

Nested Phylogeographical Clade Analysis of *Trochodendron aralioides* (Trochodendraceae) in Taiwan

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ABSTRACT: Nested clade phylogeographical analysis (NCPA), developed by A. R. Templeton and his colleague, was used to infer the evolutionary events that shaped the spatial genetic structure of *Trochodendron aralioides* in Taiwan. Genetic variation of two intergenic spacers of chloroplast DNA (*petG-trnP* and *trnA-psbJ*) among 24 populations, in which two from Japan, two from the Ryukyus and 20 from Taiwan, was examined. The NCPA suggests that the populations in Taiwan were first isolated from those of Japan proper. Since then, the spatial genetic structure in Taiwan was attributable to restricted gene flow.

KEY WORDS: cpDNA, nested clade phylogeographic analysis, Taiwan, *Trochodendron aralioides*.

INTRODUCTION

Population structure of a species is usually governed by recurrent evolutionary forces that occur in each generation, such as gene flow, system of mating, and genetic drift. As a population is in equilibrium, the net growth (birth minus death) of the population approximates zero, and F_{st} can be used to estimate the amount of the gene flow (Templeton, 1998). When the gene flow is restricted, the model of "isolation by distance" is predicted (Wright, 1943). However, in the evolutionary history of a species, the distribution of populations may be constricted or expanded due to the environmental changes, and leads to a state of non-equilibrium. In consequence the net growth of the population is either decreased or increased. When the population is constricted, gene flow between populations is constrained while the effects of genetic drift and inbreeding within populations increase. In contrast, when a population expands, gene flow between populations increases while the genetic drift and inbreeding within population decrease. Evolutionary events usually leave imprints in the spatial genetic structure of a species. To determine the roles of these events in shaping the present spatial genetic structure of a species, the nested clade phylogeographic analysis (NCPA) was developed (Templeton et al., 1987, 1992, 1993, 1995; Templeton, 1998, 2004). This technique combines the information

of the spatial distribution and the genetic structure of the population to test alternative phylogeographic hypotheses. This method utilizes the haplotype genealogy in a hierarchical manner (Templeton et al., 1993). For example, under a model of restricted gene flow, the parameters of the geographical data such as the geographical range of a particular clade, D_c , and the degree of a particular clade relative to its closest sister clades, D_n , are then tested to examine if they are significantly deviated from the random pattern via random permutation (Templeton, 1998). If they do, the biological interpretations, such as restricted gene flow, fragmentation and expansion, are proposed to explain such patterns. Technically, based on the values of D_c and D_n that are significantly smaller or larger than the random pattern within a clade, the possible events that shape the spatial genetic structure of the clade can be inferred (Templeton et al., 1995). Since a clade is considered younger than its next high-level one, the events that shaped the present spatial genetic structure are in time sequence, thus an evolutionary history of the species or species complex may be inferred (Templeton, 2004).

The phylogeography of *Trochodendron* has been reconstructed (Huang et al., 2004). Possible refuge in north-central Taiwan was proposed. *Trochodendron* started to colonize Taiwan via the Ryukyus about 5 million years ago (My) as land bridges linked Taiwan and Japan. Populations in the Ryukyus were derived from Taiwan's by recent recolonization (Huang and Lin, 2006). However, how are the Taiwan populations genetically structured remains unknown. In this study, we used NCPA to infer the possible events that shaped the present spatial genetic structure of *T. aralioides* in Taiwan.

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MATERIALS AND METHODS

Six haplotypes (Table 1, modified from Huang et al., 2004) were recognized by the variation of the nucleotide sequences of two intergenic spacers of chloroplast DNA (*petG-trnP* and *trnA-psbJ*) among 95 individuals in 24 populations, of which two from Japan, two from the Ryukyus and 20 from Taiwan (Table 2). Gene genealogy was reconstructed based on the genetic variation by using TCS program (Templeton et al., 1992) following the instruction of Templeton et al. (1995). The computer program GeoDis 2.0 (Posada et al., 2000) was carried out to calculate the parameters D_c and D_n , and to examine the deviation from the random pattern. Inference keys provided by Templeton (2004) were then followed to determine the possible events.

RESULTS

A nested cladogram was reconstructed in Figure 1. Haplotypes C and D comprise the clade 1-1, haplotypes A and G comprise the clade 1-2, and haplotypes H and I comprise the clade 1-3. Clades 1-1 and 1-2 together form the clade 2-1. Haplotype H is the most ancient type based on the outgroup comparison taking the most related species *Tetracentron sinensis* as a reference (Huang and Lin, 2006). The significance of D_c and D_n was given in Table 3. The past events inferred by following the inference keys were listed in Table 4. Clade 1-1 was considered of no geographical association while clades 1-2 and 2-1 were inferred to be of restricted gene flow. Clade 1-3 was inferred to be inadequate to discriminate between the possible events due to

Table 1. Six haplotypes of *Trochodendron aralioides* were recognized based on the distribution of substitution sites in the intergenic spacers of *petG-trnP* and *petA-psbJ* cpDNA. (modified from Huang et al., 2004: Table 2).

Haplotype	<i>petG-trnP</i>				<i>petA-psbJ</i>		Distribution
	162	239	252	376	143	570	
A	G	C	T	G	T	A	Taiwan, Ryukyu
C	G	C	C	G	T	A	Taiwan
D	G	C	C	A	T	A	Taiwan
G	G	C	T	G	T	C	Taiwan
H	G	T	T	G	C	A	Japan
I	C	T	T	G	C	A	Japan

Numbers indicate the position of the gene sequence submitted in Genbank

Table 2. Sampling localities chosen for the population study of *Trochodendron aralioides* accompanied with sample sizes and their haplotypes.

Locality and population no.	Latitude-longitude	Altitude (meter)	Sample size	Haplotype (sample no.)
Japan				
1. Asiu	35°00'N-135°42'E	700	4	H(4)
2. Chomonkyo	34°23'N-131°20'E	150	4	I(4)
Ryukyus				
3 Amami	28°24'N-129°42'E	500	3	A(3)
4 Iriomote	24°25'N-123°45'E	100	4	A(4)
Taiwan				
5 Yangmingshan	25°10'N-121°34'E	700	4	A(4)
6 Shihting	24°59'N-121°40'E	400	4	A(4)
7 Nanchatienshan	24°46'N-121°24'E	1600	4	C(3)D(1)
8 Chilanshan	24°41'N-121°20'E	1550	4	C(3)D(1)
9 Hsiakeluoshan	24°34'N-121°12'E	2000	4	C(4)
10 Hakannishan	24°32'N-121°02'E	1600	4	A(1)C(3)
11 Taipingshan	24°30'N-121°32'E	1800	4	C(4)
12 Ssuyuan	24°24'N-121°21'E	1900	4	C(3)D(1)
13 Hoping	24°18'N-121°39'E	1200	4	C(4)
14 Piluchi	24°13'N-121°17'E	2400	4	A(3)D(1)
15 Meifeng	24°06'N-121°11'E	2000	4	A(2)C(2)
16 Tanta	23°46'N-121°07'E	2450	4	A(3)G(1)
17 Chitou	23°40'N-120°47'E	1700	4	A(4)
18 Zueshui	23°32'N-121°16'E	1500	4	A(4)
19 Tatachia	23°30'N-120°52'E	2500	4	A(4)
20 Takuanshan	23°30'N-120°52'E	2500	4	A(4)
21 Hsiangyang	23°15'N-120°58'E	2350	4	A(4)
22 Tuona	22°53'N-120°46'E	1800	4	A(4)
23 Tahanshan	22°25'N-120°43'E	1500	4	A(4)
24 Lilongshan	22°10'N-120°43'E	900	4	A(4)

Table 3. Results of nested clade analyses of the geographical distance of cpDNA haplotypes of *Trochodendron aralioides*.

Haplotype and Clade	Type of geographical distance		Exact Contingency Test Probability
	Within clade (D_c)	Nested clade (D_n)	
Clade 1-1			0.29
C (Interior)	25.74	25.73	
D	23.44	23.46	
Interior-Tip	2.30	2.26	
Clade 1-2			0.86
A (Interior)	188.52	187.88	
G	0.00	76.88	
Interior-Tip	188.52 ^L	111.00	
Clade 1-3			0.04
H (Interior)	0.00 ^S	201.98 ^S	
I	0.00 ^S	202.71 ^L	
Interior-Tip	0.00	-0.73 ^S	
Clade 2-1			0.00
Clade 2-1	25.43 ^S	68.13 ^S	
Clade 1-2 (Interior)	80.77	91.76 ^L	
Interior-Tip	55.34	23.64 ^L	
Total clade			0.00
Clade 2-1	138.84 ^S	225.58 ^S	
Clade 1-3 (Interior)	202.34	1274.55 ^L	
Interior-Tip	63.50	1274.55 ^L	

An "S" superscript indicates significantly smaller than random pattern at 5%; an "L" superscript indicates significantly larger than random pattern at 5%.

Table 4. Past events inferred from the nested clade phylogeographical analysis inference keys, provided by Templeton (2004), which possibly shape the present distribution pattern of the genetic structure of *Trochodendron aralioides*.

Haplotype Clade	Chain of inference	Inference events
Clade 1-1	1 No	the null hypothesis of no geographical association of haplotypes cannot be rejected
Clade 1-2	1-2-3-4 No	restricted gene flow
Clade 1-3	1-19-20 No	inadequate geographical sampling
Clade 2-1	1-2-3-4 No	restricted gene flow
Total clade	1-19 No	allopatric fragmentation

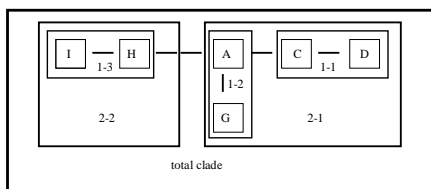


Fig.1. The nested cladogram based on six haplotypes recognized in Table 1. The haplotype H is the most ancient type predicted by outgroup comparison explained in the text. Each line indicates one substitution.

insufficient geographical sampling. The total clade was considered resulting from allopatric fragmentation. In summary, the genetic structure of *Trochodendron* in Taiwan (represented by the clades 1-1, 1-2, 2-1 and the total clade) was shaped by allopatric fragmentation followed by restricted gene flow.

DISCUSSION

Allopatric fragmentation that was inferred based on NCPA may have occurred about 5 million years ago when a land bridge connected Japan, the Ryukyus and Taiwan (Huang and Lin, 2006). Plants with haplotype A may be derived subsequently and became widespread in Taiwan. The event of restricted gene

flow was the main cause following allopatric fragmentation. The event of restricted gene flow is supposed to be generated in an equilibrium state based on the inference key (Templeton, 1998). This implies that the population size in Taiwan is not strongly influenced by changing climate. In consequence, habitats in Taiwan should be sufficient for the populations to migrate along altitudinal gradient in response to the fluctuation of climate. As a result, the haplotype A mutated to other haplotypes and dispersed to other areas such as the Ryukyus when a land bridge became available. The restriction of newly derived haplotypes C and D to the north central Taiwan and that of haplotype G to the center of the Central Mountain Ridge are examples. Haplotypes C and D have a wider distributing range than the haplotype G, indicating longer existence of the haplotype C (Willis, 1922). The ancient population in the Ryukyus could have perished about 1 million years ago when the islands were submerged (Kimura, 2000). Thus plants with the haplotype A in the Ryukyus were derived from those of Taiwan (Huang and Lin, 2006). This could have happened about 50,000 years ago when the temperature was 10°C lower than today (Tsukada, 1966) and a landbridge existed between Amami and Taiwan (Kimura, 2000).

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昆欄樹之巢狀支序分析

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摘 要

本文以 Templeton 及其同仁所研發出來之巢狀支序分析法推測形成昆欄樹在臺灣目前族群遺傳結構之可能原因。本文以葉綠體 DNA 之片段變異 (包括 *petG-trnP* 及 *tranA-psbJ* 基因間隔區) 檢視臺灣 20 族群，琉球 2 族群及日本 2 族群之基因型分布。巢狀支序分析推測臺灣之族群首先與日本族群分隔，各自演化。隨後族群遺傳結構由距離造成分隔 (isolation by distance) 現象所形塑。

關鍵詞：葉綠體 DNA、巢狀支序分析、臺灣、昆欄樹。

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