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Molecular evaluation of interspecific hybrids between *Acer albopurpurascens* and *A. buergerianum* var. *formosanum*

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ABSTRACT. *Ex situ* conservation is a common strategy for the restoration of endangered species. Botanical gardens play an important role in the restoration of plants; however, hybridization could happen if transplantation is not considered carefully. Two naturally allopatric taxa *Acer albopurpurascens* and *A. buergerianum* var. *formosanum*, grown in the Taipei Botanical Garden, hybridized spontaneously. Hybridization was shown by the presence of intermediate phenotypes and genetic data. To confirm the direction of pollen flow, indirect evidence of maternally inherited chloroplast DNA indicated that *A. buergerianum* var. *formosanum* was the pollen receiver. The hybrids shared parts of ancestral genetic variations with their parents. Furthermore, several novel haplotypes that were slightly different from their parents were cloned, which revealed higher genetic diversity of the hybrid population than the parental populations. Such a phenomenon is called the rare allele phenomenon. In comparison with haplotypes of parents and hybrids, and according to the estimation of minimum recombination events and the likelihood ratio tests, the genetic variations were brought about by recombination events, such that the rare allele phenomenon might be related to the recombination. The hybridization that occurred in the botanical garden underscores the importance of spatial isolation when carrying out *ex situ* plantations.

Keywords: *Acer*; *Ex situ* conservation; Hybridization; Rare allele phenomenon; Recombination.

INTRODUCTION

Hybridization is commonly observed between closely related species (e.g. Kirk et al., 2004; Edwards et al., 2006) and even between distantly related species (e.g. Dunn and Lindstrom, 2008). In fact, hybridization may be viewed as an "invasion of the genome" from a genetic viewpoint (Mallet, 2005), and hybridization is potentially of evolutionary significance in the formation of species complexes (Rieseberg, 1995). The invasion of the genome promotes genetic evolution, and the rearrangement of parental species genomes may widen the adaptive range for survival (heterosis). Through this process, life becomes more adaptive and diversified, especially in plants (Erickson and Fenster, 2006; Smith and Baum, 2006; Ansell et al., 2007).

Speciation by hybridization is a popular issue among botanists exploring plant diversity; however, an important feature of speciation by hybridization is the formation of reproductive barriers between hybrids and parental species (cf. Rieseberg et al., 1995; Petit et al., 2004). An alternative consequence of hybridization is a wide range in genetic variation, resulting in the formation of a hybrid swarm (e.g. Wiens et al., 2006; Wachowiak and Prus-Glowacki, 2008). This may offer the potential for widening of ecological niches (Choler et al., 2004). In other words, successful hybridization may lead to either rapid speciation or elevated genetic variation. Several studies have shown that novel alleles might evolve in the context of hybridization (Schilthuizen et al., 1999; Schilthuizen et al., 2001; Smith et al., 2003). These novel alleles were widespread in the hybridized population

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but rare, even absent, in the parental populations. Such a phenomenon was called the "rare allele phenomenon" (Sage and Selander, 1979). The cause of this phenomenon was postulated to be intragenic recombination (Golding and Strobeck, 1983) or simple point mutations (Bradley et al., 1993; Hoffman and Brown, 1995). The genus *Acer* is a highly diversified taxon and such variability is commonly considered to result from hybridization (Grimm et al., 2007). However, the high ability to hybridize shown by sympatric, coexisting *Acer* species (even those that form hybrid swarms) logically seems to be a consequence of rapid diversification (cf. Wiens et al., 2006). The causes of rapid speciation and hybridization ability are difficult to test and verify.

Interestingly, two naturally allopatric taxa, *Acer albopurpurascens* and *Acer buergerianum* var. *formosanum*, both endemic to Taiwan, grown in a botanical garden for conservation and educational reasons, were spontaneously hybridized. The hybridization event was not artificial, and this allowed us to explore the causes of hybridization and rapid speciation in *Acer* species. In addition, genetic changes after hybridization (e.g., recombination) could be simultaneously explored.

Acer albopurpurascens and *A. buergerianum* var. *formosanum* show an allopatric distribution in Taiwan. *A. albopurpurascens* grows in low-altitudinal forests while *A. buergerianum* grows around the coasts of Northern Taiwan. These two taxa were thought to be closely related phylogenetically (Tzeng, 2007), but natural hybridization has never been observed in the field because of the allopatry of the two species (Wang et al., 1996). However, the two taxa grown together in the Taipei Botanical Garden, spontaneously hybridized, and their F1 hybrids revealed phenotypes that were in between those of the two parents (Wang et al., 1996). For example, the leaf morphology of the F1 hybrids varies widely, from oblong-lanceolate to shallowly three-lobed, compared to *A. albopurpurascens* (oblong-lanceolate) and *A. buergerianum* var. *formosanum* (broadly three-lobed or shallowly three-lobed near the apex; or entire, rounded, or slightly cordate at the base) (Figure 1).

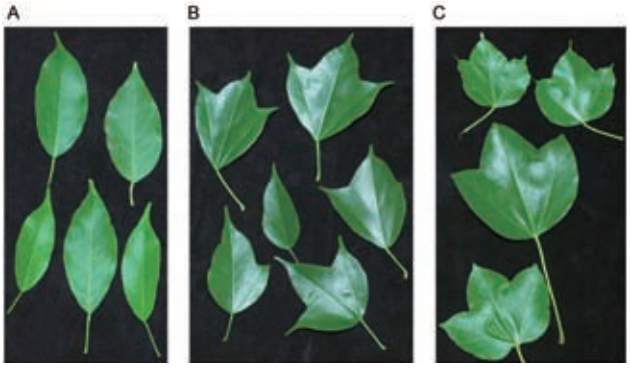

Figure 1. The morphological patterns of leaves of *Acer buergerianum* var. *formosanum*, *A. albopurpurascens*, and their F1 hybrids. (A) Leaves of *A. albopurpurascens*, (B) leaves of an F1 hybrid, (C) leaves of *Acer buergerianum* var. *formosanum*.

Spontaneous hybridization between *A. albopurpurascens* and *A. buergerianum* var. *formosanum* was confirmed by the random amplified polymorphic DNA (RAPD) technique (Wang et al., 1996), but the direction of pollen flow was unclear using this technique alone. Hence, chloroplast DNA markers (indicating maternal inheritance) were used to identify the direction of pollen flow, and the internal transcribed spacer (ITS) of the ribosomal RNA gene (rDNA) was cloned and sequenced to confirm hybridization events.

In this study, the rDNA ITS recombination was explored through analysis of spontaneous hybridization between two normally allopatrically distributed *Acer* species that were grown in a botanical garden. In addition, we focused on the phenomenon of hybridization and genetic variation in *Acer* species. This study provides insights into the contribution of hybridization to diversification, and the evolutionary mechanism of recombination by hybridization, in *Acer* species.

MATERIALS AND METHODS

Species and sampling

To identify the spontaneous hybridization in two allopatric *Acer* taxa, *A. albopurpurascens* and *A. buergerianum* var. *formosanum*, planted in the Taipei Botanical Garden, were collected and the seedlings were germinated in the nursery of the garden. These seedlings were considered the F1 hybrids described in the Results and Discussions section below. Young leaves from six F1 seedlings were collected for DNA analysis. Leaf samples from six *A. albopurpurascens* and five *A. buergerianum* var. *formosanum* parental shrubs, originally planted in the garden, were collected for DNA analysis.

DNA extraction, amplification, and sequencing

Dry leaf tissue was ground to powder in liquid nitrogen and stored at -70ºC. Genomic DNA was extracted from the powdered tissue following the cetyltrimethylammonium bromide (CTAB) procedure (Murray and Thompson, 1980). The *atp*B-*rbc*L noncoding spacer of plastid DNA and the ribosomal DNA internal transcribed spacer (rITS) of nuclear genomic DNA were amplified and sequenced using the two universal primers for the *atp*B-*rbc*L noncoding spacer designed by Chiang et al. (1998), and the two universal primers for rITS of nuclear genomic DNA described by White et al. (1990), respectively. PCR amplifications were carried out in a volume of 50 μL using 10 ng of template DNA, 10 μL of 10X reaction buffer, 10 μL $MgCl_2$ (25 mM), 10 μL dNTP mix (8 mM), 10 pmol of each primer, 10 μL of 10% (v/v) NP-40, and 2 U of Taq polymerase (Promega, Madison, WI). Reactions were run on a Biometra T-Gradient Thermal Cycler. The protocol consisted of one cycle of denaturation at 94ºC for 4 min, 30 cycles of 1 min denaturation at 94ºC, 1 min 15 s of annealing at 52ºC for plastid DNA *atp*B-*rbc*L spacer or 1 min 15s at 50ºC for rITS of nuclear genomic DNA,

and 1 min 30 s of extension at 72ºC, followed by a 10 min extension step at 72ºC. PCR products were purified by electrophoresis in 1.0% (w/v) agarose gels using 1X Tris-acetate-EDTA (TAE) buffer. Gels were stained with ethidium bromide. The targeted DNA bands were cut out and eluted using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA), and the DNA was ligated to vectors using the pGEM-T Easy Vector System (Promega). Five clones were selected randomly from each individual cloning procedure. DNA was purified using a QIAprep Spin Miniprep Kit (QIAGEN), and quantified. Purified, cloned plasmid DNAs were sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) on an ABI 3130 automated sequencer (Applied Biosystems), using the T7 and SP6 universal primers that bind to the pGEM-T Easy Vector.

Data analysis

After obtaining DNA sequences, alignments were performed using Clustal_X (Thompson et al., 1997), and checked for proper assignment of nucleotides based on peaks and alignment manually with the assistance of BioEdit v. 7.0.5 software (Hall, 1999). All sequences were submitted to GenBank (accession nos. FN651659-FN651752). After sequence alignments, the number of polymorphic sites, the haplotype diversity (*Hd*), and both nucleotide diversity measurements π and θ were estimated for each population. All indices described directly above were estimated with the assistance of Arlequin v. 3.0.1 (Excoffier et al., 2005).

To confirm and elucidate paternal and maternal relationships with the hybrids, and determine the relative positions of parents and hybrids (F1), phylogenetic relationships were constructed by neighbor-joining (NJ) and the Bayesian inference (BI) approaches using cpDNA and rITS sequences. The NJ tree was built using MEGA 4 (Tamura et al., 2007) with the settings of the Kimura two-parameter substitution model, pairwise-deletion treatment of gaps, and resampling 1,000 times to estimate the relative support for a branching pattern using the bootstrap method. The BI trees were constructed using MrBayes 3 (Ronquist and Huelsenbeck, 2003). The GTR+I+G model was selected by a model generator (Keane et al., 2006) within TOPALi v. 2.17 (Milne et al., 2004) and used to generate hypothetical trees. Two runs of over 10 million generations each with pseudo-sampling every 100 steps were employed. The pseudo-sampling consisted of "burn-ins" at 1,500 and 15,000 generations for the cp *rbcL-atpB* tree and the rITS tree, respectively. The lengths of burn-ins were sufficient to reach convergence for constructing the consensus trees.

The minimum number of recombination events (Rm) were estimated with the assistance of DnaSP 4.0 software (Rozas et al., 2003), and the recombination tests were performed using TOPALi v. 2.17 using the likelihood scores (Milne et al., 2004). The estimations of Rm according to the four-gamete test identify the probable variable sites in recombination. Furthermore, the approximate maximum likelihood analysis by estimating neighbor joining trees from parental species and hybrids was performed and the log likelihoods for these NJ trees were estimated. Using a likelihood ratio test (LRT) for comparing to the no recombination model provides more convincing evidence of the recombination events. Using LRT sliding windows, polymorphic regions with shared variations would have lower likelihood scores that are smaller than the significant threshold of the no-recombination model, and polymorphic regions without shared variations would be higher. According to the likelihood scores produced by the sliding windows, the regions that have higher probabilities of recombination could be detected.

Theoretically, chloroplast DNA (cpDNA) from F1 hybrids is all contributed by the maternal donor (plastid DNA lacks recombination events), and rITS sequences should be equally contributed by both paternal and maternal species. However, the genetic distances of hybrid populations between paternal or maternal species may not be equal if recombination occurs within some highly polymorphic fragments. Hence, we estimated the genetic differences between each parental species and its offspring. These differences were calculated as the pairwise F_{ST} and the average number of nucleotide substitutions per site between populations (Dxy) (Nei, 1987), using Arlequin v. 3.0.1 (Excoffier et al., 2005). By comparing the information of recombination and genetic differences between parents and hybrids, we tried to explore the phenomenon of genetic skewness in nuclear ITS after hybridization.

The allelic-frequency distribution of the hybrid population was assessed by Tajima's *D* (1989) test. A negative *D* value indicates abundant accumulation of rare alleles. Thus, we used this test to examine whether the hybrid populations have novel alleles. This test was performed by DnaSP 4.0 (Rozas et al., 2003). The observed value was compared with coalescent simulations repeated 50,000 times using an intermediate recombination rate model (R=0.01).

RESULTS AND DISCUSSION

Phylogenetic relationships between parents and F1 hybrids

To confirm the direction of parental inheritance, maternal phylogenetic relationships between the two parental species and their hybridized offspring were constructed. Although the topologies of the NJ tree and the BI tree of the chloroplast *atp*B-*rbc*L spacer had slight differences, both trees revealed the same grouping pattern, indicating that the F1 hybrids were well grouped into *A. buergerianum* var. *formosanum* (Figure 2). The complete exclusivity of the *A. albopurpurascens* chlorotypes (chlorotypes IV and V) in hybrids indicates that maternal

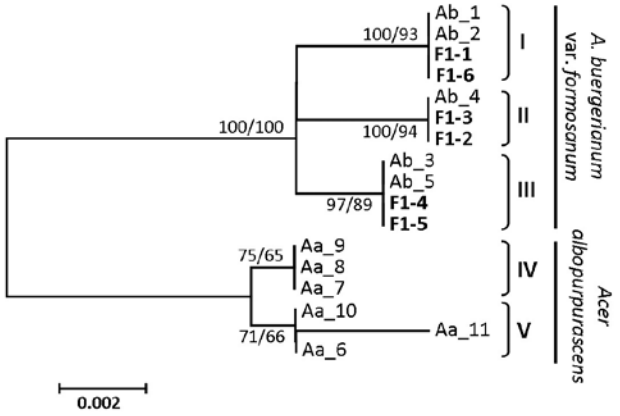

Figure 2. The phylogenetic relationships of the cpDNA *atp*B-*rbc*L spacer between *Acer buergerianum* var. *formosanum*, *A. albopurpurascens*, and their F1 hybrids, based on the neighbor-joining (NJ) method and Bayesian inference (BI). Numbers near the branches are supporting values of likelihood for the BI ratio (first number) and bootstrap supporting values after 1,000 replicates of NJ (second number). Supporting values are given only when support >50%. According to the cpDNA phylogenetic tree, the maternal parent is *Acer buergerianum* var. *formosanum*.

inherited cpDNAs were donated from the *A. buergerianum* var. *formosanum* to its offsprings; *i.e. A. albopurpurascens* is the paternal species and *A. buergerianum* var. *formosanum* is the maternal species (Table 1).

In contrast to data produced using cpDNA, the phylogenetic relationship constructed by rITS analysis revealed quite complicated patterns in both the NJ and BI trees. In the phylogeny constructed using ITS sequences, clones of any particular sample were not well grouped. On the contrary, such clones showed mosaic positioning, as grouping was not well supported either by bootstrap values (NJ tree) or likelihood ratios (BI tree) (Figure 3). This means that *A. albopurpurascens*, *A. buergerianum* var. *formosanum*, and the F1 hybrids showed incompletely differentiated relationships in rITS genomic copies. From the results of the phylogenetic analysis in rITS, it is clear that the hybrids are not identical to either one of their parents in ITS, suggesting the occurrence of recombination during the process of gamete production (meiosis). The recombination events would cause higher genetic diversity within the offspring population than in either of the parental species (Latta et al., 2007).

Genetic diversity

In the cpDNA *atp*B-*rbc*L spacer, three haplotypes were obtained from *A. albopurpurascens*, *A. buergerianum* var. *formosanum*, and the F1 hybrids, with estimated haplotype diversities of 0.73 (±0.16), 0.80 (±0.16), and 0.80 (±0.12), respectively (Table 2). The chlorotypes of the F1 hybrids were all the same as those of the maternal donor *A. buergerianum* var. *formosanum*; however, the chlorotypes of the paternal parent, *A. albopurpurascens*, were completely different from those of *A. buergerianum* var. *formosanum* and the hybridized F1s. With rITS, 23, 16, and 29 haplotypes were obtained from *A. albopurpurascens*, *A. buergerianum* var. *formosanum,* and the hybridized F1, respectively. The haplotype diversities of rITS were 0.99 (±0.02), 0.89 (±0.06), and 1.00 (±0.01) for *A. albopurpurascens*, *A. buergerianum* var. *formosanum*, and hybridized F1, respectively (Table 2).

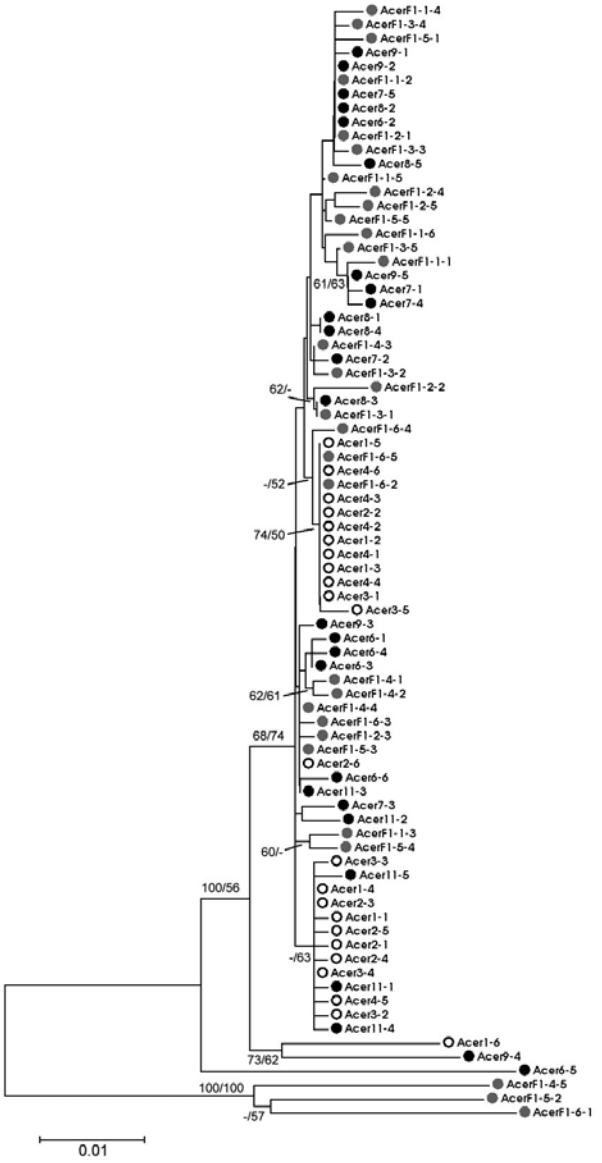

Figure 3. Phylogenetic relationships shown by rITS clones between *Acer buergerianum* var. *formosanum*, *A. albopurpurascens*, and their F1 hybrids, based on the neighbor-joining (NJ) method and Bayesian inference (BI). Numbers near the branches are supporting values of posterior probability of the BI ratio (first number) and bootstrap supporting values after 1,000 replicates of NJ (second number). Supporting values are given only when support >50%, and a dash indicates a supporting value <50%. White circles refer to *Acer buergerianum* var. *formosanum*; black circles to *A. albopurpurascens*; grey circles to F1 hybrids. The data show that the two parental species are of similar haplotype composition, leading to an unresolved, mosaic, tree topology; the F1 hybrid clones show a similar pattern.

In general, chloroplast DNA showed relatively low genetic diversity. For example, the diversity index θ ranged from 0.00219 to 0.00384 and the pairwise differences (π) per sequence varied from 0.00220 to 0.00427 (Table 2). Although small sample sizes might explain low genetic diversity of cpDNA, the rITS sequences did reveal higher genetic diversity, with θ ranging from 0.00786 to 0.02653 and π from 0.00377 to 0.01343 (Table 2). The patterns of genetic diversity estimated from the ITS are quite different from those estimated using cpDNA: with cpDNA, the genetic diversity of the F1 hybrids was similar to *A. buergerianum* var. *formosanum* and greater than *A. albopurpurascens*; however, in the analysis of ITS sequences, the hybrids had the highest genetic diversity but the *A. buergerianum* var. *formosanum* had the lowest (Table 2).

The genetic divergence data reveal a relatively lower nucleotide divergence (Dxy) (Nei, 1987) between the two parental species than between the parents and the F1 hybrids. However, the index of population differentiation (F_{ST}) revealed a relatively higher difference between the two parental species than between parents and F1 hybrids (Table 3). These contrast results due to two differentiation indices demonstrated that the two parental species were not very different in nucleotide composition (being phylogenetically related species), but that recombination caused by hybridization resulted in a more disparate nucleotide composition when parents and offspring were compared. However, because the F1 hybrids shared the same (or similar) haplotypes with both parents (especially with the paternal parent *A. albopurpurascens*), low F_{ST} values were obtained between parents and F1. This result suggests that the hybrid population might be composed of diversified gene pools that are contributed form random segregation and recombination of genes. The species boundary might then be broken if the hybridization continues.

Table 1. Chlorotype numbers of *Acer albopurpurascens* (Aa), *A. buergerianum* var. *formosanum* (Ab), and hybrids (F1). Chlorotypes I~V were defined according to the phylogenetic analyses.

	I	II	III	IV	V
Aa	0	0	0	3	3
Ab	2	1	2	0	0
F1	2	2	2	0	0

Table 2. Genetic diversity of cpDNA and rITS sequences of *Acer albopurpurascens*, *A. buergerianum* var. *formosanum*, and hybrids. *N*, sample size; *Hap*, number of haplotypes obtained; *Hd*, haplotype diversity (± standard deviation); $\theta(S)$, index of nucleotide diversity estimated by segregating sites (*S*) (± standard deviation); π, mean number of pairwise differences. Aa, *Acer albopurpurascens*; Ab, *A. buergerianum* var. *formosanum*; F1, hybrids.

	N	*Hap*	*Hd*	$\theta(S) \times 10^{-3}$	$\pi \times 10^{-3}$
cpDNA					
Aa	6	3	0.733±0.155	2.190±1.342	2.200
Ab	5	3	0.800±0.164	3.840±2.243	4.200
F1	6	3	0.800±0.122	3.504±1.972	4.267
Total	17	6	0.860±0.041	7.480±2.960	9.750
nrITS					
Aa	26	23	0.988±0.016	16.772±5.648	7.163
Ab	23	16	0.889±0.063	7.857±2.898	3.775
F1	31	29	0.996±0.010	26.533±8.404	13.338
Total	80	53	0.964±0.015	43.080±11.280	10.50

Table 3. Population pairwise F_{ST} with Kimura's two-parameter model (below), and average number of nucleotide substitutions per site between species (Dxy) (Nei, 1987), with the Jukes and Cantor (Jukes and Cantor, 1969) correction, estimated by the cpDNA/ITS sequences. Aa, *Acer albopurpurascens*; Ab, *A. buergerianum* var. *formosanum*; F1, hybrids.

	F1	Ab	Aa
F1	-	0.00358/0.01085	0.01658/0.01260
Ab	0.19832/0.06714*	-	0.01650/0.00691
Aa	0.84724*/0.01363	0.85309*/0.08714*	-

* $p<0.001$ of 1000 permutations for Mantel test.

Recombination and rare allele phenomenon

The paternal species *A. albopurpurascens* was estimated to have experienced two minimum recombination events (at sites 167 to 211 and 211 to 714), and the maternal species *A. buergerianum* var. *formosanum* showed no recombination event. Rather than displaying the low Rm values of their parents, the F1 hybrids had experienced at least eight recombination events (at sites 128-150, 168-179, 179-182, 284-426, 449-517, 559-604, 604-619, and 619-646). The estimation by likelihood approach helps to predict the regions of recombination that are consistent with the results of recombination events estimated by the four-gamete test (Rm). The likelihood scores of the maternal parent's (*A. buergerianum* var. *formosanum*) ITS regions did not surpass the 95% threshold while the scores of the paternal species (*A. albopurpurascens*) and the F1 hybrids did. Although only one region surpassed the 95% threshold in *A. albopurpurascens*, multiple regions surpassing it in F1 hybrids were found (Figure 4). This indicates that *A. buergerianum* var. *formosanum* has a stronger ability than the paternal parent to restrict intraspecific recombination, which might have resulted from the higher concerted rITS evolutionary rate (Koch et al., 2003) of *A. buergerianum* var. *formosanum* despite the detection of two flexible regions at the ITS1 and ITS2. In addition, the fact that the F1 hybrids showed many more regions over the 95% threshold would be predicted by the theory of Koch and

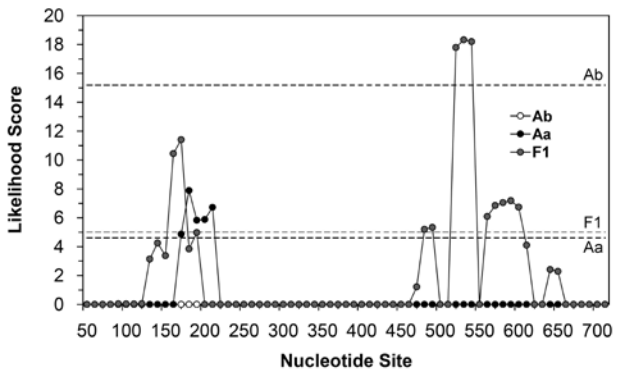

Figure 4. The 95% thresholds of likelihood scores (dash lines) showing that recombination did not occur in the maternal species *Acer buergerianum* var. *formosanum*, but it was seen in the proximal region (ITS-1) of the paternal species *A. albopurpurascens*. The F1 hybrids had more regions over the 95% recombination threshold than to parental species, indicating that recombination events were frequent after hybridization. Aa, *Acer albopurpurascens*; Ab, *A. buergerianum* var. *formosanum*; F1, hybrids.

colleagues (Koch et al., 2003) that the first hybridized generation has insufficient time to accomplish concerted evolution (Koch et al., 2003; Kovarik et al., 2005).

When ITS haplotypes of parental species and hybrids were carefully checked, a high frequency of new, rare haplotypes in the F1 hybrids (compared with the parental species) was found after hybridization (the "rare allele phenomenon"). Significant negative Tajima's *D* values (D = -2.3354, P = 0.0001 with 50,000 times coalescent simulations with a recombination rate of R = 0.01) suggested a relatively high frequency of rare alleles within the hybrid population. The "rare allele phenomenon" suggests that the increase in the frequency of rare alleles in hybrid populations is probably a result of recombination (Sage and Selander, 1979). Some hypotheses on the causes of the rare allele phenomenon have been discussed. These include intragenic recombination (Golding and Strobeck, 1983), increased rates of nucleotide substitution, positive selection of otherwise deleterious alleles, or a purifying effect of selection on unfit multi-locus hybrid genotypes that continually purges hybrid populations (cf. Schilthuizen et al., 2001). Although we cannot fit a particular mechanism, especially the selection forces, to our rather limited data, our results imply that acceleration of evolutionary rates (genetic variation) after hybridization may result from high recombination rates.

Grimm and colleagues (2007) also found several recombinant events in *Acer* rITS sequences, as revealed by nucleotide variation and fragment length polymorphism. Frequent natural hybridization or introgression has also been assumed to occur in several *Acer* species, explaining the emergence of intermediate and hard-to-distinguish morphologies, and such recombinant events might have occurred in ancestors of recently derived taxa, as indicated by the different phylogenetic positions of intra-individual rITS clones (Grimm et al., 2007). The phylogenetic malposition of intra-individual sequences also occurred in the present work (Figure 3).

Conclusions

Hybridization accompanied by recombination between two *Acer* species increased the genetic diversity of the hybrid population and might have eliminated the boundary of the two species. The present genetic survey provides good resolution to determine the pollen donor, and the expected high degree of recombination of nuclear ribosomal DNA is obtained in the hybrids. For conservation purposes, more attention should be paid to the prevention of hybridization when *ex situ* planting is ongoing. Increased anthropogenic hybridization is causing species extinction by both replacement and genetic mixing (Allendorf et al., 2001), and this would be diametrically opposed to the purpose of conservation. Spatial isolation should be carefully considered to minimize the possibility of spontaneous hybridization, especially in those endangered species in which the *ex situ* strategy for restoration is most frequently used. A recent simulation model and the case study of Maritime pine (*Pinus pinaster* Aiton) provides statistical evidence for the spatial effects (i.e. inbreeding) on an *ex situ* plantation (Fernández and González-Martínez, 2009). These studies point out the need for spatial isolation to prevent inter-specific crossing and demonstrate the importance of spatial management during *ex situ* conservation.

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樟葉槭與台灣三角楓種間雜交之分子評估

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域外保育是復育瀕危物種時經常使用的策略，植物園通常扮演了植物保育的重要功能。未經深思熟慮下進行的植物移植與種原保存，可能會導致種間雜交或是遺傳滲入問題出現，這種因人工移植造成的自然雜交現象現今發生在台北植物園的樟葉槭及台灣三角楓二近緣物種間，此二原生特有種在野外分布無重疊，在植物園種植後取得之第一子代的表型及基因型呈現中間型現象，皆證實二特有種間產生雜交現象。本研究藉由母系遺傳的葉綠體 DNA 作為分子標幟，我們確認了台灣三角楓為花粉的接受者 (母本)，樟葉槭則在雜交過程中扮演花粉提供者的角色 (父本)。藉由 ITS 序列的分析得知 F1 雜交子代除與親本共享部分祖先型的遺傳變異外，尚有許多基因型與親本在遺傳組成上有些微的不同，而擁有更高的遺傳變異度，此現象符合雜交後所致之「稀有對偶基因現象」。根據基因型的對照、最少重組事件的估計及概率比值檢測的結果證明雜交子代的遺傳變異多來自重組的發生，因此稀有對偶基因現象的發生可能與基因重組有關。本研究以分子評估的方式確認植物園內種間自然雜交的發生，並建議進行域外保育時須著重空間隔離的效應。

關鍵詞：槭樹；域外保育；雜交；稀有對偶基因現象；基因重組。