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Geographical cline of chloroplast DNA variation in *Abies mariesii*

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Abstract Where its populations are isolated in higher mountain regions, *Abies mariesii* is one of the more important conifers of Japan's alpine forest zone. In this study we tried to clarify the genetic variation of chloroplast DNA (cpDNA) in *A. mariesii*. Cones and fresh needles were collected from seven mountain regions. Total DNAs were extracted from individual seedlings, and these were digested by 15 restriction endonucleases. Southern hybridization was then done using cpDNA clones of *Cryptomeria japonica* and tobacco as probes. CpDNA variation was detected with enzyme-probe combinations: *Hin*dIII+pCS10 probe, *Hin*dIII+pCS7, and *Bgl*II+pCS7 in preliminary screening. These variations were considered to be caused by the same insertion, deletion or inversion. All populations surveyed for the combination *Hin*dIII+pCS10 resulted in only two frequency variations in each population. This indicates a gradual cline along latitude and longitude.

Key words Chloroplast DNA · *Abies mariesii* Intraspecific variation · Geographical cline

Introduction

Abies mariesii is one of the more important species found in Japan's subalpine zone. The species is distributed in the higher mountains from the Tohoku (Hakkoda Mt.) to the Chubu districts (Hakusan Mt.). Thus, *A. mariesii* forests are isolated from each other.

A genetic study of *A. mariesii* has been conducted using 23 allozyme markers (Suyama et al. 1992). Suyama (1991) reported that the genetic variation found in this species is much lower than that observed in other coniferous species. The geographical cline in the mean heterozygosity was also reported. There is a close relationship between the heterozygosity and the latitude of each population. As molecular biology techniques have progressed, extensive structural analysis has been carried out on coniferous species, especially to clarify the unique features of their chloroplast DNA (cpDNA): (1) the lack of a large inverted repeat structure (Strauss et al. 1988; Lidholm et al. 1988; White 1990a; Lidholm and Gustafsson 1991; Tsudzuki et al. 1992; Tsumura et al. 1993); (2) paternal or biparental inheritance (Neale et al. 1986; Szmidt et al. 1987; Neale and Sederoff 1989; Neale et al. 1989; Stine et al. 1989; Dong et al. 1992); (3) intraspecific variation in some species (Wagner et al. 1987, 1992; White 1990b; Ali et al. 1991). In this paper, we report on our study of the kinds and amount of cpDNA variation found in *Abies mariesii*.

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Materials and methods

Plant materials

Cones and fresh needles were collected from individual trees in seven natural populations (average number of individuals per population was 27.6 trees) that are dispersed widely across the natural distribution of *A. mariesii* (Fig. 1). Samples of the northern (Hakkoda) and southwestern (Hakusan) limit populations were also collected. Seeds of individual mother trees were extracted from cones and germinated at 25°C for 8 h under light and at 18°C for 16 h in the dark. These seedlings were grown until their fresh weights were about 100 mg. Fresh needles from individual seedlings were stored at –20°C until DNA extraction. Needles of the Yatsugatake and Chichibu populations were collected directly from individual trees and stored at –20°C until DNA extraction.

DNA manipulation

Total DNAs were extracted from each seedling's needles using the slightly modified CTAB method (Murray and Thompson 1980).

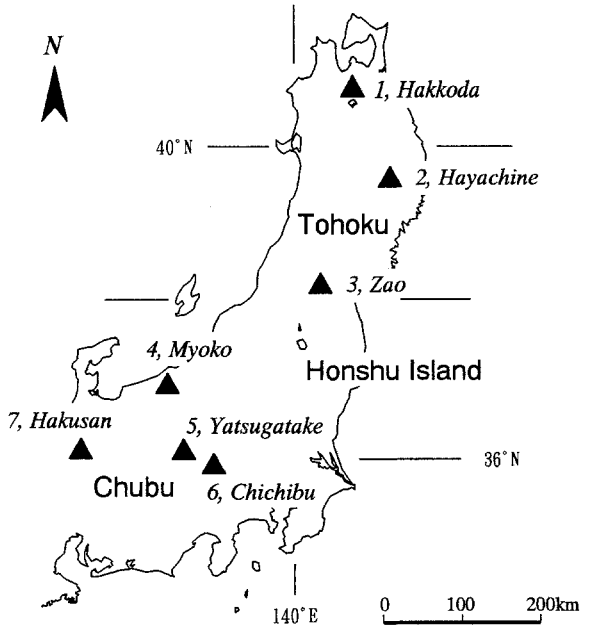

Fig. 1 Location of the seven populations of *Abies mariesii* surveyed

These DNAs were digested by 15 restriction endonucleases, *Hin*dIII, *Bam*HI, *Eco*RI, *Eco*RV, *Hae*III, *Hin*fI, *Sty*I, *Sal*I, *Sac*I, *Pst*I, *Xho*I, *Xba*I, *Bgl*II, *Kpn*I and *Pvu*II. Electrophoresis was done at 15 V for 20 h on 0.7% agarose gel in TAE buffer (40 m*M* TRIS-HCl, 20 m*M* sodium acetate and 2 m*M* EDTA, pH 8.0). After electrophoresis, DNAs were transferred to a nylon membrane (Hybond-N, Amersham) using the Southern blot method.

Southern hybridization of *Abies* DNA was done with heterologous probes to detect variation within the cpDNA in *A. mariesii*. Clones pCS3, pCS5, pCS7, pCS8, pCS9a, pCS9b, pCS10, pCS11 and pCS12 of *Cryptomeria japonica* (sugi) cpDNA (Tsumura et al. 1993) and clones pTBa3, pTS6, pTB8, pTB20, pTB25, pTB7 and pTB10 of tobacco cpDNAs (Sugiura et al. 1986) were used as probes. The probe DNAs (0.1–0.2 μg) were labeled by the digoxigenin nonradioactive labeling methods (Boehringer Mannheim). The nylon membranes were hybridized with probes for 18 h in hybridization buffer (5×SSC, 0.5%(w/v) dry skim milk, 0.1%(w/v) *N*-lauroylsarcosine sodium salt, 0.02%(w/v) SDS, 50 mg/ml salmon sperm DNA) at 68°C. Membranes were washed twice in 2×SSC, 0.1% SDS for 15 min at room temperature and then twice in 0.1×SSC, 0.1% SDS for 15 min at 68°C. Immunological detection of the hybridized fragment was conducted following the protocol of the manufacturer (Boehringer Mannheim).

Statistical analysis

To analyze correlation between the frequencies of cpDNA variants and each population's location, the logistic regression model was used. The logistic regression model is defined as $\log P_i/(1-P_i)=\alpha+\beta x_i$, where P_i and x_i are the proportions of the Type *b* cpDNA variant and the latitude. We also analyzed the correlation between the frequency of cpDNA Type *b* variation and the longitude because this species' distribution zone on Honshu Island is from the southwest to the northeast.

Results

Chloroplast DNA variation in *A. mariesii*

Sixty combinations of 15 restriction endonucleases and 15 probes were used in the Southern hybridizations. CpDNA variation was detected in 3 of the combinations: *Hin*dIII+pCS10 probe; *Hin*dIII+pCS7; and *Bgl*II+pCS7 (Fig. 2). Two types of variants were found in all combinations, Type *a* and Type *b*. In the *Hin*dIII+pCS10 combination (Fig. 2A), both types had three fragments each, two of which (4.2 kb and 0.9 kb) were identical in both types. A different and larger fragment (Type *a*=5.3 kb and Type *b*=4.6 kb) was also found whose variation was considered to be caused by insertion, deletion or inversion. The other two variants were also considered to be caused by the same structural rearrangement because pCS10 and pCS7 are in neighboring positions on a physical map of cpDNA in *C. japonica* (Tsumura et al. 1993) and because we also detected the same segregation patterns in these probe and endonuclease combinations.

Geographical cline of cpDNA variation

The cpDNA variation in the combination *Hin*dIII + pCS10 was investigated in seven natural populations. Two types of variants (*a* and *b*) were detected in each population

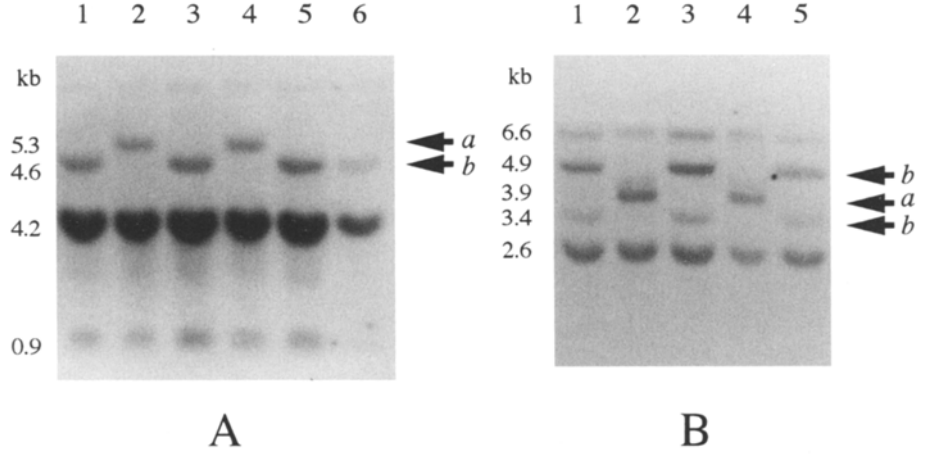

Fig. 2 Southern hybridization patterns of cpDNA variation in *Abies mariesii* using the combination of *Hin*dIII and pCS10 (**A**) and the combination of *Hin*dIII and pCS7 (**B**). *Lanes 1–6* are individual DNA samples of the Yatsugatake population. The *arrows a* and *b* indicate cpDNA variation within species. *Lanes 1, 3, 5* and *6* are Type *b* cpDNA variation, *lanes 2* and *4* are Type *a*. We consequently detected the same patterns as these shown when different probes used

Table 1 Frequency of cpDNA variation in seven natural populations of *Abies mariesii*

Population	Number of mother trees	Number of seedlings	Segregation $a : b$	Frequency[a]	
				I	$\hat{I}$
1. Hakkoda	20	34	24 : 10	0.294	0.266
2. Hayachine	23	37	30 : 7	0.189	0.197
3. Zao	28	33	15 : 18	0.545	0.554
4. Myoko	20	23	13 : 10	0.435	0.450
5. Yatsugatake	15	–	8 : 7	0.467	0.467
6. Chichibu	12	–	7 : 5	0.417	0.417
7. Hakusan	24	39	11 : 28	0.718	0.767
Total	142	193	108 : 85		
Mean	20.2	27.6		0.438	0.445

[a] Frequency I was calculated with the following formula, $b/(a+b)$; and frequency $\hat{I}$ was calculated as follows, x_i/n, where x_i is a Type b frequency in each mother tree and n is the number of mother trees

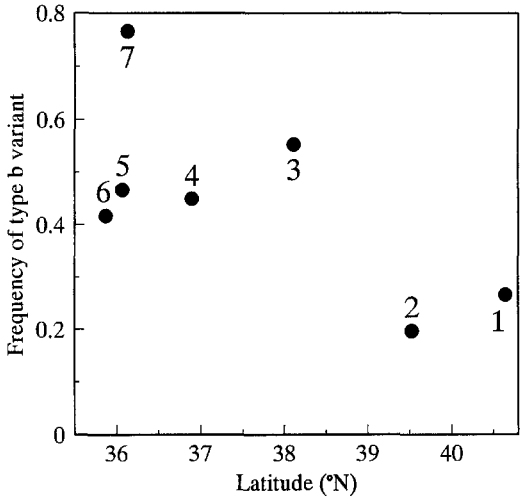

Fig. 3 Correlation between the frequency of Type b variant and latitude

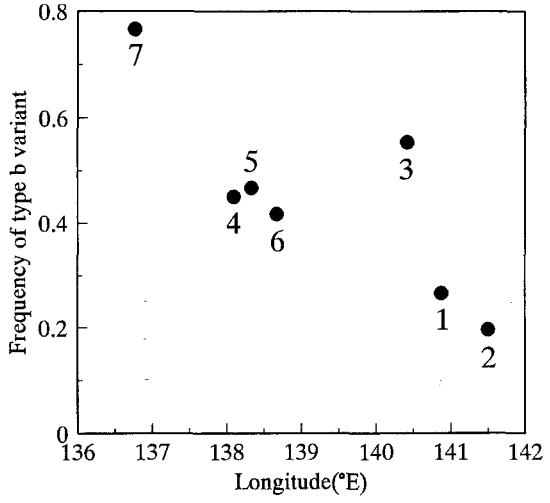

Fig. 4 Correlation between the frequency of Type b variant and longitude

(Fig. 2). The frequencies of the two variants in each population gradually changed from south to north and from west to east (Table 1, Figs. 3 and 4) and were determined to be related to population latitude and longitude. The seven populations are distributed along a southwest-to-northeast cline on Honshu Island. The frequency of Type b cpDNA in Hayachine, the northeasternmost population, was 0.197, while in the most southwestern population, Hakusan, the frequency was 0.767; the frequencies of the other populations ranged between these values. Type b frequency and latitude and longitude were closely related (Figs. 3 and 4). Because the expectation of the P_i value in the linear regression model was not always within the range of 0–1, we used the logistic regression model. The results for latitude were highly significant for the slope ($P< 0.001$); the estimates of α and β were -12.522 ± 5.100 and 0.337 ± 0.135, respectively. The results for longitude were also highly significant for the slope ($P< 0.001$); the estimates of α and β were -50.127 ± 16.520 and 0.361 ± 0.119, respectively. Consequently, Type b cpDNA frequency significantly decreases along a latitudinal and longitudinal gradient.

Discussion

Although the chloroplast genome is conservative in its evolution, intraspecific cpDNA variation has been reported in many plant species. Typically, the level of intraspecific cpDNA variation in plants is much lower than those reported for mitochondrial DNA variation in animals (Soltis et al. 1992). Intraspecific cpDNA variation in conifers has been reported in *Pinus contorta* and *Pinus banksiana* (Wagner et al. 1987); *P. contorta* (Govindaraju et al. 1988); *Pinus monticola* (White 1990b); *P. glauca* and *Picea engelmanii* (Sutton et al. 1990); *Pseudotsuga menziesii*, *Sequoia sempervirens* and *Calocedrus decurrens* (Ali et al. 1991).

CpDNA structure in conifers is different from the cpDNA typical of other plants since conifers lack a large inverted repeat structure (Strauss et al. 1988; Lidholm et al. 1988). One group of legumes also lacks an inverted repeat. In some legume species, the cpDNA is stable and unrearranged; others have undergone moderate rearrangement; while still others have sustained a complex series of

rearrangements (Palmer et al. 1987). Therefore, whether or not the degree of cpDNA instability is related to the absence of a large inverted repeat structure remains unclear.

As conifer cpDNA appears to be primarily paternally inherited and the pollen of conifers usually is dispersed a relatively long way, introgression of cpDNA is considered to occur between the closely related species. Therefore, relatively more variation might be found in conifers (Wagner et al. 1987; Sutton et al. 1990). In this study, segregation of cpDNA variation was found in 14 out of 115 mother trees. Although only 3–5 seedlings from each mother tree were investigated, two types of cpDNA were detected. This suggests that the cpDNA of *A. mariesii* might be inherited through pollen.

A geographical cline in cpDNA type has been found in the tetraploid potato between the Andes and coastal Chile (Hosaka and Hanneman 1988). The detected cpDNA variation suggests that the Andean cultivated tetraploid potato would be expected to repeatedly develop independently from cultivated diploid populations. Our study found a geographical cline in the frequencies of the two cpDNA types in *A. mariesii*, suggesting the origin and dissemination of *A. mariesii* after the last glacial period (20000 years ago). In our study, we checked two correlations in order to analyze the cline, Type *b* frequency and the latitude, and Type *b* frequency and longitude, because the species' distribution is from the southwest to the northeast on Honshu Island. We checked both clines as the dissemination of this species is thought to relate to its range of distribution from southwest to northeast. Allozyme data suggests that the center of distribution before the last glacial period was central Japan (Chubu district) (Suyama 1991), after which *A. mariesii* extended northward. Therefore, the average heterozygosity of 22 allozyme loci also indicates a clear geographic cline (Suyama 1991).

Suyama (1991) investigated 11 populations, the main distribution area of *A. mariesii*, using 22 allozyme loci. The resulting average heterozygosity (*He*) was much lower (0.054) than that found in other conifer species and the *He* values decreased toward the northeastern area. The *He* values of southwestern populations ranged from 0.091 to 0.097, while those of northeastern populations ranged from 0.002 to 0.030. The distance between the southernmost and the northernmost populations is about 600 km. These results imply that *A. mariesii* was mainly distributed in Central Japan's Chubu District (southwestern area in Fig. 2) during the last glacial period, after which the species is thought to have extended northward. The loss of some genetic variation during the northward migration is explained by the bottleneck effect and genetic drift.

The geographical cline in cpDNA variation cannot be totally explained by the bottleneck effect and genetic drift, though cpDNA variation is thought to be neutral for selection like isozyme loci and though cpDNA and the isozyme loci are thought to be independent because the latter are located on nuclear DNA. Thus, no direct relation between the cline of *He* values of the isozyme loci and the cline of cpDNA variation is thought to exist. If the bottleneck actually occurred, the *He* value usually would decrease. However, the proportion of one variant in which we found cpDNA variation also gradually decreased toward the northeast. Thus, there is still the possibility of some selection for one type of cpDNA variation. However, this cline is considered to have at least some biological meaning; that is, its relation to the dissemination of *A. mariesii*, the historical change of distribution and species differentiation.

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