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Source Populations of *Quercus glauca* in the Last Glacial Age in Taiwan Revealed by Nuclear Microsatellite Markers

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Abstract

In this work, we attempted to study genetic differentiation between populations of *Quercus glauca* in Taiwan using nuclear microsatellite markers to infer the potential refugium in the last glaciation stage. Four microsatellite loci for 20 individuals each in 10 populations of Taiwan were analyzed. We found that *Q. glauca* has relatively high within-population diversity (H_E = 0.741) and low population differentiation (F_{ST} = 0.042) but shows isolation by distance. The most divergent populations, according to the average F_{ST} for individual populations in comparison with every other population, were found in populations Cy, Sa, and Hy in southern Taiwan and Pa in north-central Taiwan. Moreover, populations Cy, Sa, and Pa were recognized as being the source populations for gene recolonization after the last glaciation stage. In addition, the three sites of Wu, Ym, and Cy exhibited the highest gene diversities that coincided with populations with the highest chloroplast DNA variations. This may have resulted from an admixture of colonization routes. In conclusion, observations of the most divergent populations and source populations suggest that southern and probably north-central Taiwan may have potentially been refugia for *Q. glauca* in the last glaciation. This agrees with the possible refugium in southern Taiwan revealed by a previous study using chloroplast DNA markers.

The distribution of genetic variation within a species is shaped by processes such as ecological events, selective forces, patterns of genetic exchange, mating systems, and patterns of historical relationships (Schaal et al. 1998). The levels of genetic diversity, gene flow, genetic differentiation, and postglacial colonization in subtropical trees are of central interest to subtropical conservation biologists. In recent years, there has been an increase in the number of studies looking not only for present-day population genetic structure but also for the history of plant migration, isolation, and divergence among population lineages since the last glaciation in Taiwan (Cheng et al. 2005; Chiang et al. 2004; Huang et al. 2002, 2004; Hwang et al. 2003). Although limited knowledge has been obtained from these studies, some preliminary results point to a common evolutionary history for various plant species. For example, isozyme studies have revealed that the highest level of genetic diversity was found in Nantou County in central Taiwan, which was a major diversity center harboring the greatest genetic variation for many plant species (Lin 2001) in several studies including *Cunninghamia konishii* (Lin et al. 1998), *Trochodendron aralioides* (Wu et al. 2001), *Alnus formosana* (Sue et al. 2000), *Taiwania cryptomerioides* (Lin et al. 1993), and *Castanopsis carlesii* (Cheng YP, Hwang SY, and Lin TP, unpublished data).

Lying between the Asian continent and Philippine Sea basin, the island of Taiwan is commonly regarded geographically as a constituent of the island-arc system along the western edge of the Pacific Ocean. Geologically, this island connects the Ryukyu Island-arc to the north and the Philippine Island-arc to the south. The Central Mountain Range (CMR), the lofty backbone range runs basically following the axis of the island (see Figure 1, the area above altitude of 2000 m). Within this range are numerous peaks above 3000 m in elevation (Editorial Committee of the Flora of Taiwan, 2nd ed 1994). The pollen record from a lake core at an altitude of 745.5 m from Jih-Yueh Tan (23°49′E, 120°53′N) in central Taiwan during the last glacial maximum suggested that the temperature during the maximal last glacial stage was 8.0°C–11.0°C cooler than today. Lowland vegetation was dominated by cool-temperate forest composed of conifers mixed with deciduous hardwood species (Tsukada 1966, 1967). The evidence of paleontology indicated a once-downward migration of tree species during glacial maximum from higher altitude. Events of recolonization

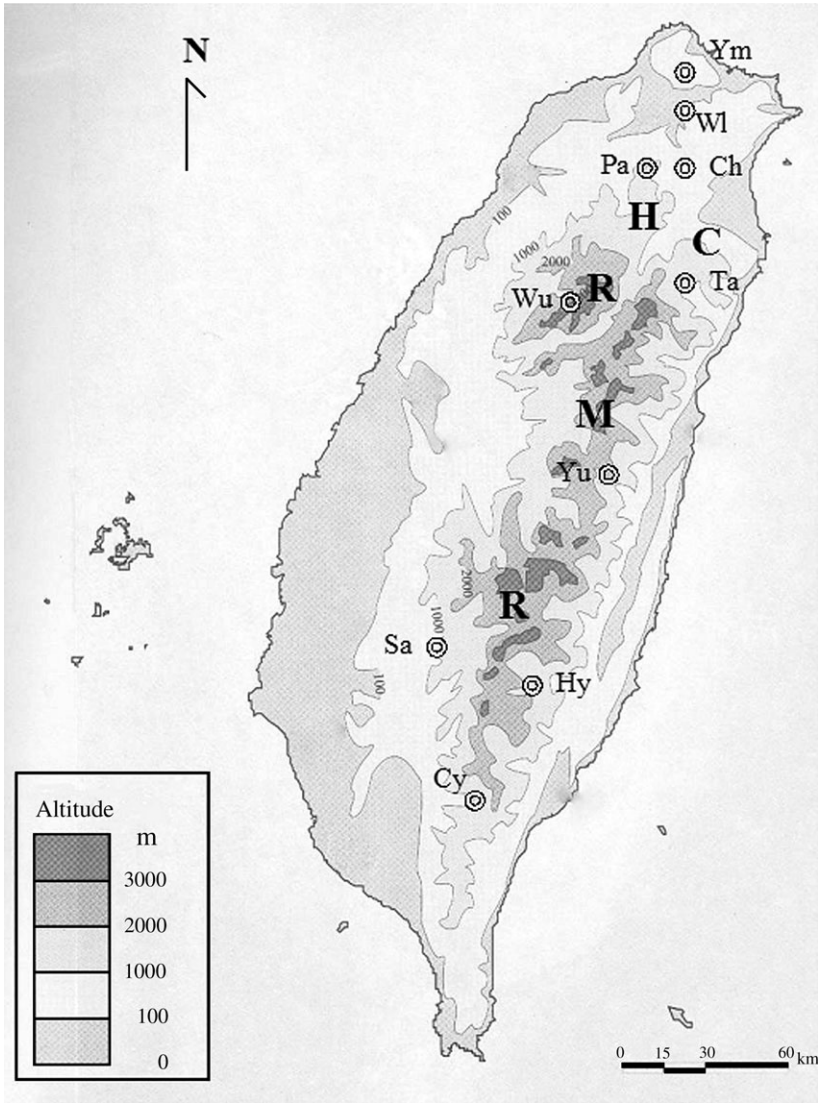

Figure 1. Map of Taiwan showing the locations of sampling sites. For population abbreviations see Table 1. Elevations of 100, 1000, 2000, and 3000 m are shown. CMR, Central Mountain Range; HR, Hsueshan Range.

of higher altitude after glacial maximum are possible. Therefore, *Quercus glauca* (or *Cyclobalanopsis glauca*, Fagaceae), a warm-temperate species, could be scarce or extinct in central Taiwan at that time. Migration of *Q. glauca* to southern Taiwan is expected.

Q. glauca is an evergreen broadleaf tree found not only in Taiwan but also in China, Korea, and Japan. *Q. glauca* is the most commonly occurring plant among the 50 native species of the family Fagaceae in Taiwan. *Q. glauca* is a medium-sized evergreen tree growing up to 20 m high and 0.6 m in diameter breast height. *Machilus-Castanopsis* is one of the two major types of evergreen broadleaf forests in Taiwan occurring at 400–1500 m. A large number of tree species appear in these forests, the most prominent among them belonging to the families Fagaceae and Lauraceae. *Q. glauca* is found from sea level up to 1700 m and is one of the dominant species of *Machilus-Castanopsis* forests (Hsieh et al. 1994). No pure stand has been found, but populations may form aggregates on steep hillsides as one of the pioneer species. With respect to the usage of *Q. glauca*, aboriginal people have used it in construction, in mushroom culture, and for making household items, such as wheels, various poles, etc. The only man-made plantations were established in mixed forests in selected sites in order to build fire-deterrent belts in areas with pine forests and a long dry season. *Q. glauca* is selected as study material because of its island-wide distribution and representative as a dominant subtropical tree species; in addition, the methodology and primers for the exploitation of *Quercus* spp. are available and have been tested in numerous European oak species. Moreover, the population genetics of *Q. glauca* using nuclear markers have never been studied in Taiwan. Using chloroplast DNA, Huang et al. (2002) examined the phylogeography of *Q. glauca*. Together with the highest haplotype and nucleotide diversities, they suggested

Table 1. Genetic variation in individual *Quercus glauca* populations or in each group of population analyzed using microsatellite loci. A_R, allele richness; H, genetic diversity; F_{IS}, Wright's inbreeding coefficient; and S, private allele. The Geo-group refers to population groupings according to geographic location

Population	Location	Sample size	No. of alleles per locus	S	A_R	H	F_{IS}
1. Wushe (Wu)	121.08°E, 24.03°N	20	7.25	1	6.42	0.765	0.215**
2. Paling (Pa)	121.23°E, 24.38°N	20	6.75	0	5.82	0.721	0.299**
3. Yuli (Yu)	121.15°E, 23.19°N	19	6	0	5.45	0.747	0.236**
4. Chilan (Ch)	121.33°E, 24.38°N	20	6.75	0	5.58	0.703	0.287**
5. Taroko (Ta)	121.37°E, 24.11°N	20	6.75	0	5.70	0.729	0.282**
6. Shanping (Sa)	120.40°E, 23.00°N	20	7	1	6.24	0.739	0.443**
7. Chinshuiying (Cy)	120.43°E, 22.25°N	20	6	0	5.54	0.758	0.492**
8. Hungyeh (Hy)	121.03°E, 22.53°N	20	6.5	2	5.77	0.733	0.422**
9. Wulai (Wl)	121.33°E, 24.53°N	20	5.75	0	5.08	0.682	0.392**
10. Yangmingshan (Ym)	121.33°E, 25.08°N	12	6	1	6.00	0.756	0.367**
Geo-group							
A (1, 2, 4)	North-central	60	8.75	—	7.78	0.731	0.122**
B (5, 3, 8)	Eastern	59	8.25	—	7.68	0.741	0.132**
C (6, 7)	South-western	40	8	—	7.65	0.753	0.286**
D (9, 10)	Northern	32	7.25	—	7.25	0.704	0.179**

* Significant at the 5% level, ** Significant at the 1% level.

the possibility that the southeastern part of Taiwan could have been a refugium during the last glaciation. A starlike genealogy was characterized in *Q. glauca*, as a general outcome of population expansion from a bottleneck.

The aims of this work were (1) to estimate the genetic diversity within and between populations of *Q. glauca* in Taiwan and to test, in particular, whether central Taiwan is the area with the highest genetic diversity and (2) to examine patterns of genetic divergence for each population in order to infer the possible refugia in the last glaciation.

Materials and Methods

Plant Material

Samples of *Q. glauca* consisting of mature leaves were collected from an average of 20 nonadjacent trees on 10 sites in Taiwan (Table 1, Figure 1). The population sites mostly overlapped with the populations used in a previous study (Huang et al. 2002), but the individuals were not the same. Leaves were enclosed in a sealed polyethylene bag and carried to the laboratory. After cleaning with water and drying on a bench top, leaves were conserved at −30°C prior to DNA extraction. In some cases, leaves were dried in silica gel in the sealed polyethylene bag.

DNA Extraction

Total DNA was extracted using the cetyltrimethyl ammonium bromide procedure (Murray and Thompson 1980) from 500 mg of fresh or dried leaves ground by hand. The quantity of DNA was measured by a spectrophotometer (Beckman Coulter, Fullerton, CA). DNA was diluted to 10 ng/μl.

Nuclear Microsatellite Primer Design and Analysis

Four polymorphic nuclear microsatellites (Table 2) were selected among the nine that were originally designed for *Quercus myrsiniflolia* (Isagi and Shuhadono 1997). Polymerase chain reaction (PCR) products of these primers were sequenced to find the simple sequence repeat (SSR) for each locus. We redesigned the primer pair for fluorescence labeling, and the primer sequences are presented in Table 2. PCR amplifications were performed in 10-μl reactions containing 1 × PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 100 μM of each dNTP, 0.02% Triton X-100, and 0.01% gelatin], 15 mM $MgCl_2$, 0.25 U *Taq* polymerase, 0.2 μM of each primer, and 5–10 ng of template DNA. The PCR conditions were 3 min at 94°C and 30 cycles of 30 s at 94°C, 30 s at a primer-specific annealing temperature (50°C), and 30 s at 72°C, with a final extension at 72°C for 7 min. The

Table 2. Core sequence for each microsatellite locus and primer sequence. The annealing temperatures for all were 50°C

Locus	Core sequence	Primer sequence
Mic57	(CCA)n	5′-GCTAAGATTTATCGCAGCCATAGG-3′ 5′-TGAGGAGGTTGGTGGAGAAAA-3′
Mic67	(CCA)n + (CCG)n + (CCT)n	5′-TGGCTTATCCAATGTTTGTGATT-3′ 5′-CGGCTTAGAGATTGGTGTCAAAG-3′
Mic51	(CA)n + (TA)n	5′-CAAAAACCTAAACCTACAAACGCTAAA-3′ 5′-AATAGCAAGAGAGAAGATGTTGCAAC-3′
Mic69	(TGG)n + (TTG)$(TGG)_2$CGG$(TGG)_2$	5′-CACAATCTGCCCACATCATC -3′ 5′- GGATGGACGAAGAGAAAAGAT-3′

PCR products were separated using a 3100 Genetic Analyzer with GeneScan software (Applied Biosystems, Foster City, CA). About 40% of total samples (72 of 181 individuals) were selected to examine the variation in the flanking and core sequences. Three to five clones were sequenced for each individual by a cloning technique (Promega pGEM-T Easy Vector System I, Madison, WI).

Data Analysis

For genetic diversity analysis, number of alleles per locus, allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E), genetic diversity (H) (Nei 1987), and inbreeding coefficient (F_{IS}) were included. These statistics were calculated for each locus, each population, and for each group of populations. Genetic differentiation, F_{ST}, was tested using FSTAT V. 2.9.3. (Goudet 1995). The levels of significance were adjusted for multiple tests according to the Bonferroni criteria. We computed Wright's inbreeding coefficient (F_{IS}) with FSTAT and tested the deviation of the genotypic frequencies from Hardy-Weinberg proportions with the program GENEPOP v. 3.4 (Raymond and Rousset 1995) to check whether inbreeding had occurred. A permutation procedure (1,000 times) was employed to test the significance of the F_{IS} deviation from a random mating system. Computation of allelic richness for specified sample sizes was based on the rarefaction method developed by Hurlbert (1971). Allelic richness corresponds to the number of different alleles found in the specified sample size. It is sampled at the locus in question and was measured as described by El Mousadik and Petit (1996). Briefly, the rarefaction method is used to estimate allelic richness at a locus for a fixed sample size.

The spatial genetic structure was investigated by testing for a pattern of isolation by distance. A Mantel test (Mantel 1967) with 1,000 random permutations was performed between the matrix of pairwise genetic differentiations between populations, using $F_{ST}/(1 - F_{ST})$ and the matrix of the natural logarithm of the geographic distance. These analyses were performed with PASSAGE (Rosenberg 2001) on the entire data set. The conventional genetic distance, F_{ST}, according to allelic frequency for population subdivision was estimated using the ARLEQUIN program (Schneider et al. 2000). In addition, the level of divergence for each population from the remaining populations was calculated as mean values of pairwise F_{ST} for each population against the rest of the populations. To identify the source population in the last glaciation, a partial Mantel test was carried out using the software zt (Bonnet and Van de Peer 2002). The concept of source population is derived from the study of fulmar (Burg et al. 2003; Fisher 1966). Fisher (1966) envisaged a "stepping stone" model of expansion of fulmar, relying on the assumption that a new colony was founded by individuals from the nearest existing colony. F statistics were then used to examine the levels of population differentiation and to indirectly obtain rough approximations for the levels of gene flow between sites.

Population relationships were analyzed by drawing dendrograms with Nei's standard genetic distance (Tamura and Nei 1983) and Dmμ (Goldstein et al. 1995). These dendrograms were drawn using either unweighted pair-group method using arithmetic averages (UPGMA) or the Neighbor-joining (NJ) method (Saitou and Nei 1987) and were constructed using POPULATIONS version 1.2.28 software (http://www.cnrsgif.fr/pge/bioinfo/populations/). Data were bootstrapped 2,000 times to estimate the node significance of the tree.

Results

Microsatellite Variations

Traditionally, length variations of microsatellites are the most conspicuous and usually the sole criterion employed to characterize allelic diversity as they display variable numbers of tandem repeats. Fine structural analyses of variations among microsatellite alleles have shown unexpected complexities in length variations. Genetic diversity in the microsatellite region of many species has been found not to be solely due to variations in the tandem repeat motif. Nucleotide substitutions and indels in the region flanking the repeat motif also contributed to the electrophoretic size variation detected on sequence gels (England et al. 2002; Garza et al. 1995; Grimaldi and Crouau-Roy 1997; Guillermo et al. 1997; Matsuoka et al. 2002; Orti et al. 1997). Thus, microsatellite alleles of identical size are not necessarily identical within species.

We analyzed the flanking region and found substitutions and indels. Four of 144 alleles (in 72 individuals) showed variations in length. One allele has a 2-bp deletion in the flanking region (181 bp) of a SSR from the 5′ end of locus *Mic69* in the 19 individuals of the Yuli population. In addition to this, we found that one sample has an additional TTG in the core sequence of *Mic69* of the Yuli population. In the case of flanking sequence variations, only the length of the core sequence was considered. Providing for 2.8% length variations in flanking regions of 72 individuals (40% of the total samples), less than 2% of the total alleles may have been wrongly identified after subtracting the four alleles mentioned above.

Microsatellite Diversity within Populations

If the population was grouped using geographical approximations, it could be divided into group A (Wu, Pa, and Ch), group B (Yu, Ta, and Hy), group C (Sa and Cy), and group D (Wl and Ym) (Table 1). North-central group (A), including populations Wu, Pa, and Ch, is located within the Hsueshan Range (HR). Populations Yu, Ta, and Hy (B) are located on the eastern side of the CMR, while populations Sa and Cy on the southwestern side of the CMR. Group C of southern Taiwan harbored the highest genetic diversity but not the greatest number of alleles or allelic richness. The fixation indices statistically deviated from zero at the group level due to Wahlunds' effect.

Averaged over the four loci, the number of alleles per locus was 5.75–7.25 at the population level and 7.25–8.75 at the group level (Table 1). Allelic richness (A_R) was calculated for gene copies; there was a maximum value of 6.42 in

Table 3. Allelic diversity of microsatellite loci scored in *Quercus glauca*. Size distribution (nt), range of sizes of PCR products; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, Wright's inbreeding coefficient; and F_{ST}, relative differentiation according to allele identity

Locus	Size distribution (nt)	No. of alleles	No. of heterozygotes[a]	H_O	H_E	F_{IS}	F_{ST}
Mic67	220–262	17	115	0.604	0.643	0.028*	0.033*
Mic57	145–166	9	109	0.572	0.767	0.221**	0.042*
Mic51	250–268	8	88	0.461	0.737	0.332**	0.063**
Mic69	221–251	11	154	0.811	0.820	−0.019	0.030*
Mean	—	—	116.5	0.612	0.741	0.140	0.042

[a] The number of plants showing heterozygotic alleles in a total number of 191 individuals.

* Significant at the 5% level, ** Significant at the 1% level.

the Wu population and a minimum value of 5.08 in the Wl population. Coincidently, the Wu population harbored the highest H value (0.765), while population Wl exhibited the lowest value (0.682). The fixation index, a measure of heterozygotic deficiency, statistically deviated from zero in all populations. Only a few private alleles, the alleles unique to one population, were found, and the south populations, Hy and Sa, have 50% of total private alleles.

The four SSR loci investigated in the present study were polymorphic in all populations. The number of alleles observed per locus in samples of 191 individuals ranged from 8 to 17, with an overall total of 45 alleles scored over the four loci (Table 3). Among the four loci, *Mic67* was the most polymorphic. The size ranges of PCR products corresponding to these alleles were roughly between 145 and 268 nucleotides (Table 3). Overall genetic diversities (H_E) were similar for each locus and ranged from 0.643 to 0.820. The average genetic diversity (H_E, 0.741) was higher than H_O (0.612). This was also reflected in the positive F_{IS} values indicating the existence of more homozygotes in the population than expected under Hardy-Weinberg equilibrium (Table 3). A low variation in F_{ST} and only 4.2% of the total variation existed among populations.

Genetic Differentiation and Isolation by Distance

Genetic differentiation between populations was low as revealed by SSR markers, and F_{ST} varied from 0.011 to 0.041 (data not shown). The unexpectedly high level of gene flow as revealed by the low F_{ST} value was found between Pa and Wu, Wl and Ym, Yu and Ch, and Yu and Ta. Interestingly, through the calculation of the average F_{ST} for individual populations in comparison with every other population, we found that Cy was genetically the most distinct population, followed by Sa and Hy (Figure 2), all situated in the south. The Pa population in the north-central area had a value comparable to Hy.

A Mantel test for SSRs also showed a significant correlation between population differentiations measured as $F_{ST}/(1 - F_{ST})$, and this significantly increased with the natural logarithm of the geographical distance between populations ($r = 0.5652$; $P = .007$ with 1,000 permutations). To test the correlation between geographical distances and genetic distances for a specific population against the remaining populations, the r value (correlation coefficient) was used to find the source population (Burg et al. 2003). We have obtained R^2 values from calculations of the simple Mantel test generated by zt, a software tool for performing simple and partial Mantel tests (Bonnet and Van de Peer 2002). According to the R^2 value for each population, all the regressions were significant. We found that four populations, i.e., Pa, Cy, Sa, and Wl, had values $\geq$0.8 (Table 4) and can be considered source populations for gene recolonization after the last glaciation stage. Populations Pa and Wl are located in the northern portion of the CMR, and Sa and Cy are in the south.

Genetic Relationships of Populations

The population NJ trees according to Nei's D (Tamura and Nei 1983) and Goldstein's Dmμ (1995) are shown in Figure 3a and b, respectively. The first major cluster included populations Hy, Sa, and Cy. The second cluster included populations Ch and Yu. The third included Wl, Ym, Wu, Pa, and Ta.

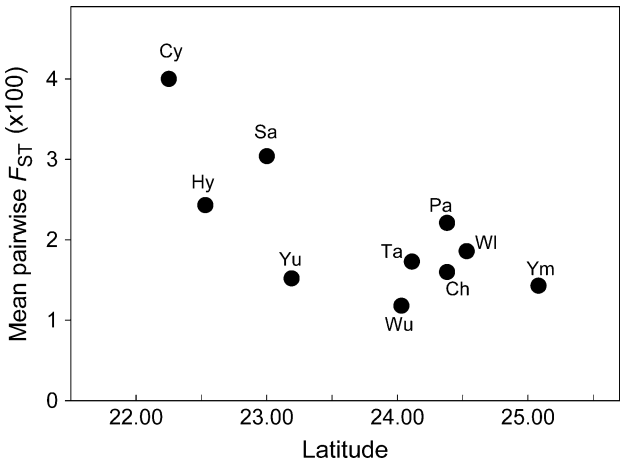

Figure 2. Plot of the average pairwise F_{ST} values for each population in comparison with the remaining populations against latitude in *Quercus glauca*. Cy (22.25°N, population no. 7) was genetically the most distinct population, followed by Sa (23.00°N, no. 6) and Hy (22.53°N, no. 8), all located in southern Taiwan. For population abbreviations see Table 1.

Table 4. Isolation by distance correlations for 10 populations, calculated separately based on the genetic distance (F_{ST}) and geographical distances between a given population and all other populations. The R^2 values were derived from calculations by simple Mantel test generated by zt, a software tool for simple and partial Mantel tests (Bonnet and Van de Peer 2002)

Population	R^2 value	*P* value
Wu	0.7263	<.001
Pa	0.8603	<.001
Yu	0.3879	.002
Ch	0.7662	<.001
Ta	0.7104	<.001
Sa	0.8374	<.001
Cy	0.7965	.002
Hy	0.6092	<.001
Wl	0.9444	<.001
Ym	0.7712	<.001

These two dendrograms, however, are not the same in terms of population grouping, and no high bootstrap values exist.

Some alleles present in both eastern and western populations of the CMR can be observed in Table 5. For example, in locus *Mic57*, allele 223 is present in populations Yu, Wl, and Ym; allele 226 is present in populations Sa and Hy; and allele 232 is present in populations Pa, Ta, and Cy. Similar conditions could be observed for the three other loci (data not shown). This indicated that the CMR is not a barrier to gene flow among populations. However, this pattern also could be due to convergence because of the high mutation rates of the microsatellite loci.

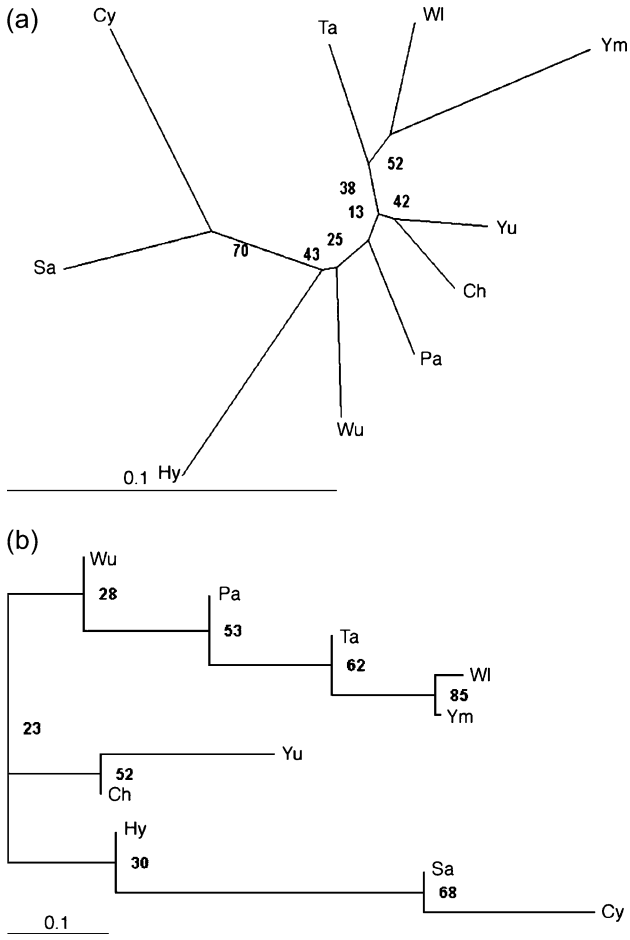

Figure 3. Unrooted NJ tree of the 10 *Quercus glauca* populations drawn using (**a**) Nei's *D* (Tamura and Nei 1983) and (**b**) Goldstein's $D_{m\mu}$ (Goldstein et al. 1995) by NJ methods. The numbers in the figures are percentage values over 2,000 bootstrap replicates. Only bootstrap values over 50% are presented.

Discussion

Null Allele Might Cause the Deviation of Fixation Index

F_{IS} for each population significantly deviated from zero (Table 1). Possible causes for deviation from Hardy-Weinberg equilibrium may be the locus being under selection, presence of null alleles in the level of inbreeding (mating among relatives), and the presence of population substructure leading to Wahlunds' effect. Although the vast majority of microsatellites are believed to be neutral, linkage of those markers to selected loci is present. In addition, strong selective pressure may cause the fixation of alleles in different populations. However, this is unclear in the loci studied in this paper. Inbreeding may be common in the population. If inbreeding is frequent in *Q. glauca*, differentiation among population will be strengthened. On the contrary, very low differentiation was observed between populations (Table 3). Inbreeding should have the same effect on every locus tested. This is also not supported by our data.

Null alleles may be present which are leading to a false observation of excess homozygotes. Locus *Mic51* and followed by *Mic57* but not the other two loci have a strong F_{IS}, suggesting that putative null alleles might occur at these two loci. It could be short allele dominance as a source of heterozygote deficiency as has been reported previously (Wattier et al. 1998).

Genetic Relationships of Populations

These two dendrograms of NJ trees based on Nei's *D* and Goldstein's Dmμ (Figure 3) do not agree completely with the geographic groupings in Table 1. Only groups of populations Sa and Cy in the south and Wl and Ym in the north were closely related geographically. The UPGMA method also generated different groupings of populations except populations Sa and Cy that were always grouped together (data not shown). This indicated that the south group was slightly isolated, and the rest of the populations have gene interchange from different directions. Nuclear gene interchanges have occurred between populations despite the presence of the CMR as low genetic differentiation (F_{ST}) was observed for *Q. glauca* (Figure 2). The pollen-seed flow ratio was estimated for the species of *Quercus* to be from 190 to 500 (Squirrell et al. 2001). In consequence, the dendrograms were not highly supported, and spatial genetic pattern was less clear than that revealed by chloroplastic DNA markers (Huang et al. 2002).

Table 5. Representatives of allele-size distributions for locus *Mic57* in each population of *Quercus glauca*

Locus *Mic57* allele size	Population									
	Wu	Pa	Yu	Ch	Ta	Sp	Cy	Hy	Wl	Ym
220	0	0	0	0	0	0.025	0	0	0	0
223	0	0	0.053	0	0	0	0	0	0.05	0.083
226	0	0	0	0	0	0.075	0	0.05	0	0
229	0.15	0.05	0.026	0.125	0.075	0.05	0.3	0.15	0.025	0.083
231	0.025	0.05	0	0	0	0	0	0.1	0	0
232	0	0.05	0	0	0.025	0	0.025	0	0	0
235	0	0.025	0.026	0	0.025	0.025	0.05	0	0.025	0.042
238	0.325	0.25	0.421	0.55	0.55	0.5	0.35	0.325	0.525	0.375
241	0.2	0.175	0.105	0.05	0.025	0.075	0.025	0.075	0.075	0
244	0.075	0.275	0.289	0.2	0.2	0.125	0.1	0.175	0.15	0.292
247	0.1	0.05	0.053	0.025	0.025	0.1	0.1	0.025	0.1	0
250	0.025	0.025	0.026	0.025	0.075	0	0.05	0.05	0	0.042
253	0.1	0	0	0	0	0.025	0	0	0	0
256	0	0.05	0	0	0	0	0	0	0	0.042
259	0	0	0	0.025	0	0	0	0	0.05	0
261	0	0	0	0	0	0	0	0.05	0	0
262	0	0	0	0	0	0	0	0	0	0.042

The NJ method does not assume a constant rate of evolution (Nei 1987). Longer branches in the NJ tree suggested by populations Cy, Sa, Hy, and Ym might indicate a faster evolutionary rate, higher differentiation, and possibly population fragmentation.

Potential Refugia of *C. glauca* Predicted from Microsatellite Analysis

The degree of the average F_{ST} value of each population in comparison with the remaining populations can be used to examine the consequence of historical and contemporary geographical population subdivisions on evolutionary processes (Johnson et al. 2000) and is important for reconstructing the phylogeographical history that has evolved during pre- and postcolonization events (Grant and Grant 1997). In the European oak [*Q. petraea* (Matt.) Liebl.], the highest differentiation values were found among the most ancient populations, close to refugia (Zanetto and Kremer 1995). It was found that in the common ivy (*Hedera* sp.) in Europe, differentiation of each population from the remaining ones revealed a latitudinal pattern, with populations from the south being significantly more differentiated from the pooled remaining populations than the central or northern populations (Grivet and Petit 2002). Thus, population divergence or genetic differentiation can be a useful criterion for locating a region of glacial refugium. Petit et al. (2003) tested the hypothesis that glacial refuge areas harbor a large fraction of intraspecific diversity. They concluded that plant populations in refuge areas have high genetic divergence and uniqueness rather than a high number of haplotypes.

Considering the existence of unrelated haplotypes and the highest nucleotide diversities, Huang et al. (2002) proposed that the southeastern part of Taiwan (in the region around the Taimali, Hy, Cy, and Laiye populations) was a potential refugium for *Q. glauca* in the last glaciation. This was further supported by this study as we found that the populations in southern Taiwan (populations Sa, Cy, and Hy) were the most genetically divergent among the populations tested (Figure 2). The south probably provided a warmer environment for *Q. glauca* to survive in the last glaciation stage. Unfortunately, no climatic/historic or pollen record data are available to support this observation. In southern Taiwan, group C (populations Sa and Cy) was found to have relatively high genetic diversity among the groups of populations (Table 1). A second refugium might have been located in the north-central part (Paling population) to the west of the CMR because a peak F_{ST} value was observed in this area (Figure 2). This was not mentioned in the chloroplast DNA study (Huang et al. 2002).

Moreover, according to R^2 of the Mantel analysis for each population, four putative source populations for gene flow, i.e., Cy, Sa, Pa, and Wl, were identified which coincide with the refuge populations mentioned above except population Wl. Wl, in fact, has the lowest allele per locus, A_R, and H. The two dendrograms do not support this conclusion either. Wl also had a low genetic divergence when chloroplastic DNA data (Huang et al. 2002) was calculated as the average F_{ST} of each population in comparison with the remaining (data not shown). The high R^2 of Wl at the present time is unclear. The dendrograms (Figure 3b) imply that colonization from the source population Pa in north-central Taiwan to the west of the CMR might have proceeded to population Wu and northward, even to Ta population, the east of the CMR, while source populations from the south might have proceeded to populations Yu and Ch along the east side of the CMR.

Among the 10 populations in this study, Wu, Cy, and Ym had the highest genetic diversity, and they were found to have high allelic richness. These three same populations coincided with the ones with the highest genetic diversity in terms of cpDNA sequences (Huang et al. 2002). In Wu and probably Ym, high gene diversity might have resulted from

admixtures of colonization after the retreat of the last glaciation because Wu and Ym had the lowest average genetic differentiation in comparison with the remaining populations (Figure 2). Wu and Ym also have a low average pairwise F_{ST} according to cpDNA markers. Thus, microsatillite data are consistent with the conclusions from cpDNA markers. In fact, Wu is located in Nantou County, central Taiwan, which is considered to be the major diversity center for several forest tree species (Lin 2001).

In conclusion, in this paper we investigated nuclear microsatellite variations of *Q. glauca* in Taiwan. We found that the southern part of Taiwan showed the highest population genetic divergence compared to all the others, which suggests the existence of a potential major refugium. This is in agreement with a previous report according to chloroplastic markers (Huang et al. 2002). Even though a much higher gene flow was revealed by nuclear markers among populations, this study implies that a correlation still exists between maternal lineage (cpDNA) and nuclear marker variations of *Q. glauca* in Taiwan.

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