Journal of Plant Biology, December 2006, 49(6) : 491-497

Molecular Evidence for Hybridization of *Ilex* ×*wandoensis* (Aquifoliaceae) by RAPD Analysis

Nam Sook Lee[1]*, Sung Hee Yeau[2], Jae Ok Park[3], and Mark S. Roh[4]

[1]Division of Molecular and Life Sciences, College of Natural Sciences, Ewha Womans University, Seoul 120-750, Korea
[2]Department of Biological Education, College of Education, Ewha Womans University, Seoul 120-750, Korea
[3]Subtropical Fruit Crops Experiment Station, Jeonnam Agricultural Research and Extension Services, Wando 537-801, Korea
[4]US Dept. of Agriculture, Agricultural Research Service, National Arboretum, Floral and Nursery Plants Research Unit, Beltsville, MD 20705, USA

Based on the presence of intermediate morphological characters, such as serrated leaf margins and flower structures, *Ilex* ×*wandoensis* was initially described as a putative natural hybrid between *I. cornuta* and *I. integra*, and was formally described as a new hybrid species, *I.* ×*wandoensis* C. F. Mill., and M. Kim. However, using molecular markers generated via random amplified polymorphic DNA (RAPD), we have now discovered hybridization in populations of the *I.* ×*wandoensis* complex collected from Wando and Jeju Islands, Korea. Marker bands of the putative parent taxa also were found in some populations of *I.* ×*wandoensis*, confirming its hybrid origin. Morphological variability within and among those populations was confirmed by model-based clustering methods, using multilocus genotype data. Phenograms generated from RAPD bands indicated that some accessions of *I.* ×*wandoensis* clustered with one of the parental species. This implied the occurrence of hybridization and recurring backcrosses of the hybrid to both parents, resulting in various hybrid derivatives because of the segregation and recombination of traits. *Ilex* ×*wandoensis* was more closely related to *I. cornuta* than to *I. integra*, suggesting that it backcrossed more with the former than with the latter.

Keywords: Aquifoliaceae, *Ilex cornuta*, *Ilex integra*, *Ilex* ×*wandoensis*, RAPD, STRUCTURE

Ilex L. is the largest genus of Aquifoliaceae, comprising 780 evergreen and 30 deciduous taxa (Galle, 1997) in both tropical and temperate regions. Along with *I. integra* Thunb. and *I. cornuta* Lindle and Paxton reported from the southern parts of the Korean peninsula (Lee, 1992, 2002), a third evergreen species, *I.* ×*wandoensis*, has been discovered at Wando, Korea (Dudley and Yinger, 1987a, b, 1988) (Fig. 1), and has been proposed as a putative hybrid between *I. cornuta* and *I. integra* based on its morphological characters. Although the name *I.* ×*wandoensis* has been attributed to "C. F. Mill. ex T. R. Dudley" and cited in the literature and the trade ever since, it has been taxonomically described instead as *I.* ×*wandoensis* C. F. Mill. & M. Kim (Miller and Kim, 2002).

Inter-taxa hybridization in plants, including introgressive hybridization and divergent evolution, can play important roles leading to genetic variation, diversification, and speciation (Rieseberg, 1995; Morrell and Rieseberg, 1998; Setoguchi and Watanabe, 2000; Rieseberg et al., 2003). Documentation of hybridization has traditionally been based on morphological characters that are intermediate between the parental types (Grant, 1981). For example, *Ilex* ×*wandoensis* has serrated leaf margins and cymes of 5 to 6 female flowers (Miller and Kim, 2002), compared with the spiny leaf margins and 7 to 10 female flowers from *I. cornuta* and the entire leaf margins and 3 to 5 female flowers of *I. integra*.

Along with those manifestations, the leaves of *I. integra* are thin and flexible, with 1- to 2-cm-long petioles, and young and newly emerged leaves that are reddish-bronze and sometimes with very tiny (0.1- 0.2-cm-long) teeth (Lee, 1992, 2002). In contrast, the leaves of *I. cornuta* are thick and dark, glossy green, with two or three sets of strong spines (teeth), or only one spine at the leaf apex, as well as shorter petioles (0.4 to 0.8 cm). Although *I.* ×*wandoensis* has intermediate characters, its leaf shapes are variable, with a range of tooth numbers and sizes, and some trace of a bronze color. These differences make it difficult to assign some accessions to any one of the three taxa groups, because some leaves are similar to *I. integra* and others to *I. cornuta* (Fig. 1). This may be due to genetic variation for leaf shape among the hybrids (Dudley and Yinger, 1987a). However, this current investigation did not employ molecular techniques to determine whether *I.* ×*wandoensis* is a natural hybrid or simply a variety of one of those other species.

Molecular data are widely considered to be better suited than morphological information for hybridization research because intermediate trait values are more easily accounted for in hybrid species (Rieseberg and Ellstrand, 1993; Rieseberg et al., 2003). Molecular markers, such as nuclear DNA and chloroplast DNA restriction site variations, nucleotide sequencing, and PCR-generated markers, have been applied to demonstrate hybridization and introgression (Rieseberg and Brunsfeld, 1992; Rieseberg and Ellstrand, 1993; Rieseberg and Wendel, 1993; Rieseberg, 1995; Raina et al., 2001). Among these, RAPD markers that show dominant inheritance have been used to evaluate genetic diversity (Lee et al., 2004), to construct genetic maps, and to examine patterns of hybridization in cultivated and natural populations (Williams et al., 1990; Joung and Roh, 2005; Roh et al., 2006).

To study the introgression and hybridization of hybrid individuals, we applied a model-based clustering method that incorporated multilocus genotype data to infer population structure and assign individuals to those populations (Pritchard et al., 2000; Anderson and Thompson, 2002; Falush et al., 2003). Through RAPD molecular markers, data

*Corresponding author; fax +82-2-3277-2385
e-mail namsook@ewha.ac.kr

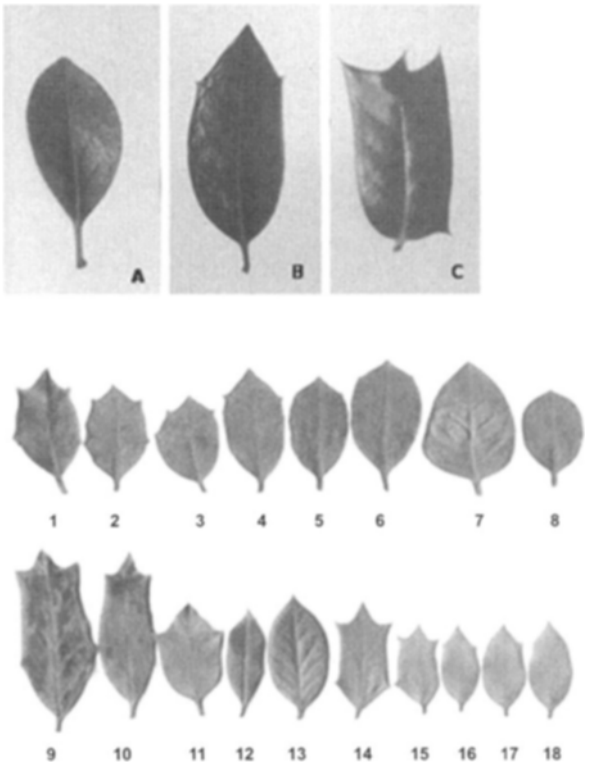

Figure 1. Typical leaf shapes of *Ilex integra* (**A**), *I.* ×*wandoensis* (**B**), and *I. cornuta* (**C**); and leaf variations within *I.* ×*wandoensis* (1 through 18).

analysis, and a multilocus genetic data program (STRUCTURE), we then attempted to assess the degree of hybridization of *Ilex* ×*wandoensis* between *I. integra* and *I. cornuta*.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Leaves from a total of 122 accessions of *Ilex* ×*wandoensis*, *I. integra*, and *I. cornuta* were obtained from natural and transplanted sites in Korea (Table 1; Fig. 2). Additional samples were gathered from seedlings maintained at either the US National Arboretum, Washington, DC, or the Woody Landscape Germplasm Repository, Glenn Dale, Maryland, USA. For the natural sites, leaves were collected from individuals at least 2 m apart, except for Accessions 50 and 57, to reduce the possibility of genetic similarity. Total genomic DNA was extracted from fresh, frozen, or dried leaves, using the DNeasy Plant Mini Kit (QIAGEN, USA) according to the manufacturer's instructions.

DNA Amplification

The concentration of each extracted DNA was determined by comparing the brightness of the DNA band with a standard lambda DNA marker band on a 0.7% agarose gel stained with ethidium bromide (EtBr). The resulting DNA extracts were diluted to equal concentrations based upon visual inspection on the agarose gel and the DNA quantification of several samples with various intensities on the gel.

A total of 20 primers from Kits A, B, and C (Operon Technologies, USA) were screened, and 14 primers (A04, A05, A08, A14, A18, B04, B06, B12, B18, C01, C02, C09, C15, and C16) were selected for analysis. Amplification of the RAPD markers was performed with approximately 10 ng of template DNA, 200 nM of primers, and Ready-To-Go PCR Beads (Amersham Pharmacia Biotech, USA), for a total reaction volume of 25 μL. A PTC-100 programmable thermal

Table 1. Collection data for *I. cornuta*, *I. integra*, and *I.* ×*wandoensis* complex. Identities were assigned based on morphological characters.

Taxa-accession number	Locality
I. cornuta	
C-5	Byunsan, NA[(a)]56770-006
C-6	Byunsan, NA56771-003
C-8	Byunsan, NA56642-001
C-9	Byunsan, NA56659-001
C-11	Byunsan, NA56771-004
C-20, 22~23,	Jeju-Do, Hyeobje
C-29~32, 35~39	Jeju-Do, Halla Arboretum
C-50	Wando, Daemun-Ri (hill)
C-52	Wando, Restaurant-town
C-62~63, 68, 79~80, 84~86	Byunsan-north [(b)]
C-72	Koshikawa, Japan
C-73~75	Jeju-Do, Hyeobje
C-87~93, 96, 98~101, 103, 107, 109~110, 112	Byunsan-south [(b)]
C-113	Bogildo
C-149	Chollipo (81-271)
C-150	Chollipo (Byunsan Lyre)
C-151	Chollipo
C-156, 162, 163	Wando, Galmun-Ri
I. ×*wandoensis*	
W-2	Wando, NA56756-011
W-3	Wando, NA56757-002
W-4	Wando, NA56757-006
W-7	Wando, NA56746-009
W-12	Wando, NA56770-008
W-15	Wando, NA56740-001
W-16~19, 34	Jeju-Do, Jeocheong
W-55, 58	Wando, Bulmok-Ri
W-56	Wando, Wando Park
W-57	Wando, Daemun-Ri (hill)
W-59	Wando, Daehan NC [(c)]
W-76~77	Jeju-Do, Halla Arboretum
W-143	Chollipo (80-1142)
W-144	Chollipo (80-420)
W-145	Chollipo (79-1274)
W-146	Chollipo (87-46)
W-147	Chollipo (79-1276)
W-148	Chollipo (81-701)
W-152	Chollipo (80-839)
W-155, 157, 159~161	Wando, Galmun-Ri

Table 1. Continued.

Taxa-accession number	Locality
I. integra	
I-14	Wando, NA56757H
I-24, 26, 40	Jeju-Do, For. Expt. Sta.[d]
I-41-45	Jeju-Do, Halla Arboretum
I-46	Wando, NA56757H
I-60	Japan, Kagoshima
I-78	Jeju-Do
I-114~121, 123~127	Bogildo, (Mountain)
I-128, 130, 134, 136~140,	Bogildo, (Reservoir side)
I-153	Chollipo (76-0044, Jeju-Do)
I-154	Wando, Galmun-Ri

[a]Transplanted in National Arboretum, USA, from Byunsan, Korea.
[b]North and South: Natural populations of *I. cornuta* from north and south sides of the monument.
[c]Daehan NC: Daehan Nursery, Gurye, Jeollanam-Do.
[d]Forest Experiment Station, Seoguipo, Jeju-Do.

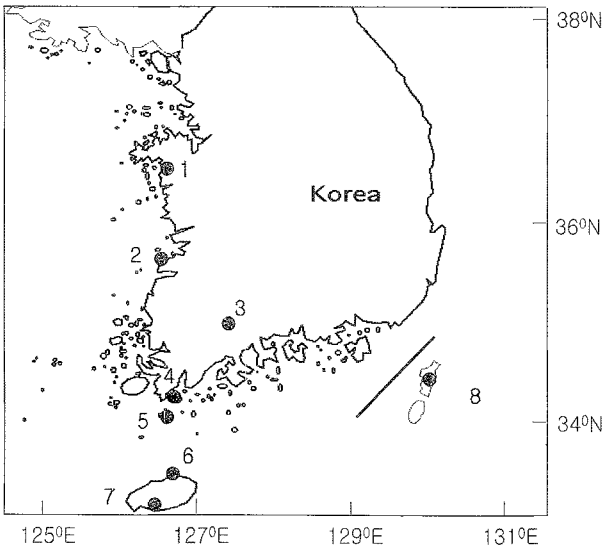

Figure 2. Collection sites: 1, Chollipo Arboretum; 2, Byunsan; 3, Gurye; 4, Wando Island (Wando); 5, Bogildo Island (Bogildo); 6, Halla Arboretum, Jeju-City; 7, Hyeobje, Jeju-Do; 8, Kagoshima, Japan.

cycler (MJ Research, USA) was used to run our PCR program, which was set for an initial cycle of 3 min at 94°C; then 36 cycles of 5 s at 94°C, 1 min at 37°C, 1 min at 72°C; followed by a final 3 min at 72°C.

Banding Pattern Analysis

PCR products were separated on 1.7% agarose gels and visualized by staining with EtBr. These gels were documented digitally with an Image Analyzer (Alphalmager 2000; Alpha Innotech, USA), and electronically scored using ONE-Dscan software (Scanalytics, USA) before being corrected manually. The molecular size of each fragment was estimated based on 100 bp DNA Ruler Plus (Fermentas, MD). Banding patterns were scored conservatively for a presence/absence (1/0) matrix, which considered only distinct bands with small differences in staining intensities among samples.

Data Analyses

From the resulting 1/0 data matrix, we calculated a similarity matrix (Jaccard's coefficient) by comparing only the polymorphic markers. A phenogram was generated by the unweighted pair-group method, using the arithmetic averages (UPGMA) clustering analysis. This UPGMA analysis was performed with the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc, version 2.1, Exeter Software; Applied Biostatistics, USA; Rohlf, 2000). We then examined the total number of bands and the distribution of bands across taxa, as well as the number of polymorphic bands, number of fixed bands, average number of bands per primer for each taxon, and bands shared among taxa. The percentage of polymorphism at a specific level was calculated as the proportion of polymorphic loci to the total number of loci scored in all accessions. Categories for marker bands were used in these calculations to estimate the degree of gene flow. When certain bands were present in only one taxon, they were considered taxon-specific private bands; when they were present in 2 or 3 taxa, and the percentage was >25% in one taxon and <5% in the others, they were considered marker bands (Wolfe et al., 1998).

Analysis for Multilocus Genotype Data

Using the admixture model in the STRUCTURE program (Pritchard et al., 2000), we generated the scores for the proportions of each individual (Q scores) for the RAPD markers. This model assumed that individuals may have mixed ancestry. Each individual was assigned to a population according to its species identification prior to analysis by STRUCTURE. The resulting Q scores were used to generate a posterior probability graph showing species contributions for each individual.

RESULTS

From 14 arbitrary primers, 244 bands were scored for 122 individuals. Our three *Ilex* taxa shared 160 marker bands

Table 2. Distribution and percent polymorphism for *Ilex* taxa using 14 RAPD Primers.

	I. cornuta	*I.* ×*wandoensis*	*I. integra*
Number of accessions	55	30	37
Total bands scored across populations	242	244	242
Fixed bands scored across populations	25	14	17
Polymorphic bands (%) across all populations	90	94	93
Marker bands (species-specific marker)	5(1)	10(1)	8(3)

Table 3. Distribution of RAPD bands among taxa, arranged by taxa and in alpha-numerical order of primers and loci. Abbreviations C, W, and I represent *I. cornuta*, *I. ×wandoensis*, and *I. integra*, respectively.

Band shared among Taxa	Primer-locus	Marker	C		W		I	
			55[a]	C%	30	W%	37	I%
W/I	B18-750	I	0	0	1	3	29	78
C/W/I	A05-575	C	14	26	1	3	2	5
	A04-600	I	1	2	2	6	11	30
	A04-1050	W	4	6	11	34	1	3
	B04-550	W/I	3	6	15	47	20	54
	B06-660	W/I	3	6	8	25	35	95
	B06-700	C/W	50	90	23	72	1	3
	B06-1020	C/W	41	75	18	56	1	3
	B18-340	I	3	6	1	3	14	38
	C01- 250	C/W	37	67	16	50	1	3
	C01-1440	W/I	3	6	9	28	22	60
	C02-1950	W/I	2	4	13	41	32	87
	C16-700	W/I	2	4	12	38	18	49
	C16-3400	C/W	45	86	23	72	1	3

[a]Number of accessions.

(66%). In addition, one (A05-575; primer–estimated size in base pairs), one (A04-1050), and three (A04-600, B18-750, and B18-340) species-specific marker bands were recorded for *I. cornuta*, *I. ×wandoensis*, and *I. integra*, respectively. *Ilex ×wandoensis* shared four marker bands with *I. cornuta*, and five with *I. integra* (Table 2, 3).

Our UPGMA analysis, based on pairwise Jaccard's coefficients, produced a phenogram that demonstrated most accessions clustering together according to their initial taxa assignments, as determined by morphological characters. The exceptions were a few *I. ×wandoensis* accessions, which were interspersed. Two *I. ×wandoensis* accessions (W-7 and W-56) intermingled with *I. cornuta* while several misidentified *I. integra* accessions (I-14, I-24, I-26, and I-118) intermingled with those of *I. cornuta* and *I. ×wandoensis*. Accessions C-156, C-162, and C-163, which were difficult to assign to either *I. cornuta* or *I. ×wandoensis* based on morphology, clustered with *I. cornuta* (Fig. 3).

The posterior probabilities of specific taxa frequency categories for each individual revealed that some accessions (i.e., I-40 through I-45, and I-117, I-120, and I-126) had high probabilities (>0.90) of being *I. integra*, and could be considered pure and true to the taxon. Only I-14 and I-24 had the highest probability of each being *I. ×wandoensis* (Fig. 4), and perhaps might instead have been considered hybrids or introgressants, rather than misidentified accessions. Furthermore, I-118 could probably have been identified as *I. cornuta*, based on a posterior value of >85%. In contrast, 18 *I. cornuta* accessions, including C-88 and C-92 (which each had >90% posterior probabilities), could be classified as pure taxa, while C-30, C-52, C-62, and C-163 could be considered introgressants between *I. cornuta* and *I. ×wandoensis*. In *I. ×wandoensis*, W-146, W-152, and W-159 among others could be considered introgressant between *I. integra* and *I. ×wandoensis*. Many *I. ×wandoensis* accessions, including W-59, W-76, and W-143, could be categorized as introgressants between *I. cornuta* and *I. ×wandoensis*. Finally, Accessions W-16, W-17, W-18, and W-19 could be declared to be pure *I. ×wandoensis* due to their >95% posterior probability values.

DISCUSSION

The presence of *Ilex ×wandoensis* populations within the range of *I. integra* and *I. cornuta* suggests that this taxon is a natural hybrid derived from the other two (Dudley and Yinger, 1987a, b, 1988). Such hybrids often display a mosaic of parental and/or intermediate characters, rather than solely intermediate forms. Morphological variations in the *I. ×wandoensis* leaves (Fig. 2) clearly showed those mosaic patterns, making it very difficult to assign certain accessions to any established species. Furthermore, the presence of teeth on the leaves, a key trait for taxon identification, depends on the particular developmental stage, again complicating the assignment of accessions based on that character. The mosaic combination pattern found here implied that *I. ×wandoensis* could be backcrossed to either or both of its parents (Dudley and Yinger, 1987a). Accumulated genetic changes over time and the high genetic similarity between taxa make the detection of ancient hybrids more difficult (Gallez and Gottlieb, 1982). Therefore, germplasm should be acquired and characterized from both large hybrid seed populations and the parent plants (Dudley and Yinger, 1988). This is particularly important when seeds are produced by open pollination, and also when the possibility of natural hybridization exists due to sympatric growth, as was found with these *Ilex* taxa. In addition, artificial or synthetic crosses should be made to reconstitute the hybrid in order to study ecological transitions, as has been reported with two species of *Helianthus* (Rieseberg et al., 2003). Nevertheless, we did not attempt here to create such an artificial

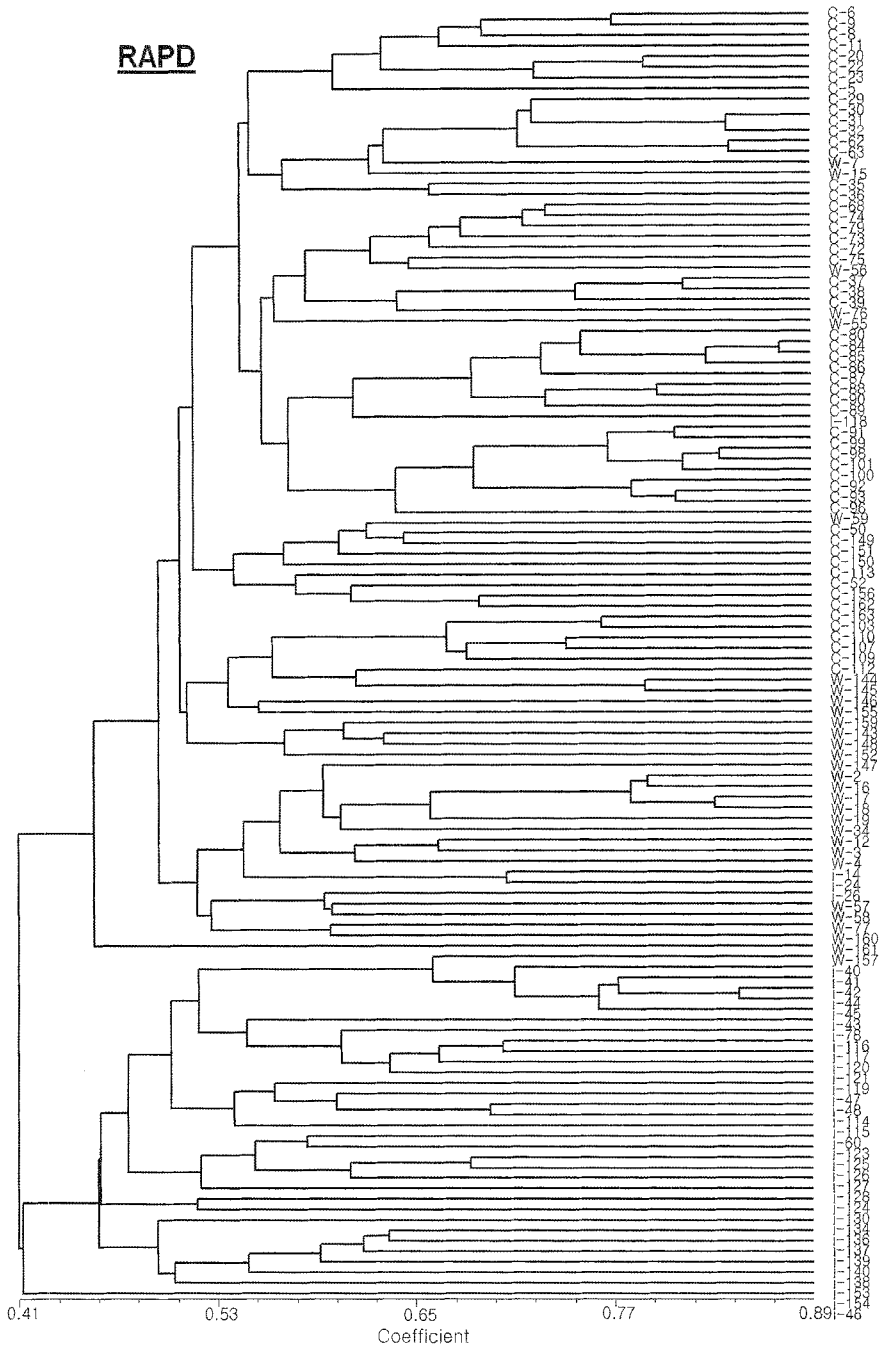

Figure 3. UPGMA phenogram using Jaccard's coefficient by RAPD markers for 122 accessions in *Ilex* hybrid complex. *I. cornuta* (C), *I.* ×*wandoensis* (W), and *I. integra* (I) precede accession numbers. Refer to Table 1 for details.

Ilex hybrid, i.e., *I.* ×*wandoensis*, Likewise, we did not obtain F_2 generations or a backcrossed generation of *I.* ×*wandoensis* with either of the putative taxa because of the time limitation for acquiring flowering, mature plants. Therefore, unlike in that research with *Helianthus*, it was difficult to assess the role of hybridization in our study of phenotypic and genomic comparisons between ancient and synthetic hybrids in *Ilex*.

If *I.* ×*wandoensis* has indeed resulted from the sympatric speciation of two parental taxa (Dudley and Yinger, 1987a) and then backcrossed with either one of the parents, significant variations in leaf morphology would be expected, which might be referred to as transgressive segregation (Rieseberg et al., 2003). We did observe this in a few accessions -- e.g., C-156, C-162, and C-163 - that initially were difficult to assign to either *I. cornuta* or *I.* ×*wandoensis* based on their morphological characters. Those could be introgressants between *I.* ×*wandoensis* and *I. cornuta*.

Some accessions of *I.* ×*wandoensis* (W-7, W-15, and W-55) belonged to a cluster intermingled with *I. cornuta* in the UPGMA phenogram. Their placement in that phenogram suggested a hybrid origin, despite some concerns with the high degree of variability among the three taxa (Rieseberg and Ellstrand, 1993), and clearly implied that introgressive

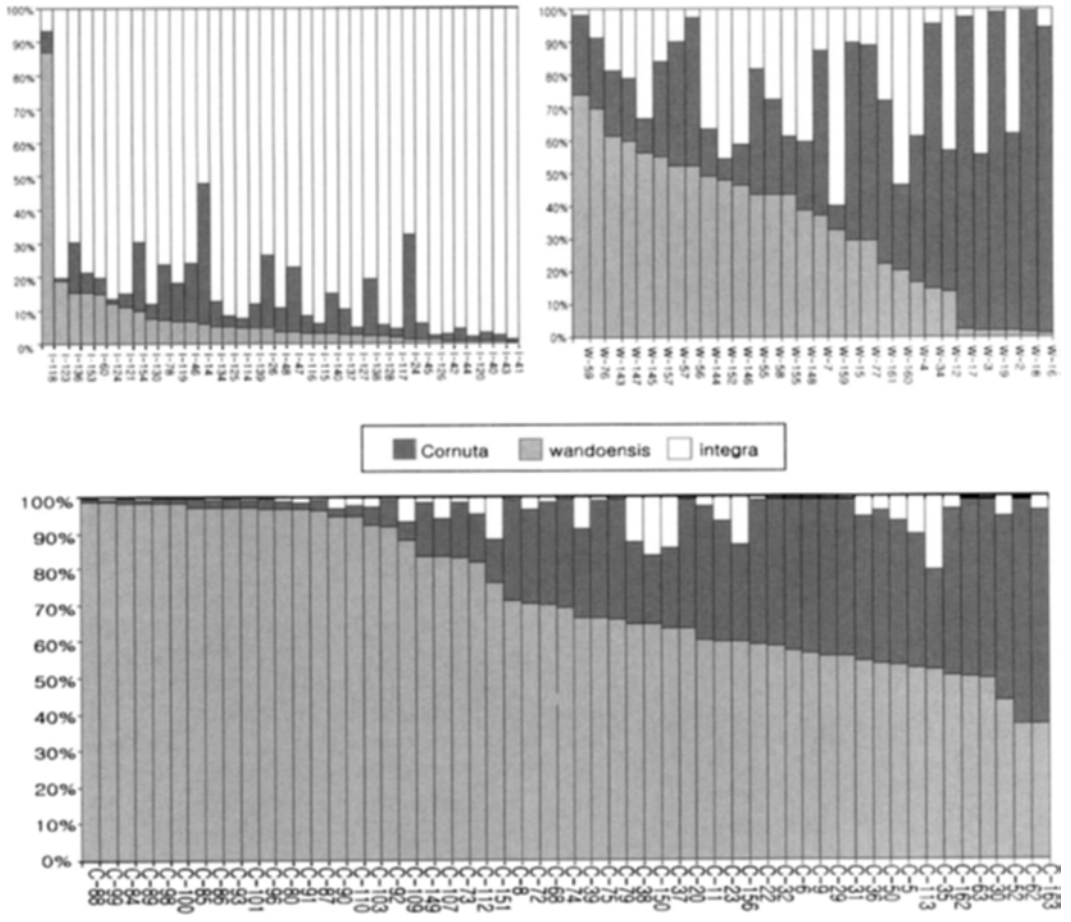

Figure 4. Posterior probabilities of specific taxa frequency categories for each individual in given taxa of *I. cornuta* (C), *I.* ×*wandoensis* (W), and *I. integra* (I), using STRUCTURE program based on RAPD markers. Heights of different patterns within columns denote posterior probability of each accession belonging to one of three taxa frequency classes.

hybridization among them had occurred. Due to extensive introgression and significant morphological variation, some individuals might have been misidentified, thus initially being assigned to the wrong taxon. Our RAPD markers were able to differentiate most accessions, consistent with their morphological characters, with *I.* ×*wandoensis* accessions clustering closer to *I. cornuta* than to *I. integra* (Fig. 3).

Hybridization also can be examined based on the distribution of marker bands, under various established criteria that determine private, marker, or shared-band status (Howard et al., 1997; Wolfe et al., 1998). Because *I.* ×*wandoensis* possessed marker bands of *I. cornuta* and *I. integra*, this supports the theory that it originated from both. In addition, the RAPD-associated marker band of *I.* ×*wandoensis* at 11 accessions from 4 populations for A04-1050 suggested introgressive hybridization between *I. cornuta* and *I. integra*. The higher number of shared bands between *I. cornuta* and *I.* ×*wandoensis* than between *I.* ×*wandoensis* and *I. integra* also implied that *I.* ×*wandoensis* introgressed more to *I. cornuta* than to *I. integra*. The fact that the three taxa were highly polymorphic and had many shared marker bands leads us to believe that the hybrid swarm has existed for considerable time (Rieseberg and Brouillet, 1994).

Based on the posterior probabilities of specific taxa frequency categories that were obtained for each individual via the STRUCTURE program, we can conclude that specific accessions represent pure lineages for *I. integra* (I-40, I-41, I-42, I-43, I-120, and I-126), for *I. cornuta* (C-84, C-88, C-89, and C-99), and for *I.* ×*wandoensis* (W-16, W-17, W-18, W-19).

In summary, the data from our molecular analyses with RAPD support the recent proposal that Korean *I.* ×*wandoensis* originated from hybridization between *I. cornuta* and *I. integra*. The expected intermingling of some accessions with each of the parental species demonstrates hybridization and complex backcrossing to both parents. *Ilex* ×*wandoensis* is most likely derived from an initial cross of *I. cornuta* and *I. integra*, which subsequently introgressed largely with *I. cornuta* or, to a lesser degree, with *I. integra*. This has resulted in a wide range of morphological characters, followed by ecological transitions based on the posterior probabilities of two parental taxa for the hybrid individuals. The assignment of most accessions, as determined by their morphological characters, generally agrees with the assignment based on molecular markers, except for a few. Therefore, most of the accessions should be considered as pure or true to the taxa, while some could be introgressant, although their level of hybridity could not be

identified here. Further investigation is being conducted to examine the relationship between *I.* ×*wandoensis* and its putative parental taxa, to understand this degree of introgression. Using restriction enzymes to analyze the sequences of chloroplast or mitochondria genes and their fragment sizes, we hope to determine the true accessions of both maternal and paternal parents of this putative hybrid.

ACKNOWLEDGEMENTS

This research was supported in part by a grant from the Holly Society of America to M. Roh, and was conducted out during N.S. Lee's sabbatical leave at the US Department of Agriculture, Agricultural Research Service. We thank Deric Picton for his laboratory work and data processing, Detiger Dunams for her laboratory work, and Dr. H. Ikeda, Kagoshima, Japan, for providing the *I. integra* leaf samples. We are also grateful to Drs. Kim Hummer and John Wiersema for their careful reading and critical comments, and to Martin Morgan for his interpretation of the data and many valuable suggestions.

Received August 29, 2006; accepted December 1, 2006.

LITERATURE CITED

Anderson EC, Thompson EA (2002) A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160: 1217-1219

Dudley TR, Yinger BR (1987a) 1985 U.S. National Arboretum Plant Exploration in the Republic of Korea, Southwest Coast and Islands Final Report: Part II. Holly Soc J 5: 10-16

Dudley TR, Yinger BR (1987b) 1985 U.S. National Arboretum Plant Exploration in the Republic of Korea, Southwest Coast and Islands Final Report: Part III. Holly Soc J 5: 4-13

Dudley TR, Yinger BR (1988) 1985 US National Arboretum Plant Exploration in the Republic of Korea, Southwest Coast and Islands. Final Report: Part IV. Holly Soc J 6: 5-13

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics 164: 1567-1587

Galle FC (1997) Hollies. The Genus *Ilex*. Timber Press, USA

Gallez GP, Gottlieb LD (1982) Genetic evidence for the hybrid origin of the diploid plant *Stephanomeria diegensis*. Evolution 36: 1158-1167

Grant V (1981) Plant Speciation. Columbia University Press, New York

Howard DJ, Preszler RW, Williams J, Fenchel S, Boecklen WJ (1997) How discrete are oak species? Insights from a hybrid zone between *Quercus grisea* and *Quercus gambelii*. Evolution 51: 747-755

Joung YH, Roh MS (2005) Mapping characterization of *Pinus sylvestris* var. *sylvestriformis* based on chloroplast DNA microsatellite markers. For Genet 12: 89-98

Lee S, Ma S, Lim Y, Choi HK, Shin H (2004) Genetic diversity and its implications in the conservation of endangered *Zostera japonica* in Korea. J Plant Biol 47: 275-281

Lee TB (1992) Illustrated Woody Plants of Korea. Forestry Research Institute, Forestry Administration

Lee YN (2002) Flora of Korea. Kyo-Hak, Seoul

Miller CF, Kim M (2002) *Ilex* ×*wandoensis* C.F. Miller & M. Kim, a new hybrid species of *Ilex* (Aquifoliaceae) from Korea. Kor J Plant Tax 32: 293-299

Morrell PL, Rieseberg LH (1998) Molecular tests of the proposed diploid hybrid origin of *Gilia achilleifolia* (Polemoniaceae). Amer J Bot 85: 1439-1453

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959

Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Dearumath RM (2001) RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome 44: 763-772

Rieseberg LH (1995) The role of hybridization in evolution: Old wine in new skins. Amer J Bot 82: 944-953

Rieseberg LH, Brouillet L (1994) Are many plant species paraphyletic? Taxon 43: 21-32

Rieseberg LH, Brunsfeld SJ (1992) Molecular evidence and plant introgression, *In* PS Soltis, DE Soltis, JD Doyle, eds, Molecular Plant Systematics. Chapman and Hall, New York

Rieseberg LH, Ellstrand NC (1993) What can molecular and morphological markers tell us about hybridization? Crit Rev Plant Sci 12: 213-241

Rieseberg LH, Raymond O, Rosenthal DM, Lai Z, Livingstone K, Nakazato T, Durphy JL, Schwarzbach AE, Donovan LA, Lexer C (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301: 1211-1216

Rieseberg LH, Wendel JF (1993) Introgression and its consequences in plants, *In* R Harrison, ed, Hybrid Zones and the Evolutionary Process. Oxford University Press

Roh MS, Lee AK, Suh JK, Slovin J (2006) Confirmation of cross-pollination of *Ardisia crenata* by sequence-characterized amplified region (SCAR) markers. Scient Hort 109: 361-367

Rohlf FJ (2000) NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System. version 2.1, Exeter Software, Setauket

Setoguchi H, Watanabe I (2000) Intersectional gene flow between insular endemics of *Ilex* (Aquifoliaceae) on the Bonin Islands and the Ryukyu Islands. Amer J Bot 87: 793-810

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res 18: 6531-6535

Wolfe AD, Xiang QY, Kephart SR (1998) Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. Mol Ecol 7: 1107-1125