LOSS OF GENETIC DIVERSITY AND ERRONEOUS PHYLOGEOGRAPHICAL INFERENCES IN *LITHOCARPUS KONISHII* (FAGACEAE) OF TAIWAN CAUSED BY THE CHI-CHI EARTHQUAKE: IMPLICATIONS FOR CONSERVATION[1]

Kuo-Hsiang Hung,[2,5] *Tsai-Wen Hsu,*[3,5]
Barbara A. Schaal,[4] *and*
Tzen-Yuh Chiang[2,6]

ABSTRACT

The effect of natural catastrophic events, such as volcanic eruptions and earthquakes, on the genetic structure of species has been infrequently studied. The 1999 Chi-Chi Earthquake of Taiwan resulted in population extinction of *Lithocarpus konishii* (Fagaceae), an endangered species, and provides a case study for assessing impacts of natural catastrophes on genetic diversity. Genetic diversity of the *atp*B-*rbc*L intergenic spacer of cpDNA and the intron 2 of NADH dehydrogenase subunit 7 gene of mtDNA in *L. konishii* was determined before and after the earthquake. Loss of rare polymorphisms due to extinction of an entire population at the earthquake epicenter contributed to the reduction of genetic diversity within species in both cpDNA and mtDNA. Star-like phylogenies and significantly negative Tajima's D statistics suggest a history of recent expansion of the species, which, however, is not confirmed by a nested clade analysis. Phylogeographical inferences were made before and after the earthquake; differences in the phylogeographic analysis suggest that long-range dispersal inferences may be misidentified due to loss of haplotypes and clades in geographical regions of population extinction. Tests of genetic and ecological exchangeability indicate that *Lithocarpus* populations should be recognized as different conservation units that should be managed separately.

Key words: Chi-Chi Earthquake, cpDNA, genetic diversity, *Lithocarpus konishii*, minimum spanning network, mtDNA, NCA, phylogeography.

Population genetic studies over the last decade have revealed that many plant or animal species can be structured into phylogenetic units that often correspond to geographical regions associated with vicariance and glaciation (e.g., Avise et al., 1987; Avise, 1992; Riddle, 1995; Manos et al., 1999). Phylogeographical patterns of many taxa that evolved through glacial cycles or vicariance events have been well demonstrated based on genealogical information of organelle DNAs, e.g., *Pinus* (Latta & Mitton, 1997), *Abies* (Tsumura & Suyama, 1998), *Cycas* (Huang et al., 2001), *Fagus* (Demesure et al., 1996), *Quercus* (Petit et al., 1997), *Alnus* (King & Ferris, 1998), *Kandelia* (T. Y. Chiang et al., 2001), and *Beta* (Desplanque et al., 2000). In addition to the vicariance and glaciation cycles, fire is also one of the natural disturbances in boreal forests, with a major impact on forest structure and dynamics (Moore, 1996). Until recently, fires reoccurred in boreal forests once to twice per century, annually affecting about 1% of the landscape (Esseen et al., 1997); forest fires shaped the population structure and fates of species (cf. Parker et al., 2001; Johannesson et al., 2001). Like forest fires, earthquakes have the potential to change geographical structure and alter habitats, leading to fluctu-

ations in genetic diversity and population structure of species within a short time span. Nevertheless, to date, few studies have assessed the influences of such catastrophes on genetic diversity in natural populations.

On 21 September 1999, a devastating earthquake (7.5 on the Richter scale) with three large aftershocks (6.2–6.4) occurred at Chi-Chi in central Taiwan and caused extensive casualties and damage to an extent not experienced in the recent history of Taiwan (Kao & Chen, 2000). This earthquake is referred to as the 921 Earthquake or Chi-Chi Earthquake. The island of Taiwan is at the boundary of the Eurasian and Philippine tectonic plates and is a constituent of the island-arc system along the western edge of the Pacific Ocean. Geologically, Taiwan connects the Ryukyu Island-arc to the north and the Philippine Island-arc to the south (Vita-Finzi, 2000). Along the island-arcs in East Asia, earthquakes occur frequently due to unstable geotectonic structures. As is typical of large earthquakes in steep mountainous regions, well over 1000 landslides were recorded in the region affected by strong ground movement. Landslides on Mt. Juofenger at the earthquake epicenter denuded large regions of vegetation and caused local extinction of many species, including an endemic species of Taiwan, *Lithocarpus konishii* (Hayata) Hayata (Fagaceae).

Lithocarpus konishii, a dominant species of broadleaf forests, grows at low elevations in central and southern Taiwan (Liu & Liao, 1976). *Lithocarpus* trees are 4–9 m in height. Leaves are oblong to obovate with revolute margins and obtuse apices; fruits are rounded nuts in cupules with tomentose scales. Many lowland broadleaf forests with their constituent species have been lost due to human activities (T. Y. Chiang et al., 2004). Like many other lauraceous and fagaceous plants of the island, the number and size of *L. konishii* populations have dramatically decreased. Based on previous collection records (Liao, 1996) and herbarium specimens, many populations of *L. konishii* have disappeared from the wild, especially those of East Taiwan. This oak species is now restricted to sporadic populations on governmental and public lands. *Lithocarpus konishii* has low economic value as a wood source; no human cultivation has been recorded.

In general, plant populations in central and southeastern Taiwan have the highest levels of genetic heterozygosity on the island (Lin, 2001). Lin (2001) found that *Michelia formosana* Masam. & Suzuki populations of central Taiwan harbor more genetic polymorphisms than populations in other regions. Huang et al. (2002) identified glacial re-

fugia for *Fagus* in central Taiwan based on mtDNA, indicating possible hot spots in central Taiwan for the conservation of genetic diversity. The landscape destruction of the Chi-Chi Earthquake in the center of Taiwan could have substantial impact on both the survival of many plant populations and also on the genetic diversity maintained within those populations.

Molecular markers of organelle DNA with low rates of recombination are useful for resolving phylogeographical patterns, population structure, and genetic diversity and assessing the range expansion and migration of species (Avise, 1994; Ouborg et al., 1999; Ennos, 2001; Provan et al., 2001). In plants, chloroplast DNA in general tends to evolve slowly, but recently moderate to high levels of genetic variation have been detected between closely related species and between populations (Ohsako & Ohnishi, 2000). Because one or both organelle genomes are often maternally inherited in plants (Birky, 1995; Mogensen, 1996), with occasional paternal leakage, organelle genomes are particularly well suited for investigating processes associated with dispersal and range expansions (Cruzan & Templeton, 2000; Petit et al., 2001) and the contribution of seed movement to total gene flow (McCauley, 1994, 1995; Cain et al., 2000; Orive & Asmussen, 2000). As in most plants, organelle DNAs in oaks is maternally inherited, and it allows a direct study of seed-mediated dispersal and gene flow and is consequently particularly useful to infer recolonization routes (Rebound & Zeyl, 1993; Dumolin et al., 1995; Lumaret et al., 2002; T. Y. Chiang et al., 2004). Seed dispersal in *Lithocarpus konishii* is mediated by foraging of rodents, e.g., squirrels (Hung et al., in press). Ecological observations found that most migration of the seed carriers is restricted to within populations, given the substantial geographical barriers of mountains and human-dominated landscapes that exist between forest remnants (Hung et al., in press). Because gene flow (both seed and pollen) is restricted, we expect *L. konishii* to have an isolation-by-distance population structure.

In the current study, five relict populations of *Lithocarpus konishii* were sampled from central and southern Taiwan. During the course of botanical inventory, one population on Juofenger Mountain was sampled one week before the Chi-Chi Earthquake. We used the *atp*B-*rbc*L intergenic spacer of cpDNA and intron 2 of the NADH dehydrogenase subunit 7 gene of mtDNA as molecular markers to examine the impact of the Chi-Chi Earthquake on genetic diversity and population structure of *L. konishii.* The phylogeographical structure of genetic varia-

tion among populations of *L. konishii* was determined before and after the earthquake.

MATERIALS AND METHODS

POPULATION SAMPLES

Due to recent human over-exploitation, many previously recorded populations, such as those of eastern Taiwan (Liao, 1996), have gone extinct from the wild. Five extant populations (117 individuals) of *Lithocarpus konishii,* which are 5 to 260 km apart, were sampled (Table 1, Fig. 1). The KB population at the Mt. Juofenger was sampled before the Chi-Chi Earthquake. Young leaves were collected in the field and dried with silica gel.

DNA EXTRACTION, PCR, AND SEQUENCING

Leaf tissue was ground to powder in liquid nitrogen and stored at −70°C. Genomic DNA was extracted from leaf tissue following a CTAB procedure (Murray & Thompson, 1980). The *atp*B-*rbc*L noncoding spacer of cpDNA and intron 2 of the NADH dehydrogenase subunit 7 gene of mtDNA were amplified and sequenced. PCR amplification was carried out in 100 μL reaction 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 pmole of each primer, 10 μL of 10% NP-40, and 2 U of *Taq* polymerase (Promega, Madison, U.S.A.). The reaction was programmed on an MJ Thermal Cycler (PTC 100) as one cycle of denaturation at 95°C for 4 min., 30 cycles denaturation at 92°C for 45 sec., annealing at 52°C for 1 min. 15 sec., and extension at 72°C for 1 min. 30 sec., followed by another extension at 72°C for 10 min. A pair of universal primers for cpDNA *atp*B-*rbc*L spacer (T. Y. Chiang et al., 1998) or the intron 2 of NADH dehydrogenase subunit 7 gene (Demesure et al., 1995), dNTP, and *Taq* polymerase were added to the above ice-cold mix. Reaction was restarted at the first annealing at 52°C.

PCR products were purified by electrophoresis in 1.0% agarose gel using 1 X TAE buffer. The gel was stained with ethidium bromide and the desired DNA band was cut and eluted using agarose gel purification (QIAGEN). Purified DNA was ligated to a pGEM-T easy vector (Promega). Five clones of the plasmid DNA were selected randomly and purified using a plasmid mini kit (QIAGEN). Purified plasmid DNAs were sequenced in both directions by standard methods using the *Taq* dye deoxy terminator cycle sequencing kit (Perkin Elmer) on an Applied Biosystems Model 377A automated sequencer (Applied Biosystems). Primers for se-

Table 1. Populations of *Lithocarpus konishii* samples for DNA sequencing. The number of individuals sampled and sequenced is indicated. Nucleotide diversity (π), haplotype numbers, and haplotype diversity (h) are indicated.

| Locations	Coordinate	Symbol	Sample size	Nucleotide diversity (π) (%)		Haplotype numbers		Haplotype diversity (h)	
				cpDNA	mtDNA	cpDNA	mtDNA	cpDNA	mtDNA
Overall			117	2.469 ± 0.793	0.160 ± 0.099	24	27	0.697	0.666
Overall (excluding KB)			100	2.153 ± 0.752	0.144 ± 0.087	16	17	0.689	0.608
Sun Moon Lake	23°50'N, 120°55'E	KA	12	0.101 ± 0.076	0.127 ± 0.080	4	3	0.682	0.318
Mt. Juofenger	23°56'N, 120°50'E	KB	17	4.335 ± 1.660	0.259 ± 0.174	10	11	0.794	0.904
Carp Lake	24°21'N, 120°48'E	KC	44	1.935 ± 0.973	0.252 ± 0.157	7	9	0.462	0.759
Tahan Forest Road	22°24'N, 120°38'E	CD	12	6.877 ± 1.698	0.073 ± 0.038	3	3	0.621	0.727
Chingshuiying	22°25'N, 120°45'E	CE	32	0.141 ± 0.065	0.053 ± 0.042	5	5	0.708	0.601
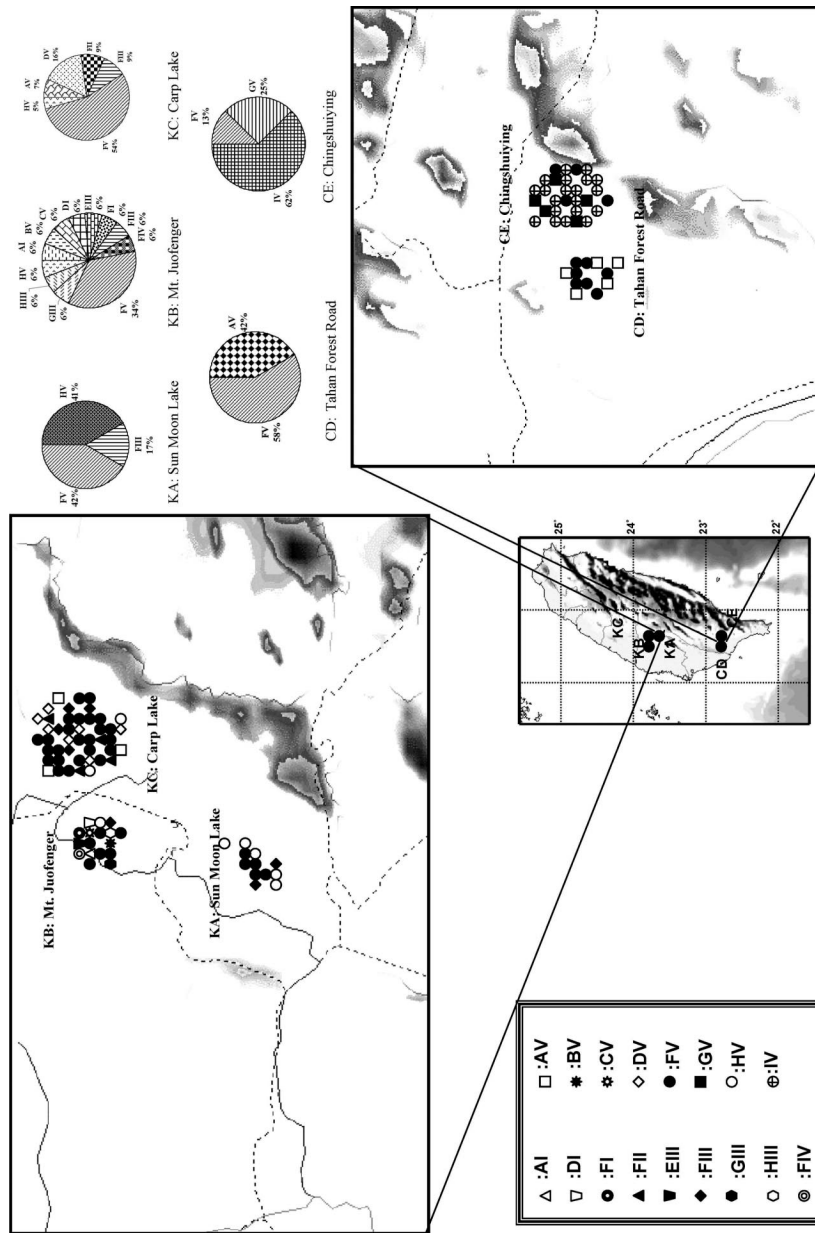
Figure 1. Map showing the spatial distribution and genetic composition (chlorotype-mitotype associations) of populations of *Lithocarpus konishii*. KA: Sun Moon Lake, KB: Mt. Juofenger, KC: Carp Lake, CD: Tahan Forest Road, CE: Chingshuiying.

quence determination were T7-promoter and SP6-promoter located on the p-GEM-T easy Vector termination site.

Nucleotide sequences were aligned with the program CLUSTAL V (Higgins et al., 1992) and later adjusted manually. Neighbor-joining (NJ) analysis was performed with the software MEGA (Kumar et al., 1993) by calculating Kimura's (1980) two-parameter distance and excluding the gaps. Confidence of the resulting tree was tested by bootstrapping (Felsenstein, 1985) with 1000 replicates using unweighted characters. The number of mutations between haplotypes in pairwise comparisons, calculated using MEGA (Kumar et al., 1993), was used to construct a minimum spanning network with the aid of the MINSPNET (Excoffier & Smouse, 1994). After linking haplotypes into a clade, closely related clades were linked further to form a higher-level group via hierarchical linking, and a nested network was generated (cf. T. Y. Chiang & Schaal, 1999). In this study, the use of the term "clade" follows Templeton et al. (1995).

Levels of inter- and intra-population genetic diversity were quantified by indices of haplotype diversity (h) (Nei & Tajima, 1983) and pairwise estimates of nucleotide divergence (π) (Jukes & Cantor, 1969) using DnaSP (Vers. 3.0, Rozas & Rozas, 1999). Patterns of geographical subdivision and gene flow were also estimated hierarchically with the aid of DnaSP. A measure of gene flow within and among regions (populations), F_{st}, was obtained from Nm, the number of female migrants per generation between populations. It was estimated using the equation $F_{st} = 1/(1 + 2\,Nm)$, where N is the female effective population size and m is the female migration rate (Slatkin, 1993).

Geographical associations of haplotypes and clades within the minimum spanning network were tested by using program GeoDis (Posada et al., 2000). Two major statistics were calculated: the clade distance (Dc), a value of the geographical spread of a clade, and the nested clade distance (Dn), a value of the geographical distribution of a clade relative to other clades in the higher-level nesting category. These measures of geographical distribution were used to infer the historical processes following the key of Templeton et al. (1995) and Templeton (2004).

RESULTS

The sequence length of *atp*B-*rbc*L intergenic spacer of cpDNA varied from 809 to 871 bp. In total, 902 bp of the consensus length was aligned. The intron 2 region of the NADH dehydrogenase subunit 7 gene of mtDNA in *Lithocarpus konishii* was also amplified and sequenced, with a length varying from 1327 to 1337 bp and a consensus length of 1369 bp. In total, 24 and 27 haplotypes were detected in cpDNA and mtDNA, respectively.

The KB population from the earthquake epicenter in central Taiwan had the highest haplotype number in both organelle genes, namely 10 for cpDNA and 11 for mtDNA (Table 1). As expected, haplotype and nucleotide diversity of cpDNA ($h = 0.697$, $\pi = 0.02469$) were higher than those of mtDNA ($h = 0.666$, $\pi = 0.00160$) in *L. konishii*. The highest levels of haplotype and nucleotide diversities of cp- and mt-DNAs were detected in the KB population (Table 1). Compared to the genetic composition of populations of *L. konishii* before the earthquake, eight cpDNA haplotypes and ten mtDNA haplotypes disappeared from the wild due to the extinction of population KB at the epicenter. After the Chi-Chi Earthquake, levels of haplotype and nucleotide diversities declined more in cpDNA ($h = 0.697$ vs. 0.689, $\pi = 0.02469$ vs. 0.02153) than in mtDNA ($h = 0.666$ vs. 0.608, $\pi = 0.00160$ vs. 0.00144) (Table 1).

In order to address the relationships of haplotypes in *Lithocarpus konishii*, a minimum spanning network was recovered based on the mutational changes between haplotypes (Fig. 2). The chloroplast DNA sequences of *L. konishii* are distinct from those of *L. formosanus* (Skan) Hayata and *L. dodonaeifolius* (Hayata) Hayata (T. Y. Chiang et al., 2004) with more than 108 nucleotide sites different. In contrast, all populations are polymorphic for cpDNA. Two major clades, separated by 101 mutations, and nine clades were identified: clade 3–1 consisting of clades A, B, and C, and clade 3–2 consisting of clades D–I. Clade F is abundant among the nine clades, with an overall frequency of 50.6% (Table 2). Geographically, clades B, C, and E were found only in the KB population and were lost from the species after the earthquake, while clade I was only present in the CE population (Table 2; Fig. 2). The extinct KB population contained haplotypes from all clades, except for clade I (Table 2).

A spanning network was reconstructed based on the variation of intron 2 of NADH dehydrogenase subunit 7 gene of mtDNA (Fig. 3). As for cpDNA, populations of *Lithocarpus konishii* were polymorphic for mtDNA, except for CD and CE populations. Five major clades, I–V, were identified. Haplotpes from clade V (84.5%) were most frequent and were widely distributed in all populations of *L.*

Figure 2. Minimum spanning network of *Lithocarpus konishii* based on the mutational changes between sequences of cpDNA. Clades A to I, and replicate number of each haplotype are indicated.

konishii (Table 2); while clades I and IV were found only in the KB population and became extinct after the earthquake.

For resolving the phylogeographical ambiguities, a nested clade analysis was conducted based on the hierarchical relationships between haplotypes and between clades in the networks. A significant geographical pattern was detected at 2-step and 3-step clade levels in the cpDNA data. Two clades at net-

Table 2. Distributions and frequencies of cp- and mt-clades in *L. konishii*.

DNA	KA	KB	KC	CD	CE	Total
cpDNA-clade						
A		1	3	5		9 (7.6%)
B		1				1 (0.9%)
C		1				1 (0.9%)
D		1	7			8 (6.7%)
E		1				1 (0.9%)
F	7	9	32	7	4	59 (50.6%)
G		1			8	9 (7.6%)
H	5	2	2			9 (7.6%)
I					20	20 (17.2%)
mtDNA-clade						
I		3				3 (2.7%)
II			4			4 (3.3%)
III	2	4	4			10 (8.6%)
IV		1				1 (0.9%)
V	10	9	36	12	32	99 (84.5%)
Total	12	17	44	12	32	117 (100%)

work tips, clades 1-3 and 1-9, were restricted to unique geographical regions, while interior clades 1-1, 1-6, and 1-8 were widespread. Values of the clade distance (Dc), an estimate of the geographical spread of a clade, and the nested clade distance (Dn), an estimate of the geographical distribution of a clade relative to other clades in the higher-level nesting category, for each clade were determined from the geographical distribution of haplotypes. These values were then used to interpret historical and ongoing processes following the key of Templeton et al. (1995) (Table 3). Restricted seed movement with isolation by distance was identified in clades 1-6, 1-8, 2-2, 2-3, and 3-1, while restricted gene flow coupled with occasional long-distance dispersal contributed to the spatial distribution of haplotypes in clades 1-1 and 3-2. Interestingly, after removing the extinct KB population from the nested clade analysis, phylogeographical patterns became undetectable in clades 1-1, 1-6, and 2-2, and restricted gene flow with long-distance dispersal was identified as the phylogeographical pattern for clade 3-1 (Table 3).

Based on mtDNA data, restricted gene flow with isolation by distance was indicated as a major pattern in clades 2-1 and 3-2, while restricted gene flow coupled with occasional between-population dispersal was identified as a force shaping clade 1-5. No conclusive patterns can be determined for the total cladogram (Table 4). For the cladogram without the KB population, a phylogeographical

Figure 3.   Minimum spanning network of *Lithocarpus konishii* based on the mutational changes between sequences of mtDNA. Clades I to V, and replicate number of each haplotype are indicated.

pattern of occasional long-distance dispersal was identified in clade 2-1.

Low to medium levels of genetic differentiation at the cpDNA intergenic spacer were detected among populations of *Lithocarpus konishii*. Pairwise comparisons revealed that most *Nm* values deduced from the cpDNA sequence are greater than 1, with a range from 0.84 (between KA and CE) to 73.90 (between KB and KC). Likewise, mtDNA sequences revealed no geographical structuring, as indicated by high *Nm* values (1.77 to 58.65) and low to medium F_{st} values (0.22020 to 0.07292) between populations (Table 5). High *Nm* values deduced from both organelle sequences might reflect frequent gene flow between oak populations.

DISCUSSION

HISTORICAL AND CURRENT MIGRATION IN *LITHOCARPUS KONISHII*

In this study, we investigated the genetic diversity of cp- and mtDNAs and population genetic structure in *Lithocarpus konishii*. As expected, this oak species possessed higher levels of nucleotide diversity in cpDNA than in mtDNA due to the differences in the overall rate of nucleotide substitutions for the two genomes (cf. Li, 1997). Nucleotide diversity of cpDNA of the endangered *L. konishii* was relatively high compared to other plants, e.g.,

California pine (π = 0.003) (Hong et al., 1993), *Michelia formosana* (π = 0.01063) (Lu et al., 2002), *Cunninghamia konishii* Hayata (π = 0.01018) (Lu et al., 2001), *Cycas taitungensis* C. F. Shen et al. (π = 0.01268) (Huang et al., 2001), *Dunnia sinensis* Tutcher (π = 0.0022) (Ge et al., 2002), and *Lithocarpus formosanus* (π = 0.081) (T. Y. Chiang et al., 2004). Relative to most angiosperms, the nucleotide diversity of mtDNA of *L. konishii* was also high, although still lower than that of *Cycas taitungensis* (π = 0.02637) (Huang et al., 2001) and *Dunnia sinensis* (π = 0.0019) (Ge et al., 2002). It has been known that endangered or threatened species tend to have depauperate genetic variability due to small population size (O'Brien, 1994; Taylor et al., 1994; Vrijenhoek, 1994; Mundy et al., 1997; Gibbs et al., 1998). Such unusually high levels of genetic diversity in *L. konishii* reflect that this endangered endemic species must have been shaped by other evolutionary forces in addition to random genetic drift. Populations may have residual levels of genetic variation before extirpation.

Although high *Nm* values were deduced from both organelle DNAs, they are unlikely to represent current gene flow among populations due to constraints of seed dispersal across the geographical range of the species (T. Y. Chiang et al., 2004;

Table 3.   Nested contingency analysis of geographical associations and phylogeographical inferences made from a nested haplotype analysis of *L. konishii* based on chloroplast DNA.

	Clade	Permutation-al chi-square statistic	Probability	Clade key	Inferences
Both extant and extinct populations	Clade 1-1	18.0000	0.0030	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dispersal
	Clade 1-6	12.9218	0.2550	1, 2, 11, 17, 4, NO	restricted gene flow with isolation by distance
	Clade 1-8	15.8400	0.0140	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 2-2	6.5185	0.1100	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 2-3	9.1283	0.4000	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 3-1	35.3862	0.0000	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 3-2	59.5765	0.0000	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dispersal
	Total cladogram	20.9339	0.0010	1	non-significant
Extant populations	Clade 1-8	9.0000	0.0280	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 2-3	35.0000	0.0000	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 3-1	8.0000	0.0180	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dispersal
	Clade 3-2	56.7053	0.0000	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dispersal
	Total cladogram	22.3897	0.0010	1	non-signficant

Hung et al., in press). Nm values may also be a reflection of ancestral polymorphisms or may be the result of gene flow among populations when populations were more contiguously distributed. However, the phylogeographical pattern in *Lithocarpus konishii* does not conform to an "isolation by distance" model (i.e., the correlation between degree of genetic differentiation and geographical distance), which could indicate long-distance gene dispersal in the past. In this study, a smaller genetic distance frequently existed between two geographically distant populations than between sequences within the same population, e.g., haplotypes of KB1 and KC12 (= KC33, KC34) separated by a single mutation versus KB1 and KB6 with 5 mutations in mtDNA. Such patchy distributions (Fig. 1) of closely related organelle haplotypes among populations have also been observed in many European tree species that survived glacial periods (Ferris et al., 1995; Petit et al., 1997; King & Ferris, 1998), as well as in *Cunninghamia kon-*ishii (Lu et al., 2001), *Michelia formosana* (Lu et al., 2002), and *Lithocarpus formosanus* (T. Y. Chiang et al., 2004) of Taiwan. According to geological record, since the late Pleistocene, Taiwan was the southeastern edge of the Asian continent before the formation of Taiwan Strait about 100,000 years BP (Lin, 1966; Tsukada, 1966; Kizaki & Oshiro, 1977). This continental island was linked to the mainland via a land bridge and was not completely isolated until the last glacier retreated ca. 18,000 to 20,000 years BP (Lin, 1966). Geological evidence indicates that ice ages have occurred at regular intervals of approximately 100,000 years followed by warm periods of about 20,000 years (Milankovitch cycles) (cf. Bennett, 1990; King & Ferris, 1998). During the glacial maximum, many oaks and conifers previously dominant in the northern part of eastern Asia were forced to migrate into refugia in southern China and Taiwan (T. Y. Chiang et al., 1999), which were mostly distributed at low elevations (cf. Tsukada, 1966). During the subse-

Table 4.   Nested contingency analysis of geographical associations and phylogeographical inferences made from a nested haplotype analysis of *L. konishii* based on mitochondrial DNA.

	Clade	Permuta-tional chi-square statistics	Probability	Clade key	Inferences
Both extant and extinct popula- tions	Clade 1-5	142.5075	0.0000	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dis-persal
	Clade 2-1	7.0000	0.0280	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Clade 3-2	11.7019	0.018	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Total cladogram	8.4311	0.0740	1, 2	inconclusive outcome
Extant populations	Clade 1-3	19.0000	1.0000	1, 2, 11, 17, NO	inconclusive outcome
	Clade 1-5	142.5075	0.0000	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dis-persal
	Clade 2-1	7.0000	0.0370	1, 2, 3, 5, 6, 7, YES	restricted gene flow/dispersal but with some long-distance dis-persal
	Clade 3-2	11.7019	0.0140	1, 2, 3, 4, NO	restricted gene flow with isolation by distance
	Total cladogram	8.4311	0.0780	1, 2	inconclusive

quent deglaciation, elevated global temperatures forced the lowland plants to migrate to high elevations or local peaks.

Heterogeneous composition of organelle DNAs within populations and low levels of genetic differentiation among populations suggest a likely migrant-pool model (Wade & McCauley, 1990), which describes a dispersal pattern where colonists are recruited from a random sample of all the other populations. Under this model, the number of colonists is likely to be large during recolonization and many ancestral polymorphisms are maintained within populations. In this study, the low level of genetic differentiation among *Lithocarpus konishii* populations suggests that the period of 18,000 years for isolation may not be ample for coalescence. The star-like shape of both cp- and mtDNA genealogies, with one common haplotype and many

recent single mutation derivatives (Figs. 2, 3), is consistent with a recent range expansion as are the negative Tajima's D statistics detected for both sequences (Y. C. Chiang et al., 2004; Rueness et al., 2003).

LOSS OF GENETIC DIVERSITY IN *LITHOCARPUS KONISHII* DUE TO THE CHI-CHI EARTHQUAKE

The Chi-Chi Earthquake had a profound effect on the genetic structure of *Lithocarpus konishii*. Our molecular genetic analysis revealed that the KB population in central Taiwan, like many other angiosperms in Taiwan (cf. Lin, 2001; Huang et al., 2002), possessed a large number of haplotypes of cpDNA and mtDNA (10 and 11, respectively) and high levels of genetic diversity and represented a hot spot for the conservation of genetic diversity.

The devastating earthquake eliminated from the species eight cpDNA haplotypes (33.3%), including clades B, C, and E, and 10 mtDNA haplotypes (37.0%), including clades I and IV. The Chi-Chi Earthquake had a severe impact on the levels of genetic diversity within the species. Changes of nucleotide diversity of cpDNA are evident before and after the earthquake, $\pi = 0.02469$ versus 0.02153, with 12.8.0% of the genetic diversity eliminated. Compared to cpDNA, a relatively lower percent (10.0%) of genetic diversity of mtDNA has been lost from this species, although in absolute numbers

Table 5.   Pairwise F_{st} estimates between populations of *L. konishii* based on cpDNA (above diagonal) and mtDNA (below diagonal).

	KA	KB	KC	CD	CE
KA		0.11444	0.07045	0.36223	0.37397
KB	0.00845		0.00672	0.06248	0.13482
KC	0.12579	0.03547		0.22144	0.10395
CD	0.16268	0.13479	0.20579		0.36462
CE	0.09841	0.09620	0.07292	0.22020	

more haplotypes went extinct in mtDNA. The loss of this central population due to the earthquake increases the risk of extinction for *L. konishii*; the species is now even more narrowly distributed and the loss of the most genetically diverse populations has dramatically decreased the overall levels of diversity.

PHYLOGEOGRAPHY OF *LITHOCARPUS KONISHII*

Minimum spanning networks, based on two organelle loci, were used to trace the geographical associations among alleles (Templeton et al., 1995). As has been extensively documented, climatic changes influence the distribution and evolution of species in a geographical context; fluctuating cycles of glaciation since the late Pleistocene have constrained the migration of many species (Hewitt, 1996, 1999) and strongly affected the distribution of their genetic variation (Caron et al., 2000). In resolving geographical patterns of variation, nested intraspecific gene networks usually provide greater resolution compared to conventional phylogenetic trees (Crandall & Templeton, 1993).

In *Lithocarpus konishii,* as in many other plants in Taiwan, e.g., *Cycas taitungensis* (Huang et al., 2001), *Cunninghamia konishii* (Lu et al., 2001), *Michelia formosana* (Lu et al., 2002), *Lithocarpus formosanus* and *L. dodonaeifolius* (T. Y. Chiang et al., 2004), *Trema dielsiana* Hand.-Mazz. (Y. C. Chiang et al., 2004), and *Miscanthus sinensis* Andersson (Y. C. Chiang et al., 2003), range expansion occurred after the glaciers retreated and the species spread out from their refugium; the inferences of long-distance dispersal and displacement for tip clades is consistent with range expansion (Tables 3, 4). For example, chloroplast clades/haplotypes at the tip nodes, such as clade I and haplotype KC1, which are restricted to population CE and KC (Figs. 2, 3), respectively, may represent some migrants from the putative refugium at KB during range expansion. Evidence of star-like phylogenies of both cp- and mtDNA genealogies, and significantly negative Tajima's D statistics also suggest a scenario of recent population expansion. However, a history of recent demographics was not supported by nested clade analysis (NCA). The shortcoming of NCA in detecting range expansion, which was also illustrated in a recent phylogeographical analysis of an Iberian lizard (Paulo et al., 2002), may be ascribed to the existence of widespread ancestral (interior) haplotypes in the networks, which contrasts with the prediction that ancestral haplotypes are restricted to the ancestral range while descendent (tip) haplotypes are more widespread under population expansion (cf. Templeton, 1998).

In this study, restricted gene flow with occasional long-distance dispersal, identified by phylogeographical analyses, seems to explain why a model of "isolation by distance" is not supported at the scale of overall species. Nevertheless, the discrepancy in phylogeographical inferences within the same clades before and after the Chi-Chi Earthquake implies that the NCA is sensitive to the stochastic extinction of haplotypes and clades. Although long-range seed dispersal has been recognized as one of the major mechanisms for the postglacial recolonization in European oaks (Petit et al., 1997), here we illustrated that some long-range colonization inferences, three out of eight NCA inferences of this study, may have been misidentified due to extinction of haplotypes (clades) in intervening areas, especially those geographically central to other populations (cf. Masta et al., 2003). Simulations by removing peripheral populations from this analysis, in contrast, do not reveal any additional long-range dispersal events (data not shown), indicating different roles of the populations spanning across a geographical range. Apparently, when intermediate populations are removed, historical stepping-stone migration can be erroneously interpreted as long-distance dispersal among distant, extant populations. Overestimated long-range colonization would become more serious and unavoidable, as species experience frequent and recurrent extinction-recolonization.

MANAGEMENT UNITS FOR CONSERVING *LITHOCARPUS KONISHII*

The Chi-Chi Earthquake caused a dramatic loss of genetic diversity in *Lithocarpus konishii* due to extinction of a single population located at the epicenter. The Mt. Juofenger population harbored a large number of genetic polymorphisms, making this endemic species more threatened. Various criteria have been suggested for defining evolutionarily significant units (ESUs) for species of high conservation priority, including reciprocal monophyly (Moritz, 1994), adaptive variation (Ryder, 1986), and reproductive separation (Waples, 1991). Recognizing ESUs as reciprocally monophyletic groups ensures that all evolutionary heritages within a species can be maintained and that populations of different lineages can be managed separately (Crandall et al., 2000). Nevertheless, in contrast to animal mitochondrial genes, of which reciprocal monophyly is a common characteristic due to fast evolutionary rates, plant organelle DNA

genes, especially those unrelated to speciation (cf. Ting et al., 2000), usually display paraphyly between sister species due to ancestral polymorphisms (Chiang, 2000). Examples include European oaks (Petit et al., 1997), *Lithocarpus formosanus* and *L. dodonaeifolius* (T. Y. Chiang et al., 2004), *Cycas taitungensis* and *C. revoluta* Thunb. (unpublished data), *Amorphophallus* (Chiang & Hong, 1999), *Archangiopteris* (T. Y. Chiang et al., 2002)**,** and *Cunninghamia* (Lu et al., 2001). Frequently encountered paraphyly among closely related plant species suggests that such a monophyly-based definition is not widely applicable to plants (Newton et al., 1999; Crandall et al., 2000).

If Moritz's (1994) criterion of reciprocal monophyly is used here, no single population of *Lithocarpus konishii* can be recognized as a conservation unit due to lack of reciprocal monophyly and low levels of genetic differentiation, even though all populations are geographically isolated. According to the rationale, despite the rampant paraphyly in the oak populations, "awaiting attainment of monophyly within species/populations via coalescent processes" is an explicit policy that could be adopted for conserving such a threatened species. As a consequence, many genetic polymorphisms that were maintained during the paraphyletic stages would be randomly lost from *L. konishii* before the species/populations reach monophyly. A simple dichotomy of monophyly and non-monophyly is insufficient for determining conservation units. In *L. konishii* high levels of genetic diversity within populations may lead conservation biologists to conclude a low conservation priority for this restricted, endemic species. The dramatic loss of genetic diversity due to the devastating Chi-Chi Earthquake reveals how fragile a species like *L. konishii*, with limited population number and size, can be. Although molecular data provide sufficient information for reconstructing phylogeographical patterns of *L. konishii*, defining conservation units solely on the basis of information from genetic markers should be avoided (Taylor & Dizon, 1999).

Crandall et al. (2000) developed 16 categories for identifying population distinctiveness and management practices based on rejection of, or failure to reject, genetic and ecological exchangeabilities in both recent and historical time frames. The dichotomy of historical versus recent events can be made based on particular genetic markers. In this study, both cp- and mt-DNAs indicate large *Nm* values, suggesting historical interchanges between populations. At the same time, our ecological observations reject current exchangeability (both ge-

netic and ecological). Altogether these considerations suggest that *Lithocarpus* populations represent different units for conservation (Case 5(a) of Crandall et al., 2000); populations spanning geographical regions should be managed separately. In addition to preserving adaptive diversity and evolutionary processes across the geographical range of a species, specific threats and recent disturbance in each population should be taken into account before genetic diversity is lost.

Taking genetic and demographical data into consideration in defining conservation units, as opposed to reciprocal monophyly, makes Crandall et al.'s (2000) methodologies more thorough and applicable. Nevertheless, overemphasizing the current ecological exchangeability may make one neglect the role of conservation genetics. If recent ecological exchangeability is rejected, populations would be treated as distinct conservation units; if not, an entire group of populations would be recognized as a single unit. Genetic exchangeability, seemingly, plays a minor role in determining the management action. That is, no matter how different the outcomes of testing of genetic exchangeability, they are unable to change the management units recommended by ecological exchangeability. In this study, molecular data provided sufficient information for reconstructing phylogeographical patterns, which in turn are critical for testing historical, genetic exchangeability. We therefore suggest that defining conservation units solely based on ecological measures should be avoided.

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