based on pollination experiments (T. YAHARA of Kyushu University, pers. comm.). The mean estimates of genetic-diversity parameters (*%P*, A_P, A_{eP}, and H_{eP}) within populations of *A. japonica* were similar to the means for selfing species (N = 113, 20.0%, 1.31, 1.10, and 0.074, respectively; HAMRICK and GODT, 1989). In addition, because *A. japonica* can extensively reproduce vegetatively, ample opportunities for geitonogamous pollination exist.

Since the plants of *A. japonica* are small and the primary pollinators are bees and flies, pollen flow would be limited under a forest canopy. In warm temperate broad-leaved evergreen forests in northeastern Asia fruit-eating birds (e.g., *Zosterops palpebrosa insularis*) feed on insects from spring to autumn (YUMOTO, 1987). When insect food is scarce in winter, the birds collect fruits (drupes) of a member of evergreen subshrub and woody plants (e.g, *Ardisia* spp., *Cinnamomum* spp., *Lindera* spp., *Neolitsea* spp., *Persea* spp., etc.) and nectar in the flowers of understory-flowering trees (e.g., *Camellia japonica*) (YUMOTO, 1987; CHUNG et al., 2000b; M. G. CHUNG, pers. obs.). Although birds are endothermic, it is highly likely that the frequency of their visitation to collect red drupes of *A. japonica* would be low below the dark and cold canopy (YUMOTO, 1987). Thus, populations of *A. japonica* would be colonized from a few seeds via long-distance seed dispersal by birds ("propagule-pool model of extinction-recolonization model": SLATKIN, 1977; WADE and MCCAULEY, 1988). The resulting adult population should consist of only a few reproductive adults (founders) and the population would experience reduced genetic diversity due to founder effects.

Assuming there is no linkage among the allozyme loci, stochastic processes would result in independent fixation of different alleles in different populations given enough time and no gene flow. The lack of association between geographic and genetic distances found in *A. japonica* might be a result of genetic drift due to small effective size (founder effects) coupled with a highly selfed-mating system, restricted pollen flow among local populations, and an extensive clonal reproduction. These factors could all result in the low levels of allozyme diversity within populations and a high degree of genetic divergence between populations of *A. japonica*. However, it is difficult to determine which factor played a major role in shaping population genetic structure in *A. japonica*.

It may be of interest to note that in all eight Japanese populations and two Korean populations (1 and 10: CHEON et al., 2000), the level of observed heterozygosity was higher than or similar to the expected heterozygosity (e.g., population 10). This is a reason why we do observe the significant excess of heterozygotes in Japanese populations. It is suggested that variation of fixation indices in the populations of *A. japonica* from Korea and Japan is probably due to artifacts of extensive clonal growth and founder effects (e.g., MURAWSKI and HAMRICK, 1990). Alternatively, this observation could suggest selection for heterozygous individuals in Japanese populations and two Korean populations. Selection for heterozygous genotypes in populations was also found in the small bamboo, *Sasamorpha borealis* in Korea (LEE and CHUNG, 1999).

The degree of population differentiation observed in *A. japonica* was similar to that reported for the mean values of selfing species (N = 78, F_{ST} = 0.510: HAMRICK and GODT, 1989). The broad-leaved evergreen woody species and sub-tropical or temperate plant species native to the southern Korean peninsula and Japan have been present in these regions from pre-Pleistocene times, presumably from southern China (KONG and WATTS, 1993). As glaciation in these regions was restricted to the northernmost mountains of Korea and northern and central Japan (Altitude, above sea level 2,000–3,000 m), the coastal forests were not directly affected by the glaciation (KONG and WATTS, 1993). Thus, it is highly likely that a relatively higher level of allozyme differentiation between populations of *A. japonica* in Korea may be attributed in part to the isolated island populations, which has lowered the possibility of historical gene flow between populations. Although the land connection between the southern Korean peninsula and southern Japanese archipelagos no longer existed after the last Ice Age, a very low degree of genetic differentiation was found between populations in Korea and Japan. Similar results were found in an orchid and a member of tea family of the coastal forests in northeastern Asia (e.g., *Cymbidium goeringii:* M. Y. CHUNG and M. G. CHUNG, 2000; *Eurya japonica:* M. G. CHUNG and M. Y. CHUNG, 2000).

Finally, the high total genetic diversity maintained in *A. japonica* is indicative of a potential for providing genetic diversity via long-distance seed dispersal by birds in the future.

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Variation in Outcrossing Rates and Growth in *Eucalyptus camaldulensis* from the Petford Region, Queensland; Evidence of Outbreeding Depression

By P. A. BUTCHER[1]) and E. R. WILLIAMS

CSIRO Forestry and Forest Products, Australia

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Summary

Comparison of growth rates of *Eucalyptus camaldulensis* DEHNH. in provenance/progeny trials in Thailand has revealed significant differences among families. One possible cause of differential family performance in eucalypt species with mixed mating systems is variation in the level of inbreeding. Outcrossing rates were estimated for ten trees from each of four populations in the Petford region of north-east Queensland using allozymes. They were amongst the highest recorded in eucalypts (mean t_m = 0.95) with relatively little variation among families (t_m = 0.60–1.0). Regression analyses revealed a significant association between family outcrossing rates and growth which varied among populations. A positive association was observed in one population; negative relationships in the other three populations may reflect outbreeding depression associated with hybridisation. Differences in outcrossing rates did not explain a significant level of variation in seedlot viability or survival assessed at two years of age. The high mean outcrossing rates for the four populations of *E. camaldulensis,*

[1]) Corresponding author: P. A. BUTCHER
CSIRO Forestry and Forest Products, PO Box E4008, Kingston, ACT 2604, Australia
Phone +61 2 6281 8289
Fax: +61 2 6281 8233
Email: Penny.Butcher@csiro.au