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Research note

Analysis of mitochondrial DNA of an endangered beech species, *Fagus hayatae* Palibin ex Hayata

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Abstract. Mitochondrial DNA restriction fragment length polymorphisms were used to examine cytoplasmic diversity within a relic-like population of *Fagus hayatae*, located in northern Taiwan. Fifteen trees were surveyed for three restriction endonucleases (*Bam*HI, *Eco*RI and *Hin*dIII) and five mitochondrial gene probes (*atpA*, *atp6*, *atp9*, *coxI* and *coxII*). The analysis failed to reveal any polymorphisms, an observation that suggests cytoplasmic uniformity in the *F. hayatae* population examined. It is also interesting to note that the chondriome type of our *F. hayatae* samples is very close to that characteristic of *F. crenata* populations in the southernmost area of Japan.

Introduction

The genus *Fagus* comprises two subgenera, *Engleriana and Fagus*, with the latter further subdivided into four sections: *Grandifolia*, *Longipetiolata*, *Lucida* and *Fagus* (Shen 1992, Peters 1997). Some members of the genus have received much attention in silviculture because they fulfill a wide spectrum of economic and environmental functions. *F. hayatae* Palibin ex Hayata is grouped in the section *Lucida*, together with *F. crenata* Blume, *F. lucida* Rehder et Wilson (occurs in southern China), and *F. chienii* Cheng (found in western China), and is an example of a relic tree species with a very limited distribution and in a state of regression (Hsieh 1989). It occurs only in a few locations in northern Taiwan and southern China (Hsieh 1989; Peters 1997). Knowledge of the genetic variation of this

species is of great importance with respect to the preservation of genetic resources.

Mitochondrial (mt) DNA restriction fragment length polymorphisms (RFLPs) are convenient markers for determining cytoplasmic diversity in natural populations of higher plants (Palmer 1992, Strauss et al. 1993). Koike et al. (1998) have recently examined the mtDNA RFLP pattern in two Japanese beech species, *F. crenata*, and *F. japonica* Maximowicz (assigned to the subgenus *Engleriana*), and have revealed cytoplasmic diversity among and within populations. In this paper, we have analyzed mtDNA to assess the degree of cytoplasmic variation in *F. hayatae* plants sampled in Taiwan. An attempt has also been made to estimate genetic relationships among the populations of *F. hayatae*, *F. crenata* and *F. japonica*.

Materials and methods

Young expanding leaves were collected from fifteen trees of the *F. hayatae* population within the Lalashan Nature Reserve (between 24°41′ and 24°50′ north latitude and 121°23′ and 121°30′ east longitude) in northern Taiwan. Sampled individuals were well-spaced to avoid over-representation of maternal half-sibs. Total cellular DNA was extracted from freeze-dried leaf tissue (approximately 3g) by a CTAB procedure (Ishikawa et al. 1992). The DNA was digested with each of three restriction endonucleases (*Bam*HI, *Eco*RI and *Hin*dIII), followed by electrophoretic separation in 0.8% agarose gel. The gel was Southern blotted onto nylon membranes (Hybond N^+, Amersham, Little Chalfont, U.K.) according to the manufacturer's recommendations. The membrane was hybridized overnight at 42 °C with constant shaking in the hybridization buffer [enhanced chemiluminescence (ECL) methods, Amersham, Little Chalfont, U.K.] containing the labelled probe. Then the hybridized blot was rinsed twice with the first washing solution (6M urea, 0.4% SDS and 0.5 × SSC) for 20 min at 42 °C, followed by two rinses with the second washing solution (2 × SSC) for 5 min at room temperature. Labelling of probe DNA and visualization of the probe-target DNA hybrid were carried out by the ECL methods, according to the supplier's instructions. *Hin*dIII-digested Lambda DNA and *Hae*III-digested Phi X 174 DNA were used as molecular size markers.

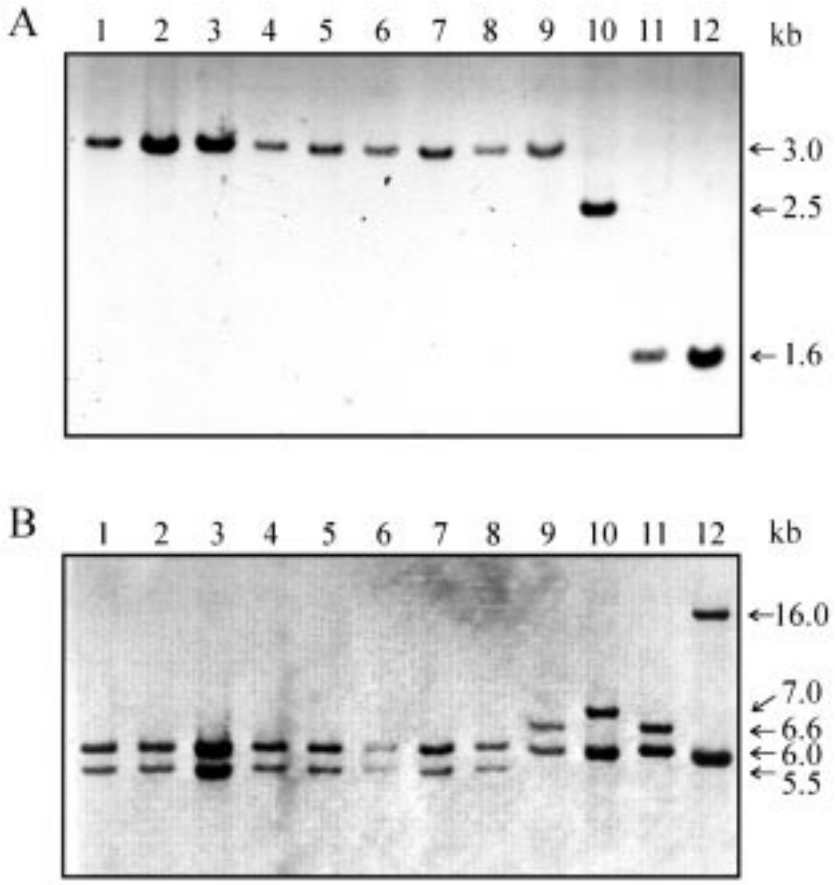

Figure 1. Southern hybridization analysis of total DNA from *F. hayatae* plants. *F. crenata* samples were also included as reference in the analysis. Total DNA was digested and probed with; (A) *Hin*dIII and the *atp9* gene; and (B) *Bam*HI and the *coxII* gene. Slot designations: 1–8, *F. hayatae*; 9, *F. crenata* (mtDNA haplotype, XI); 10, *F. crenata* (VIII); 11, *F. crenata* (VI); and 12, *F. crenata* (IV). [See Koike et al. (1998) and Table 1 in this paper for a description of mtDNA haplotype].

Results and discussion

Five known mitochondrial genes were used as probes to see whether they might display cytoplasmic differences among the *F. hayatae* individuals. The probes were: sugarbeet *atpA* [0.7-kb *Eco*RI-*Bam*HI fragment (Senda et al. 1993)], *Oenothera atp6* [1.5-kb *NheI* fragment (Schuster and Brennicke 1987)], apple *atp9* [0.3-kb cDNA (Kato et al. 1995)], sugarbeet *coxI* [1.6-kb *Eco*RI fragment (Senda et al. 1991)], and sugarbeet *coxII* [0.4-kb *Sal*I-*Hin*dIII fragment (Senda et al. 1991)]. Figure 1 illustrates representative patterns

Table 1. Comparison of mtDNA RFLP patterns between *F. hayatae*, *F. crenata* and *F. japonica*. The presence (1) or the absence (0) of a hybridization signal for the mitochondrial genes (*atpA*, *atp6*, *atp9* and *coxII*) is shown. Fragment sizes are indicated in kb. The mtDNA data of *F. crenata* and *F. japonica* are taken from Koike et al. (1998). The mtDNA haplotype VI was found in both *F. crenata* and *F. japonica*

Probe/enzyme			mtDNA haplotype in *F. crenata*											*F. japonica*		
combination	kb	*F. hayatae*	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	VI	XII	XIII
atpA/*Hin*dIII	7.7	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	7.4	1	0	0	0	0	1	1	0	0	0	1	1	1	0	0
	6.4	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	5.2	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1
	4.4	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
atp6/*Bam*HI	7.0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	4.5	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1
atp9/*Hin*dIII	5.2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	3.9	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	3.5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	3.0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	2.5	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1
	1.6	0	1	0	0	1	1	1	0	0	0	0	0	1	1	0
coxII/*Bam*HI	16.0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0
	7.0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
	6.6	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0
	6.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	5.5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2.7	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1
	2.3	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0

of hybridization. With the five probes examined, one or two fragments per enzyme hybridized and no RFLPs were noted (Table 1). Although the number of the individuals studied is not necessarily sufficient, the result suggests that the genetic variation of mtDNA in the *F. hayatae* population is limited, which may predispose this population to potential damage, such as the 1970's southern corn leaf blight epidemic (Fragoso et al. 1989).

One can address the question of whether or not the hybridization signals detected represent mtDNA because total cellular DNA was examined

throughout our experiments. The strength of hybridization appears too great for a single-copy nuclear gene. In addition, the five mitochondrial gene probes failed to hybridize with the chloroplast DNA prepared from *F. crenata* plants under the hybridization and wash conditions used (data not shown). The observations support our contention that hybridization of our blots of total DNA resolved mtDNA fragments, and not those of nuclear or chloroplastic origin.

The next aim was to gain insight into the cytoplasmic relatedness of the *F. hayatae* population and the two Japanese beech species. We have previously identified eleven and three chondriome groupings within *F. crenata* and *F. japonica*, respectively (Koike et al. 1998, see Table 1). The *F. crenata* and *F. japonica* populations analyzed represent the entire geographic range of the species. The characterization of the Japanese beech mtDNAs was based upon the same probe/enzyme combinations as used in the present study, which makes it possible to compare immediately the fragment phenotypes found in *F. hayatae* with the RFLP data from *F. crenata* and *F. japonica* (Table 1). Interestingly, the chondriome type of our *F. hayatae* samples proved to be very close to that (mtDNA haplotype XI) characteristic of three *F. crenata* populations [viz. Kouchi pop., 33°30′ north latitude (N) and 133°10′ east longitude (E); Miyazaki pop., 32°20′ N and 131°10′ E; and Kanoya pop., 31°20′ N and 130°30′ E; see Koike et al. 1998] in the southernmost area of Japan. Shen (1992) described that during the Pliocene the ancestors of *F. crenata* and *F. hayatae* co-occurred in southern Japan. It will thus be of interest to determine whether the similarity in mtDNA could be due to a common maternal ancestry or an interspecific hybridization.

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