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PARTIAL CONCORDANCE BETWEEN NUCLEAR AND ORGANELLE DNA IN REVEALING THE GENETIC DIVERGENCE AMONG *QUERCUS GLAUCA* (FAGACEAE) POPULATIONS IN TAIWAN

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Quercus glauca (Thunb. *ex* Murray) Oerst (Fagaceae) has a wide distributional range in Taiwan. In this study, the evolutionary history and the most genetically divergent sites of *Q. glauca* were studied using a nuclear gene marker, glyceraldehyde-3-phosphate dehydrogenase. Also, the consistency of the results obtained from nuclear gene and cytoplasmic loci was investigated. Using a genealogical approach (TCS software), we determined haplotypes and their relationships to one another. We used the level of divergence for each population from the remaining populations (calculated as mean values of pairwise population differentiation, F_{ST}, for each population) to locate the most genetically divergent areas in Taiwan. According to the average F_{ST} of each population in comparison with the remaining ones, a peak was found in the northern part of central Taiwan, and another was found in the southeastern region. The peak profiles of the mean F_{ST} values for all three DNA data sets (nDNA, cpDNA, and mtDNA) showed similar trends on both sides of the Central Mountain Ridge, except for the mtDNA sequence on the western side. This study suggests that two potential refugia existed in Taiwan during the last glaciation: one in the northern part of central Taiwan and another in southern Taiwan.

Keywords: glyceraldehyde-3-phosphate dehydrogenase, phylogeography, refugium, genetic divergence, Taiwan, *Quercus glauca*.

Introduction

Using molecular markers in combination with paleoecological studies for the analysis of the late Quaternary history of angiosperms in order to deduce historical information from their present-day geographical distributions has led to the recognition of glacial refugia of many species of Europe, North America, and Asia (Comes and Kadereit 1998). DNA sequences can even provide evidence of refugium sites that have not been detected by geological or fossil data (Rowe et al. 2004). The power of nucleotide sequences comes from the fact that they can be organized into hierarchically ordered networks of descent and can provide historical information that nonordered markers are unable to provide (Schaal et al. 1998). The availability of DNA sequence data and the development of coalescent-based analysis of allele genealogies can therefore form the basis of the study of intraspecific processes within a phylogenetic framework (Avise 2000) and can be used to examine the geographic distribution of genetic variations, postglacial recolonizations, and the ways in which recent evolutionary history has shaped patterns of intraspecific variations of a wide range of species (Newton et al. 1999).

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Gene flow between populations may be achieved through pollen or seeds. Pollen-mediated gene flow is not necessarily faster than gene flow by seeds; however, the rate of the flow through pollen in tree species like oaks with large heavy seeds is faster than that through seeds because pollen grains are smaller and can be carried more easily over longer distances by diverse agents including wind and insects (Ennos 1994). In addition, the chloroplast genome is haploid, whereas nuclear genomes are diploid or polyploid; thus, the effective population size of cytoplasmic genomes is half that of the diploid nuclear genome (McCauley 1995; Moore 1995). The deeper coalescence times expected for nuclear haplotypes will obscure spatial genetic patterns revealed by the plastid genomes. To test this idea, we compared the spatial genetic pattern of the plastid genome, including mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA), with nuclear DNA (nDNA) of a subtropical tree species, *Quercus glauca* (Fagaceae). In addition, we examined whether the patterns from different gene markers reflect different genetic histories, which may be integrated to postulate the possible historical events that have occurred in *Q. glauca* in Taiwan.

Quercus glauca is a member of the subgenus *Cyclobalanopsis*. *Cyclobalanopsis* is monophyletic within the genus *Quercus* according to molecular data (Manos et al. 2001). The subgenus *Cyclobalanopsis* contains ca. 122 species in tropical and subtropical eastern Asia (Luo and Zhou 2001). In consequence, *Cyclobalanopsis* is considered to be of tropical and subtropical origin (Luo and Zhou 2001). Among

Table 1

Populations and Haplotypes of *Quercus glauca* in Taiwan and Japan

Population	Location	Elevation (m)	Sample size (no. alleles)	Haplotypes (no. alleles)
Taiwan:				
1. Yangmingshan	121.51°E, 25.18°N	400	10	A (4), B (1), C (2), D (1), E (2)
2. Wulai	121.54°E, 24.86°N	450	11	A (3), B (1), C (3), D (2), F (1), B3 (1)
3. Baling	121.37°E, 24.68°N	600	11	A (4), C (3), F (1), H (2), I (1)
4. Chilan	121.60°E, 24.58°N	400	9	A (1), B (2), C (5), D (1)
5. Chingchuan	121.09°E, 24.58°N	450	9	A (3), B (1), C (2), F (1), L (2)
6. Kukuan	121.00°E, 24.21°N	700	7	B (3), C (1), D (2), C1 (1)
7. Huisun	121.03°E, 24.09°N	650	8	A (2), B (3), M (2), I1 (1)
8. Wushe	121.13°E, 24.02°N	850	12	A (3) B (2), D (1), K (2), L (1), B5 (1), B2 (1), B6 (1)
9. Liukuei	120.64°E, 23.00°N	550	10	A (4), C (1), D (2), E (1), J (1), B1 (1)
10. Chinshuiying	120.73°E, 22.42°N	1000	10	A (5), B (1), C (2), F (2)
11. Suao	121.84°E, 24.60°N	450	10	A (2), B (4), C (1), D (1), E1 (1), O2 (1)
12. Taroko	121.62°E, 24.15°N	500	9	A (3), B (3), C (1), D (1), E (1)
13. Lungchien	121.43°E, 24.00°N	400	10	A (5), B (1), C (3), E (1)
14. Hungyeh	121.04°E, 22.90°N	400	9	A (3), B (2), C (1), D (1), F (1), B4 (1)
15. Juisui	121.42°E, 23.50°N	200	9	A (3), B (3), C (2), H (1)
16. Fuli	121.27°E, 23.08°N	500	9	A (4), B (2), C (1), N (2)
17. Taimali	121.01°E, 22.67°N	100	3	A (1), E (1), J (1)
Japan:				
18. Kyushu	130.54°E, 33.67°N		8	B (1), G (4), H1 (1), B7 (1), G1 (1)
19. Kagoshima	130.43°E, 31.55°N		4	I (1), O1 (1), B8 (1), B9 (1)

these 122 species, *Q. glauca* has the widest range of distribution and extends to the northern limit of this subgenus. The species *Q. glauca* is found from the Himalayas to Indochina, via China, and has spread to the coast of the west Pacific in places such as Taiwan, the Ryukyus, Japan, and Korea. In Taiwan, *Q. glauca* is distributed from sea level up to 1700 m in elevation throughout the entire island. It is a codominant tree in subtropical evergreen forests and grows to 20 m in height. It occurs as a pioneer species that prefers open sites such as areas of landslides and windy ridges.

Taiwan is thought to have been connected to the Asian continent during the glacial maximum in the late Pleistocene (Boggs et al. 1979). A more recent land configuration proposed for the late Pleistocene (0.2–0.02 Ma) indicates that a large land bridge extended from eastern China to Taiwan, to the Ryukyus, and probably to the main islands of Japan (Kimura 1996) and should have provided the opportunity for gene flow among haplotypes. The floristic composition of Taiwan, a continental island, has high levels of endemism and species diversity. Most of the flora was thought to have originated from the Asian mainland during cycles of temperature oscillations. Although the land in Taiwan has never been covered by ice sheets except on the highest peaks, the tremendous temperature and climatic changes should have influenced species distributions and evolution. Palynological data indicate that during the last glacial maximum, most subtropical species disappeared from the lowlands of central Taiwan. At the same time, temperate species expanded their ranges of distribution from high elevations, and lowland forests were dominated by conifers (Tsukada 1966). When the ice retreated, a reverse course of events occurred, with subtropical species recolonizing from south to north and lowland forests retreating to higher elevations. The current geographic distribution of living organisms is the result of both present and past ecological and historical factors.

We indicated in a previous article that the southeastern part of Taiwan could have been a potential refugium in the last glacial maximum (Huang et al. 2002), according to chloroplastic DNA sequences. In addition to higher haplotype and nucleotide diversities, synapomorphic haplotypes were found on the eastern side of Taiwan (Huang et al. 2002). Accompanied by published palynological records of the last glaciation (Tsukada 1966), these observations favor the concept that regards the southeastern part of Taiwan as a potential refugium during the last glaciation.

No climatic/historic or pollen record data are available for the southern part of Taiwan to support this observation. Is there any other parameter that can be used for predicting the potential refugium in the last glaciation? Fortunately, the degree of average population differentiation (F_{ST}) of each population in comparison with the remaining populations (i.e., genetic divergence) can be used to examine the consequences of historical and contemporary geographical population subdivision on evolutionary processes (Johnson et al. 2000) and is important for reconstructing phylogeographical histories that have evolved during pre- and postcolonization events (Grant and Grant 1997). It was found that in the common ivy (*Hedera* sp.) of 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 were the central or northern populations (Grivet and Petit 2002). Thus, population divergence or genetic differentiation can be a useful criterion for locating regions of glacial refugia. 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. The concept of genetically highly divergent

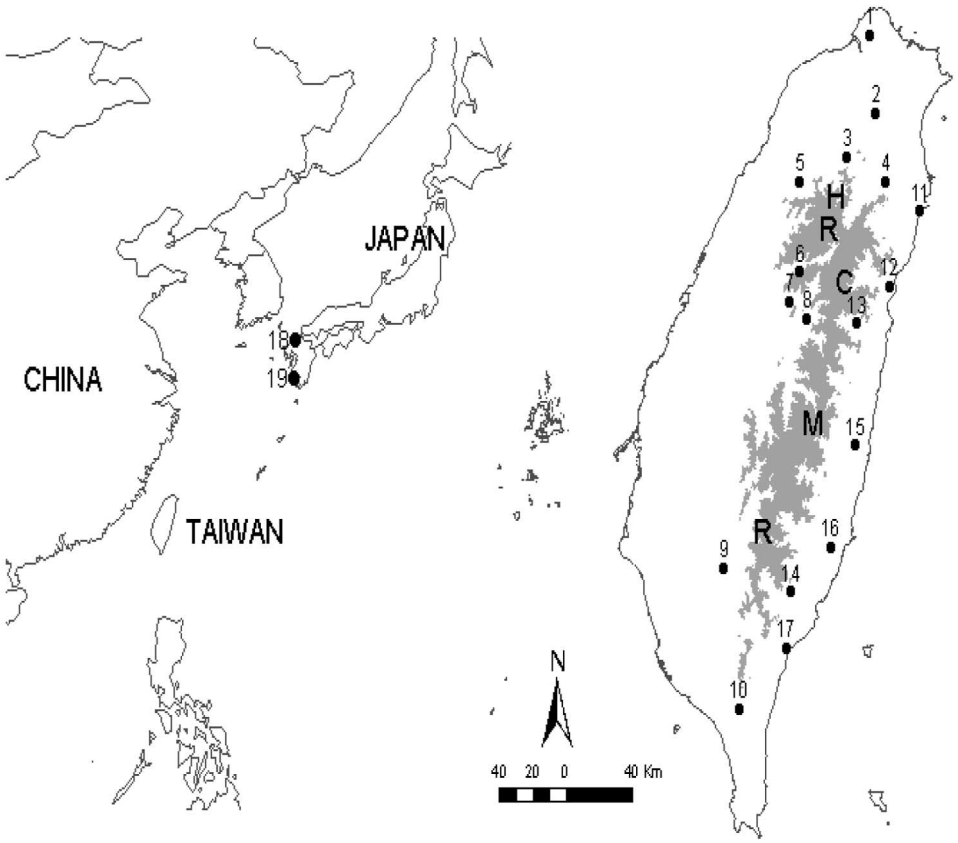

Fig. 1 Map of East Asia showing the location of Taiwan and the populations sampled for *Quercus glauca*. Only elevations above 2000 m of the mountain areas are shown. *CMR* = Central Mountain Ridge; *HR* = Hsueshan Range.

populations existing in regions of glacial refugia is also supported in the common ash (*Fraxinus excelsior*; Heuertz et al. 2004).

It is worth reexamining the potential refuge site reported using chloroplastic DNA (Huang et al. 2002). Also, this site needs to be tested with independent nuclear markers. In this article, we address whether the data obtained from nuclear genes are able to corroborate previous results based on cytoplasmic loci and to further characterize the population structure and evolutionary history of *Q. glauca* in Taiwan. The results of phylogeographical analysis in this study support those obtained from independent DNA sequences from the cytoplasmic genome in *Q. glauca*.

Material and Methods

Sampling of Plant Populations

Every stand used in this study was considered to be genetically original, as low economic interest strongly suggests their indigenous status. Most of the populations used in this study are the same as those used in a previous study of cytoplasmic data (Huang et al. 2002) but are not necessarily from the same individuals. On average, the number of alleles used in this study was 2.5 times greater than that used in the chloroplast DNA study.

In total, 19 populations, including 17 from Taiwan and two from Japan, were collected (table 1; fig. 1). In general, six individuals or fewer represented each population. Leaves from fresh or silica-gel-dried collections of each individual were deposited in a freezer at −70°C.

Polymerase Chain Reaction and Sequencing

DNA was extracted from leaves following modification of a standard protocol (Murray and Thompson 1980). The primers used by Olsen and Schaal (1999) for glyceraldehyde-3-phosphate dehydrogenase (*G3pdh*) were tested to screen the same gene in *Quercus glauca*. A polymerase chain reaction (PCR) was performed with newly designed forward (TGG AAT TGT TGA GGG TCT CAT; denoted GPD-CG-F) and reverse (TGC TGT CAC CAA TGA AGT CG; denoted GPD-CG-R) primers so that they best fit our sequence. The PCR solution was prepared as follows: 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin, 100 mM Tris-HCl (pH 8.3), 250 μM of each dNTP, 2 μM of each primer, 0.04–20 ng of template DNA, 1 μg RNase, 0.5 units of *Taq* polymerase (Amersham Pharmacia Biotech, Taipei, Taiwan), and double-distilled water to a final volume of 10 μL. Amplifications were performed with an initial denaturing of 2 min at 95°C, followed by 32 cycles of 1 min at 95°C, 90 s at 62°C, and 2 min at 72°C, ending with a 9-min extension at 72°C. We amplified a *G3pdh* gene sequence of 898 base pairs (bp) (including a partial sequence of 5′-UTR, exon A [79–176 bp], intron a, exon B [594–736 bp], intron b, and a partial sequence of exon C

Table 2

Haplotypes and Accession Numbers of *Quercus glauca* according to Mutations of the *G3pdh* Gene

Haplotype and accession no.	Mutation site of haplotype 000122222233333333334444444444555555555556666678888 467200355800013457780335666789002334447789026785024 5 946738526618972357812242678831230281466853152927484 8
B. AY780452	TTTCATAATCAATTGTATGGGTTGTTTAATAATTACCGAGCATTACTGAATT
A. AY780451	----------T----------
C. AY780453	--G-C----------C----------A----------G---
D. AY780454	C----------
E. AY780455	---------A-A----------
F. AY780456	----------C----------T--------T----------
G. AY780457	----------G----------
H. AY780458	-A-T--T----------C---A------G------A----C--T------
I. AY780459	----------G----------C----------A----------
J. AY780460	--------C-G--------A-C----------A----------
K. AY780461	---T-------G----G-C---C-C-A--A---C---AG-T--C--G---C-
L. AY780462	----G---------A---C---------G----------A----------
M. AY780463	----------G----------G-------
N. AY780464	---T-------G------C---C-C-A------C---AG-T--C--G-----
B1. AY780465	----------A----------
B2. AY780466	--G--C----------G----------A----------
B3. AY780467	--G--C----------C----------A----------
B4. AY780468	--G--C----------C-----A----------AG----------
B5. AY780469	---T----------C---C-C-A------C---AG-T-----G---C-
B6. AY780470	---T---G----------C---C-C-A------C---AG-T-----G---C-
B7. AY780471	-A-T--T----------C----------G--
B8. AY780472	-A-T--T----------C----------C---------C----------
B9. AY780473	----------A----------C----------T---
C1. AY780474	--G-C----------C-C----------A----------G---
E1. AY780475	C--------A--A----------
H1. AY780476	----------C---A------G------A----C--T------
G1. AY780477	----------G----------T-----G----------
I1. AY780478	C-------C-G----------C----------A----------
O1. AY780479	----------C----------C
O2. AY780480	----------C----------

[ca. 865 bp]) that covers the region highly homologous for the *G3pdh* gene in cassava (Olsen and Schaal 1999).

Nucleotide sequences were determined by direct sequencing of the purified PCR products on an ABI 3300 genetic analyzer with BigDye terminator cycle sequencing reagents (Applied Biosystems, Foster City, CA). This was applied to sequences of homozygotes or sequences containing one polymorphic site. For cloning of PCR products from heterozygotes, DNA fragments were ligated into a vector (pGEM-T Easy, Promega, Madison, WI) with Amp^r. The constructs were transformed to competent cells (ECOS 101, Yeastern Biotech, Taipei, Taiwan) with 45 s of heat shock at 42°C. Bacterial cultures containing X-Gal and IPTG were spread onto LB broth medium agar plates and incubated at 37°C overnight. Three clones, however, were sequenced for each individual when sequences contained two or more polymorphic sites and/or an unpaired indel (indel of different length). The two sequences of a heterozygote were separated by comparing the sequences of the PCR product and cloned sequence. Fortunately, no sequence containing more than one indel was found in the length of *G3pdh* used in this study. Because the *Taq* polymerase error was estimated to be as high as 0.1% (Okuyama et al. 2005) or even higher, the singleton was removed after the cloned sequences were compared with the sequences from direct sequencing in the forward and/or reverse directions.

Two lines of evidence suggest that the present *G3pdh* haplotypes were derived from a single gene. First, haplotype determination was made using direct sequencing of PCR products, and no more than two haplotypes were identified per individual (as would be expected for a diploid nuclear genome). Second, for one trial homozygous individual, the PCR product was cloned and then sequenced; the DNA sequences of multiple clones from this individual were identical.

Sequence Analysis

G3pdh sequences were aligned by eye. Haplotypes and their relationship were determined using the TCS program (Templeton et al. 1992) by considering gaps as missing data. Nucleotide diversity and haplotype diversity were carried out using the DnaSP program (Rozas and Rozas 1999).

Analysis of the Population Substructure

Measures of diversity and population differentiation, G_{ST}, were analyzed using the Hapstep program (Pons and Petit 1996), which employed Nei's (1977) approach to the case of haplotypes under Wright's model of population structure.

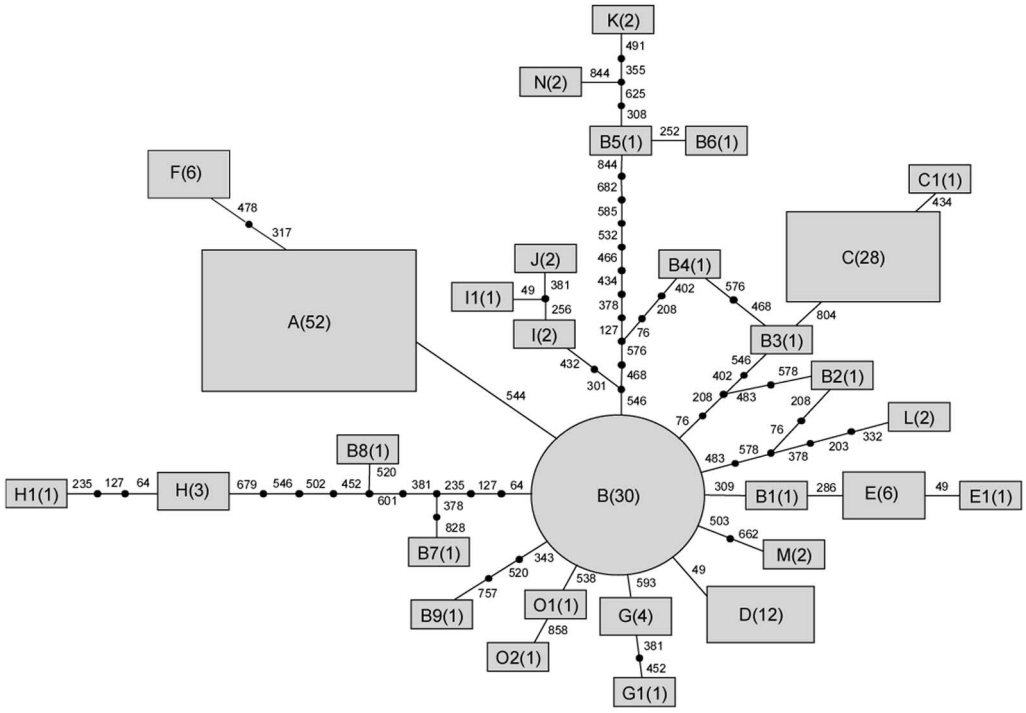

Fig. 2 Relationships of haplotypes of the nuclear gene marker, *G3pdh* (unrooted), reconstructed by the computer program TCS, version 1.04. Numbers of alleles are in parentheses. Haplotype B, indicated by a circle, is located in the center, from which the Taiwanese and Japanese haplotypes originated. The other haplotypes, indicated by squares of various sizes, reflect the number of alleles found. B4 and B2, which are connected to two internal branches, respectively, probably resulted from homoplasy or recombination. A dot represents a mutation for which a haplotype has not been found within the sample. Numbers along the lines represent the base positions of the mutations that separate the haplotypes.

The conventional genetic distance, F_{ST} (Wright 1931), according to DNA sequences for population subdivision, was estimated using the Arlequin program (Schneider et al. 2000), which provides a matrix of pairwise F_{ST} values between populations. The Mantel test, implanted in the PASSAGE program (Rosenberg 2001), was performed to test whether genetic distances correlated with geographical distances. 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 remaining populations. The contribution to total expected heterozygosity (CT) of each population was calculated using the Contrib software (Petit et al. 1998). This contribution is split into two components: one due to the diversity of the population (CS) and the other due to its differentiation from the remaining populations (CD). In this case, CS and CD for each population were computed relative to the mean population diversities and mean population differentiation, respectively. These led to either positive or negative contributions to populations (Petit et al. 1998).

Sequences of cpDNA and mtDNA of the populations from Taiwan were obtained from published data (Huang et al. 2002; Lin et al. 2003) and were used to estimate the population differentiation, F_{ST}, using the Arlequin program. Conventional F_{ST} values and the level of divergence for each population from the remaining populations were obtained as described above.

A test involving three matrices, including two matrices of mean F_{ST} values and a matrix of geographic latitude, was performed using ZT, a software tool for simple and partial Mantel tests (Bonnet and Van de Peer 2002). In these tests, we arranged the corresponding mean F_{ST} values in the matrices according to latitude.

Results

Sequence Analysis of the G3pdh *Gene*

In total, 96 individuals were sampled, and 86% of them were heterozygous. However, only 168 alleles of the total 192 *G3pdh* sequences in the sample could be identified unambiguously, and these could be assigned to one of only 30 haplotypes. Thirty haplotypes were detected from 52 substitution sites (GenBank accession numbers are given in table 2). Haplotypes, assigned from A to N, were shared by at least by two alleles, while the others were singletons. The genealogical relationship of these haplotypes is shown in figure 2, and the numbers of alleles containing such haplotypes are shown in table 1.

Distribution Patterns of Haplotypes

Mapping the geography onto the haplotype network produced a complicated pattern (fig. 3). Among the 22 haplotypes found in Taiwan, haplotypes A–C accounted for 68.6%

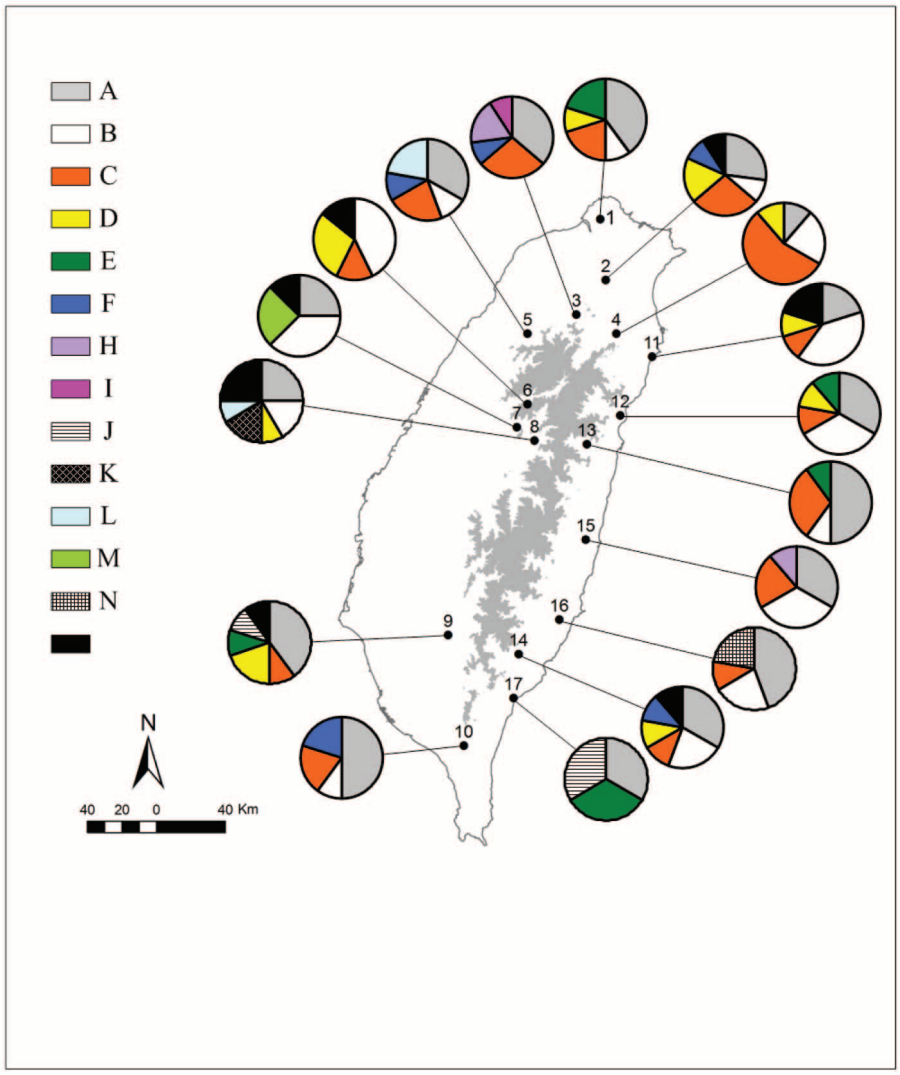

Fig. 3 Map showing the geographical distribution of *G3pdh* haplotypes and the proportion of each haplotype in each population of *Quercus glauca* in Taiwan. Twenty-two haplotypes were detected for the *G3pdh* fragments, and only haplotypes occurring in two or more alleles are presented. Ten haplotypes were singleton, and these are indicated in black.

(107) of 156 alleles. These three haplotypes were spread over Taiwan's population (table 1). Haplotype C occurred mainly in northern Taiwan, while haplotype B occupied a high proportion of populations of the northern part of central Taiwan. Haplotype B is a possible ancestral haplotype and was not found in the populations of Baling (population 3), Liukuei (9), or Taimali (17). Ten singleton haplotypes were restricted to only one allele, while haplotypes D–F and H–J were found in widely separated populations.

Many lineages were found on both sides of the Central Mountain Ridge (CMR) and had sporadic distributions; only a few haplotype lineages clearly showed a continuous geographic distribution (fig. 3). The lineage containing B1 and E occurred on the eastern side of the CMR, whereas the lineage containing B2, B3, and C1 was located in the center and north on the western side of the CMR. Haplotype D was found in many locations except southern and southeastern Taiwan.

The Japanese haplotypes were all traced to ancestral haplotype B, and some were closely related to those in Taiwan (fig. 2). For example, haplotype O1 of Japan is related to O2 of Suao (11) of northeastern Taiwan, and haplotype H1 of Japan was derived from type H, which occurs in Juisui (15), eastern Taiwan, while haplotype I was found in both Japan and northern Taiwan. This indicates that a relic connection of *Quercus glauca* existed between Japan and northern and northeastern Taiwan.

Analysis of the Population Genetic Structure

Genetic distances among populations did not correlate with geographical distances because the Mantel test was not significant (data not shown). For the *G3pdh* gene, the value of G_{ST} was 0.005, intrapopulational diversity (H_S) was 0.825 ± 0.0184, and total diversity (H_T) was 0.829 ± 0.0158. Thus, the spatial genetic pattern was very weak.

Table 3

Estimates of Haplotype Diversity (*h*) and Nucleotide Diversity (π) for Populations of *Quercus glauca* according to Mutations of the *G3pdh* Gene

Population (no.)	No. sequences	No. variable sites	No. haplotypes	Haplotype diversity (h)	Nucleotide diversity (π)
Yangmingshan (1)	10	9	5	0.78 ± 0.10	0.0034 ± 0.0008
Wulai (2)	11	9	6	0.87 ± 0.07	0.0041 ± 0.0006
Baling (3)	11	18	5	0.82 ± 0.08	0.0070 ± 0.0013
Chilan (4)	9	7	4	0.69 ± 0.15	0.0037 ± 0.0006
Chingchuan (5)	9	13	5	0.86 ± 0.10	0.0055 ± 0.0011
Kukuan (6)	7	7	4	0.81 ± 0.13	0.0036 ± 0.0011
Huisun (7)	8	8	4	0.82 ± 0.13	0.0029 ± 0.0011
Wushe (8)	12	24	8	0.92 ± 0.06	0.0097 ± 0.0016
Taroko (12)	9	9	5	0.83 ± 0.10	0.0026 ± 0.0009
Liukuei (9)	10	13	6	0.84 ± 0.10	0.0039 ± 0.0010
Chingshuiying (10)	10	8	4	0.73 ± 0.12	0.0033 ± 0.0001
Suao (11)	10	10	6	0.84 ± 0.10	0.0026 ± 0.0009
Lungchien (13)	10	8	4	0.71 ± 0.12	0.0037 ± 0.0008
Hungyeh (14)	9	11	6	0.89 ± 0.91	0.0038 ± 0.0011
Juisui (15)	9	14	4	0.81 ± 0.09	0.0049 ± 0.0016
Fuli (16)	9	17	4	0.78 ± 0.11	0.0070 ± 0.0022
Taimali (17)	3	8	3	1.00 ± 0.27	0.0060 ± 0.0020
Taiwan	157	43	23	0.82 ± 0.02	0.0049 ± 0.0005
Japan	12	21	9	0.91 ± 0.08	0.0055 ± 0.0011
Total	169	52	30	0.84 ± 0.02	0.0050 ± 0.0004

On the western side of the CMR, peak nucleotide diversity was found in the Baling (3; 0.0070) and Wushe (8; 0.0097) populations (table 3), while on the eastern side, the nucleotide diversity gradually increased toward the south and peaked in the Fuli (16; 0.0070) and Taimali (17; 0.0060) populations. If the total diversity consists of two components (i.e., genetic differentiation and genetic diversity), then the populations of Chingchuan (5), Kukuan (6), and Huisun (7) contributed most of the differentiation component to the total diversity (fig. 4*A*). The Taimali (17) and Wushe (8) populations contributed most to the diversity component of the total diversity (fig. 4*A*).

Pairwise F *Statistics according to Haplotype Frequency and Population Divergence*

In general, genetic differentiation between pairwise population comparisons indicated that the genetic distance between Taiwanese and Japanese populations was greater than that between any of the Taiwanese populations (data not shown). On the western side of the CMR, a major peak of average F_{ST} was found in the Kukuan (6; 0.06393) and Huisun (7; 0.05991) populations (fig. 5*A*). The peak decreased to low F_{ST} values for the populations of Wushe (8; 0.02935) and Wulai (2; 0.02611) to the south and north, respectively. On the eastern side of the CMR, the major peak of F_{ST} was found in southeastern Taiwan at Taimali (17; 0.0551), Chinshuiying (10; 0.04915), and Hungyeh (14; 0.04829), and another peak was found at Taroko (12; 0.0526) of east-central Taiwan, which coincides with the latitude of the major peak on the western side of the CMR.

Pairwise F *Statistics and Population Divergence Revealed by cpDNA and mtDNA Sequences*

The results showed that a major peak of average F_{ST} according to cpDNA data was found in the Huisun population (7; 0.80) on the western side of the CMR (fig. 5*B*). The peak decreased to low F_{ST} values for the populations at Wushe (8; 0.47) and Kukuan (6; 0.14) to the south and north, respectively. A second peak was found in the population at Laiye (18; 0.62), close to the population at Chinshuiying (10). On the eastern side of the CMR, a major peak of F_{ST} was found at Lungchien (13; 0.98), a population close to Taroko (12) and conciding with the latitude of the population at Huisun (7). A small minor peak was found at Taimali (17; 0.29) that coincides with the latitude of the peak of Laiye on the western side of the CMR.

According to mtDNA data, Liukuei (9; 0.800) and Mutan (0.815) in the south had the highest F_{ST} values (fig. 5*C*), but there was no detectable peak in the region covering the Kukuan and Huisun populations. On the eastern side of the CMR, a major peak of F_{ST} was found at Lungchien (13; 0.867), as well as a smaller peak to the south at Yuli (0.577) and Taimali (17; 0.468).

Correlations between the two matrices of mean F_{ST} derived from the two different DNA marker sequence data sets against latitude were tested using the partial Mantel test. Pairwise comparisons, i.e., cpDNA-mtDNA ($R = 0.895111$, $P = 0.02778$), cpDNA-*G3pdh* ($R = 0.483809$, $P = 0.036411$), and mtDNA-*G3pdh* ($R = 0.43721$, $P = 0.040278$), showed that average F_{ST} values for each population on the eastern side of the CMR were significantly correlated. However, correlations between mean F_{ST} values for each population of any two genome markers on the western side of the CMR showed no correlations. Subsequently, only the relationship between cpDNA and *G3pdh* was further tested after moving populations of cpDNA northward slightly so that population 7 of cpDNA met population 6 (Kukuan) of *G3pdh*. The Mantel test showed that the cpDNA-*G3pdh* pair was now significantly correlated with latitude ($R = 0.486740$, $P = 0.027797$). Populations at Tayuanshan (very close to population 4), Huisun (7), Lungchien (13),

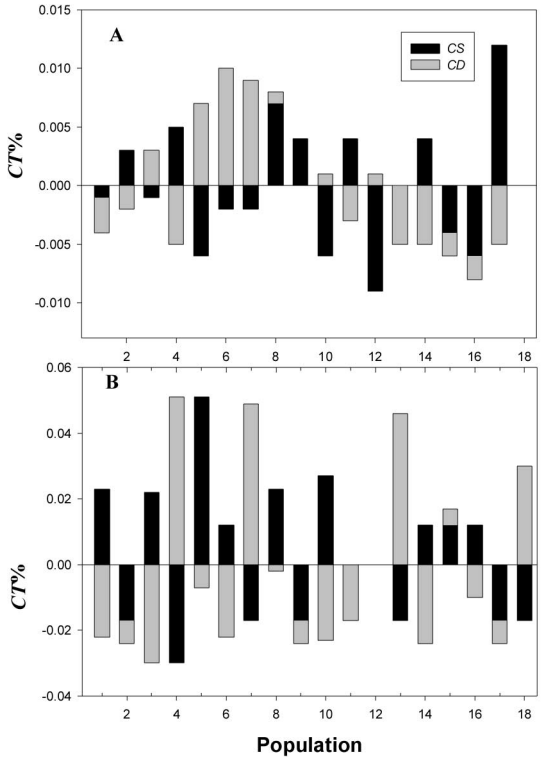

Fig. 4 Contribution to the total diversity (CT) of each population of *Quercus glauca* using *G3pdh* haplotypes (*A*) and chloroplastic sequences (*B*). Gray bars and black bars represent contributions of differentiation (CD) and diversity (CS), respectively. *A*, Populations 5–7, representing Chingchuan, Kukuan, and Huisun, respectively, contributed most to the differentiation component. See table 1 for population numbers. *B*, Populations 4, 7, 13, and 18, representing Chilan, Huisun, Lungchien, and Laiye, respectively, contributed most to the differentiation component. See table 1 for population numbers, except 18, which is designated as Laiye (120.72°E, 22.52°N).

and Laiye (very close to population 17) contributed most of the differentiation component to the total diversity of cpDNA (fig. 4*B*) because of their unique haplotypes.

Discussion

Genetic Spatial Structure of Populations

In a previous study, by sequencing three cpDNA intergenic spacer fragments, it was found that the level of differentiation among populations of *Quercus glauca* was relatively high ($G_{ST} = 0.612$; Huang et al. 2002). In this study, we found that some haplotypes were distributed in widely separated locations or on both sides of the CMR. This pattern is consistent with the extremely low G_{ST} value. The shared haplotypes between distant areas could be the result of either recent interpopulational gene exchange or shared ancestral polymorphisms (Hare 2001).

The ancestral haplotypes, e.g., haplotype B, were widely spread out, while the derived haplotypes (fig. 2) also occurred in most of the populations; thus, a clear migration route cannot be elucidated. So the temporal resolution offered by genealogies, such as chloroplast DNA markers, was unavailable through nuclear genes. The gene tree for the genealogical relationships of *G3pdh* among these nuclear haplotypes reveals nothing about the history of population divergence almost certainly because they predate the history of population divergence. However, the power of nuclear

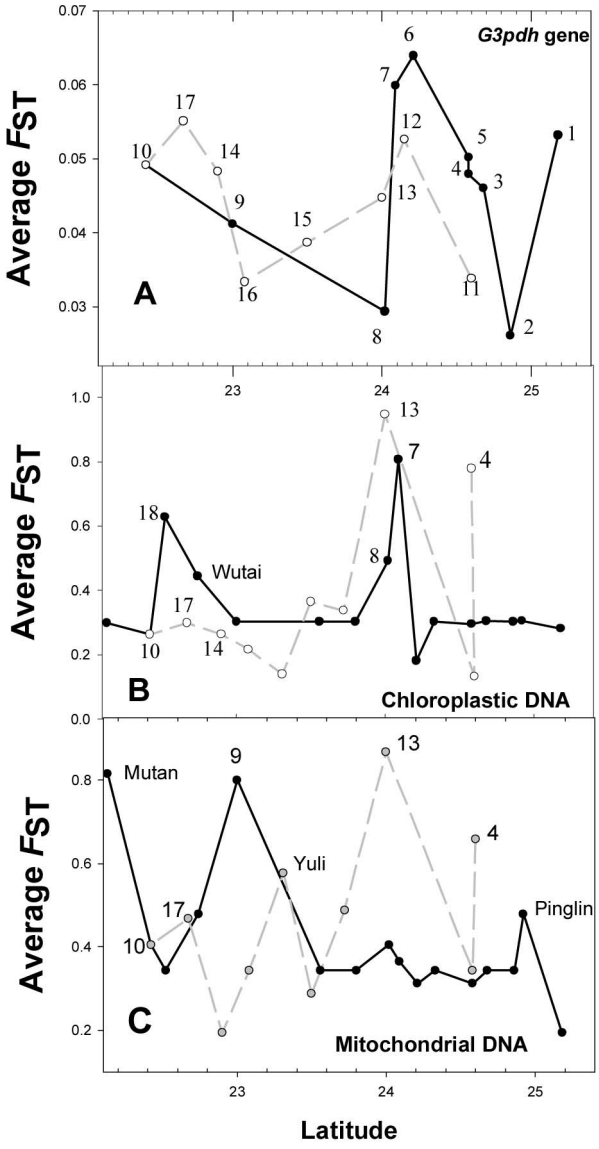

Fig. 5 Plot of the mean F_{ST} values of each population compared with every other population against population latitude in *Quercus glauca* using *G3pdh* sequence (*A*), chloroplastic DNA sequence (*B*; data taken from Huang et al. 2002), and mitochondrial DNA data (*C*; data taken from Lin et al. 2003). The solid line indicates populations on the western side of the Central Mountain Ridge (CMR); the dashed line indicates populations on the eastern side of the CMR. Arabic numbers refer to populations having high F_{ST} values. Population codes are labeled according to table 1, except 18, which is designated as Laiye. Pinglin (121.70°E, 24.92°N), Wutai (120.72°E, 22.74°N), Mutan (120.78°E, 22.13°N), and Yuli (121.25°E, 23.31°N) are indicated.

genes in providing resolution of historical events, i.e., predicting potential refugia, can still be appreciated.

Differentiation between populations (G_{ST}) can be used to calculate the pollen-seed flow ratio, which was as high as 291 (estimated by the equation $\{2 \times [(1/G_{STC}) - 1] - [(1/G_{STN}) - 1]\}/[1 - (1/G_{STC})]$, cited in Oddou-Muratorio et al. 2001, where C is the chloroplast and N is the nucleus), and was within the range of species of *Quercus* of 190–500 (Squirrell et al. 2001). The lack of clear spatial genetic patterns is far more likely to reflect incomplete lineage sorting, with nuclear genes having a greater expected coalescence time than differential gene flow by pollen.

Conformance of the Trend of Population Divergence of Three Genes (Loci)

The most significant discovery in this study is the conformance of different genes/loci having similar trends of genetic divergence for each population from the remaining populations. In terms of the mean values of pairwise F_{ST} for each population against the remaining populations, the most divergent populations were situated in two places. The first region was found in Kukuan (6) and Huisun (7) on the western side of the CMR and Taroko (12) at a comparable latitude on the eastern side of the CMR (fig. 5*A*). This region is located in the northern part of central Taiwan between 24.00°N and 24.25°N and is in proximity to the most genetically divergent areas determined for *Trochodendron aralioides* (Huang et al. 2004) and *Cunninghamia konishii* (Chung et al. 2004). Although the F_{ST} values for nDNA were fairly small (around 6%), a peak was evident. In fact, the region of the Huisun population has major peaks of F_{ST} for both nDNA and cpDNA. mtDNA data also showed that Lungchien (13) in the northern part of central Taiwan had high degrees of mean F_{ST}. An exception was found on the western side of the CMR, where a high F_{ST} value from mtDNA analysis was not detected in the northern part of central Taiwan. A second peak of F_{ST} in nDNA was found for the Chinshuiying (10), Hungyeh (14), and Taimali (17) sites, which are similar to sites with peak F_{ST} values in cpDNA of Chinshuiying (10), Hungyeh, Taimali, and Laiye (close to population 17) (fig. 5*B*). This region is located in southeastern Taiwan between 22.40°N and 22.90°N; mtDNA data also showed that the Taimali (17) and Mutan populations of southern Taiwan had high values of mean F_{ST}, reinforcing the impression of similar regions having significant differentiation from other regions. High mean F_{ST} of the population Liukuei (9) is an exception that could not be detected in cpDNA and *G3pdh*. The profile on the western side might imply that several smaller shelters existed in southern Taiwan during the last glaciation. The correlations between mean F_{ST} values for each population of cpDNA and *G3pdh* against latitude were tested using the partial Mantel test, and the results support the average F_{ST} profiles being correlated well but not being the result of chance alone.

The average F_{ST} values for each population of mtDNA are only partially in accord with those of cpDNA, and values of nDNA are unclear at present. We found that the gene genealogical tree of mtDNA was partially congruent with the cpDNA tree (Lin et al. 2003). The average F_{ST} values of mtDNA for each population against latitude differed from those of cpDNA in *Machilus kusanoi* Hay. (S. Y. Hwang, unpublished data), indicating that mtDNA has a different evolutional history from cpDNA.

The contribution of a differentiation component to total diversity (CT) of the *G3pdh* gene and chloroplastic gene of each population also supports the hypothesis that the northern part of central Taiwan contributed most of the differentiation component to the total diversity of *Q. glauca*. This was due to the specific rare alleles in each population, which resulted in high contributions to differentiation. The contribution of the differentiation component of the *G3pdh* gene of each population to CT was undetectable in the southeastern part. In the chloroplastic gene, however, the south (Laiye; close to population 17) contributed most to the differentiation component.

It is interesting to note that the Wushe (8) population has a very low average F_{ST} among all populations but the highest nucleotide diversity in both nDNA and cpDNA, indicating that the high diversity may have been derived from a mixing of different colonization routes. Wushe (8) also has a large number of derived haplotypes (fig. 2), indicating a mixed nature of its composition. Yangmingshan (1), at the northern tip of the island, also had the highest haplotype richness of cpDNA and a high average F_{ST} of nDNA and could also have been a cryptic shelter.

In this article, we predicted potential refugia using high average F_{ST} estimates. This idea is based on a study of numerous publications on European plant phylogeography and on a theoretical study of island biogeography. It may be that these cases of European plants are unique and not necessarily applicable to our study. This cannot be resolved until fossil pollen data are available in the future.

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