GENETIC DIFFERENTIATION OF ORGANELLE DNA POLYMORPHISMS IN SAGHALIN FIR FROM HOKKAIDO

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ABSTRACT

The investigation of genetic differentiation among Saghalin fir (*Abies sachalinensis* Mast.) populations was carried out with genetic markers of contrasting mode of inheritance. The expected mode of inheritance for the two organelle genomes was also confirmed. Restriction fragment length polymorphisms (RFLPs) were analyzed among 264 individuals, including plus trees, from 19 natural populations. Total genomic DNAs was digested with two restriction endonucleases (*Bam*HI and *Eco*RI) and hybridized with a cytochrome oxidase subunit II (*cox*II) probe from mitochondrial (mt) DNA of sugar beet and an ATPase α-subunit (*atp*A) probe from chloroplast (cp) DNA of Saghalin fir. Three variants were observed in *Bam*HI / *cox*II, and two in *Eco*RI / *atp*A. Nei's gene diversity within populations (H_e) was 0.232 and 0.001 respectively. Genetic differentiation is higher when using a maternally inherited mtDNA marker (G_{ST} = 0.715), than using a paternally inherited cpDNA marker (G_{ST} = 0.003). Especially southwestern populations have genetically different from the rest. The high differentiation of *cox*II gene might be the result of high evolutionary rate and low migration rate of mitochondrial genome. These results provide information for elucidating phylogeography and consequently for an application to conservation purpose of this fir.

Key words: genetic differentiation, RFLP, organelle DNAs, maternal inheritance, paternal inheritance, *Abies sachalinensis*

INTRODUCTION

Saghalin fir (*Abies sachalinensis* Mast.) is widely distributed throughout the islands of Hokkaido and Saghalin and the Southern Kurile Islands as a dominant tree species of four forest zones: the cool-temperate forests, the pan-mixed forests, the subalpine forests, and alpine forests. In the cool-temperate forests, it is associated with the broad-leaved trees represented by the beech (*Fagus crenata*) and in the subalpine forests, with *Picea jezoensis* and *Picea glehnii*. Sometimes it also forms pure forests (JAPAN FOREST TECHNICAL ASSOCIATION 1966). This fir is one of the most important forest trees in Hokkaido prefecture as an ornamental tree, a source of building materials and as a genetic resource.

Evidence concerning the mode of inheritance of chloroplast (cp) DNA has been found in progeny from intra- and inter- specific crosses for Douglas fir (NEALE *et al.* 1986), *Pinus* (WAGNER *et al.* 1987; NEALE & SEDEROFF 1989), *Larix* (STINE *et al.* 1989, SZMIDT *et al.* 1987), *Picea* (SZMIDT *et al.* 1988; SUTTON *et al.* 1991), *Abies* (ZIEGENHAGEN *et al.* 1995) and *Chamaecyparis* (KONDO *et al.* 1998). Generally, cpDNA seems to be paternally inherited in the family Pinaceae. Hence, cpDNA gene flow is considered to occur through pollen and seed dispersal in these species. Since mitochondrial (mt) DNA of Pinaceae species is predominantly maternally inherited (NEALE & SEDEROFF 1989; DEVERNO *et al.* 1993; SUTTON *et al.* 1991), its migration depends only on seed dispersal. Plant mtDNA is subject to rapid structural evolution (SEDEROFF 1987; PALMER 1992). The mtDNA variation, caused by its relatively frequent genome rearrangements, can be easily observed by restriction fragment pattern analysis. Therefore, strong population differentiation, resulting from the maternal inheritance of mtDNA and restricted seed dispersal, have been reported (DONG & WAGNER 1993; STRAUSS *et al.* 1993; TSUMURA & SUYAMA 1998). Confirmation of the mode of inheritance of organelle DNAs is an important step for further study of the population genetics of Saghalin fir.

Previous studies of the fir have revealed genetic variation in morphological and phenological traits of cone, seed and, seedling (HATAKEYAMA 1981). Seedling characters, resistance to snow-damage, freezing resistance, and winter desiccation-resistance of the species have also been well documented (OKADA *et al.* 1973; OKADA 1983; EIGA 1984). Allozyme variation

among natural populations in relation to environmental gradients in Hokkaido has been detected in a recent study (NAGASAKA *et al.* 1997). Moreover, a recent phylogenetic study of *Abies* species, in which restriction fragment length polymorphisms (RFLPs) of mtDNA were detected by *cox*I and *cox*III gene probes, showed within-population variation of two eastern populations of Saghalin fir (TSUMURA & SUYAMA 1998). No variation of mtDNA within populations was detected in one northern and two western populations sampled. Although that study showed diversity within and among five natural populations of Saghalin fir, more information concerning diversity among plus trees, and differentiation of populations from which plus trees were collected, is needed to design conservation programs. This paper reports the diversity of organelle DNAs by use of RFLPs, and confirmation of the mode of inheritance for the two organelle genomes.

MATERIALS AND METHODS

Plant materials

Figure 1 shows the location of the populations studied. In the first sample collection, flashing buds from 210 trees representing 19 natural populations were sampled at the gene conservation plantation of plus tree in Hokkaido Regional Breeding Office, west of Ebetsu City, Hokkaido, North Japan (50 m asl.; 43°04'N, 141°31'E). To supplement these samples, a second collection was made from 54 individuals representing three natural populations (in Iwanai, Kucchan, and Gamushi). The results of RFLP analysis from the

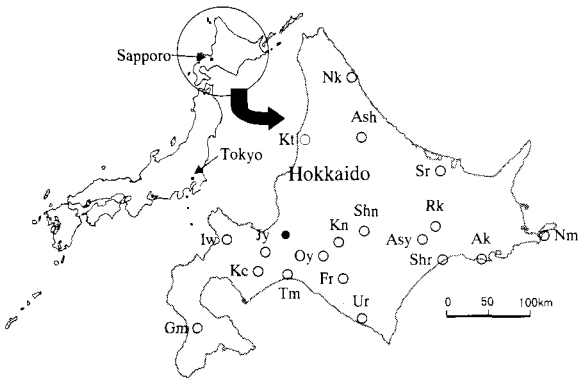

Figure 1. Sampled locations (circles) of Saghalin fir. White circles represent populations, in which plus trees were collected. Black circle represents gene conservation plantation in Hokkaido Breeding Office. Key to location abbreviations: Nk, Nakatonbetsu; Kt, Kotanbetsu; Ash, Asahi; Sr, Saroma; Nm, Nemuro; Ak, Akkeshi; Asy, Asyoro; Rk, Rikubetsu; Shr, Shiranuka; Shn, Shintoku; Oy, oyubari; Kn, Kanayama; Fr, Furenai; Ur, Urakawa, Tm, Tomakomai; Jy Jyozankei; Iw, Iwanai; Kc, Kucchan; Gm, Gamushi.

supplemental collections were combined with the data from the plus trees. A third sample collection was made at an operational progeny test plantation consisting of intra-specific crosses produced by controlled pollination. From each reciprocal tree family for which parents showed polymorphisms in *Bam*HI / *cox*II or *Eco*RI / *atp*A enzyme-probe analyses, flashing buds of fourteen individuals were collected. These samples were used in the study of inheritance of organelle DNA.

DNA analysis

For total DNA extraction, 1 g of each sampled bud was used following a modified CTAB procedure (MURRAY & TOMPSON 1980). The bud was ground in liquid nitrogen using a mortar and pestle. Total DNA was extracted from the powdered bud by a one hour digestion at 65 °C in 4 ml of CTAB buffer, 100 mM Tris-HCl (pH.8.0), 2% CTAB, 2% PVP, 0.1% mercaptoethanol, 1.4 M NaCl, and 20 mM EDTA. The digestion was followed by two chloroform-isoamyl alcohol (CIA, 24:1) extractions. The crude DNAs were precipitated at room temperature by adding 3 M sodium acetate (pH.5.2) to a final concentration of 0.3 M, and isopropanol (1 : 1 with the CIA extract). Pellets were recovered by centrifugation at 4 °C for 20 min. at 16,000 g, and were resuspended in 2 ml of TE. The solution was incubated with RNase ($1 mg \cdot ml^{-1}$) at 37 °C for 1 hour. Two buffered phenol treatments and one CIA extraction followed the RNase treatment. Purified DNA was reprecipitated, followed by centrifugation as above, dried *in vacuo*, and resuspended in 1 ml of TE.

Five μg of total DNA were digested with four restriction endonucleases, *Bam*HI, *Eco*RI, *Dra*I, and *Hin*dIII following the manufacturer's recommendations. Digested DNAs were electrophoretically separated on 0.8% agarose gels. DNA samples were transferred to nylon membranes and bound by baking. Blotted restriction fragments were visualized by enhanced chemiluminescence (ECL), following southern hybridization (SOUTHERN 1975). An ECL Gene Detection System (Amersham Life Science) was used for probe labelling and signal detection, following the protocol of the manufacturer.

The cpDNA probe used in this work was amplified by polymerase chain reaction from total DNA of Gamushi 106, a Saghalin fir plus tree, using the following primers:
5'-AGACGGAAATACCCACATTA-3' and
5'-TCAAGTAGGAGATGGCATTG-3', designed from the *atp*A sequence of *Pinus thunbergii*.

The mtDNA probes were amplified from a pUC vector containing a cytochrome oxidase subunit I [*cox*I, 1.6 kbp *Eco*RI fragment (SENDA *et al.* 1991)], a

cytochrome oxidase subunit II [*cox*II, 0.4 kbp *Sal*I-*Hin*dIII fragment (SENDA *et al.* 1991)] and a NADH dehydrogenase (*nad*9, KUBO *et al.* 1993) sequence from sugar beet (*Beta vulgaris* L.), a ATPase α-subunit gene [*atp*A, 1.4 kbp *Eco*RI-*Hin*dIII fragment (SENDA *et al.* 1993)] and a ATPase subunit 9 gene [*atp*9, 1.3 kbp *Hin*dIII-*Bam*HI fragment (MORIKAMI & NAKAMURA 1987)] from pea (*Pisum sativum*) using the following primers:
5'-CGACGTTGTAAAACGACGGCCAGT-3' and
5'-GGAAACAGCTATGACCATGATTAC-3'. Then the amplified probe DNAs were purified by phenol treatment and CIA extraction. Purified probe DNAs were precipitated by adding polyethylene glycol solution [13% (v/w), 1.6 M NaCl] of equal volume to the CIA extract, followed by centrifugation as above, and dried *in vacuo*.

In this paper, the *cox*II probe from sugar beet mtDNA and the *atp*A probe from Saghalin fir cpDNA are frequently abbreviated to '*cox*II (mt)' and '*atp*A (cp)' respectively.

Population genetic Analysis

To assess the statistical significance of the difference in variant frequencies (observed in the *cox*II gene analysis) between 4 populations locate on the southwestern peninsula and other populations, Fisher's exact test of independence (SOKAL & ROHLF 1981) was conducted. Gene diversity statistics (NEI 1978) were calculated using the derived variant frequencies. Total gene diversity (H_T) consists of the genetic variation within (H_S) and among (H_e) populations. G_{ST}, the coefficient of gene differentiation among populations was calculated using the equations $G_{ST} = H_e / H_T$, $H_e = H_T - H_S$. These estimates were compared with those of PONS & PETIT (1995). Using the FSTAT program (GOUDET 1995), the fixation index θ (WRIGHT 1965) was also estimated, together with its standard deviation, by the method of WEIR (1990).

RESULTS

Inheritance of organelle DNAs

In order to select proper enzyme/probe combinations for use in more detailed analyses, the preliminary survey was carried out on randomly selected 30 individuals. Two enzyme-probe combinations [*Bam*HI / *cox*II (mt) and *Eco*RI / *atp*A (cp)] which gave clear polymorphisms were selected. When the other enzyme-probe combinations were used, the monomorphic patterns were detected frequently. Instead, since the signals were sensitive to the extent of the stringency for membrane washing after hybridization, it was difficult to obtain polymorphisms reproducibly. In order to identify appropriate lines to study organelle DNA inheritance, RFLPs of parental trees used in crosses were investigated with both of these enzyme-probe combinations. Iwanai 106 and Gamushi 106 differed from each other in fragment variant, and when 14 trees of each reciprocal cross between these two parental trees were analyzed by *Bam*HI / *cox*II (mt), all progeny showed restriction fragments identical to the maternal tree (Fig. 2a). When analyzed by *Eco*RI / *atp*A (cp), however, all progeny showed restriction fragments identical to the paternal tree (Fig. 2b).

Diversity within and between populations

Table 1 shows restriction fragment variant frequencies of organelle DNAs in natural populations. The detection of just one fragment by each probe-enzyme combination per sample suggests that probe-homologous sequences are present in single copies. Three variants were detected in the *Bam*HI / *cox*II (mt) analysis, and two in the *Eco*RI / *atp*A (cp) tests. The *Bam*HI / *cox*II combination detected, predominantly, a fragment of 11.7 kb in samples from northern and eastern populations. The most prevalent fragment detected in two southwestern populations was 9.1 kb long, and samples from Asyoro and Shintoku populations had a rare 7.7 kb variant, distinguishing them from all other populations. No within-population variation was observed in three northern populations (Nakatonbetsu, Kotanbetsu, and Asahi), three eastern populations (Nemuro, Akkeshi, and Rikubetsu), one central population (Kanayama), and three southern populations (Furenai, Urakawa, and Tomakomai). Fisher's exact test of independence shows that the difference in variant frequencies between 4 populations (Jyozankei, Iwanai, Kucchan, and Gamushi) which locate on the southwestern peninsula and other populations is statistically significant ($p < 0.02$). In the *Eco*RI / *atp*A (cp) analysis, two variants were detected. All population showed intrapopulation variation. Nei's gene diversity values (NEI 1978) for organelle DNAs are shown in Table 2. Polymorphism of *cox*II ($H_e = 0.232$) was greater than that of *atp*A ($H_e = 0.001$). Gene differentiation coefficients among populations (G_{ST}) is higher when using a maternally inherited marker, *cox*II. The estimates of PONS & PETIT (1995) were quite close to those of NEI.

DISCUSSION

Organelle DNA diversity and differenciation

In this investigation heterologous *cox*II probes derived

Table 1. Frequencies of restriction fragment phenotypes of organelle DNAs from 19 populations of Saghalin fir.

Populations	*N*	*Bam*HI/*cox*II (mt)			*Eco*RI/*atp*A (cp)	
		11.7 (kb)	9.1	7.7	6.6	4.9
Nakatonbetsu	5	1.00	0.00	0.00	0.40	0.60
Kotanbetsu	14	1.00	0.00	0.00	0.79	0.21
Asahi	11	1.00	0.00	0.00	0.45	0.55
Nemuro	7	1.00	0.00	0.00	0.57	0.43
Akkeshi	14	1.00	0.00	0.00	0.64	0.36
Rikubetsu	17	1.00	0.00	0.00	0.53	0.47
Saroma	20	0.85	0.15	0.00	0.65	0.35
Shiranuka	17	0.94	0.06	0.00	0.53	0.47
Asyoro	20	0.95	0.00	0.05	0.40	0.60
Shintoku	18	0.94	0.00	0.06	0.67	0.33
Kanayama	10	1.00	0.00	0.00	0.50	0.50
Furenai	9	1.00	0.00	0.00	0.78	0.22
Urakawa	6	1.00	0.00	0.00	0.33	0.67
Tomakomai	8	1.00	0.00	0.00	0.50	0.50
Oyubari	13	0.92	0.08	0.00	0.54	0.46
Jyozankei	6	0.83	0.17	0.00	0.50	0.50
Iwanai	15	0.93	0.07	0.00	0.53	0.47
Kucchan	20	0.15	0.85	0.00	0.55	0.45
Gamushi	34	0.21	0.79	0.00	0.59	0.41
Total	264	0.80	0.19	0.01	0.56	0.44

ment of the subdivision of the diversity at a single locus, sampling many populations rather than many individuals per population is necessary (PONS & PETIT 1995). Secondly, differences in location of the genes studied on the mitochondrial genome might be a significant factor. The rapid structural transformation and slow rate of base sequence evolution of the mitochondrial genome have already been discussed (SEDEROFF 1987; PALMER 1992). Thirdly, there may be significant differences inherent to the gene studied. Plant *cox*II genes have an interesting feature: sugar beet (Senda *et al.* 1991), petunia (PRUITT & HANSON 1989), carrot (TURANO *et al.* 1987), and most monocotolydenous species analyzed so far (including Maize, FOX & LEAVER 1981; wheat, BONEN *et al.* 1984; and rice, KAO *et al.* 1984) have introns in their *cox*II genes. In contrast, *Oenothera*, pea and soybean (studied by HIESEL & BRENNICKE 1983; MOON *et al.* 1985; and GRABAU *et al.* 1988, respectively) do not contain introns. The probe used in this study is located at one of the two exons that occur adjacent to the intron. Although it is not clear that Saghalin fir has an intron or not in the *cox*II gene, it is possible that the large insertion in the intron may cause at least some of the intraspecific variation in RFLP variants.

Both Saghalin fir and *A. veitchii* are included in the same taxonomic section (FARJON 1990; YAMAZAKI 1995). TSUMURA & SUYAMA (1998) also indicated that these species have a genetically close relationship, sharing the same major RFLP-based mtDNA haplotype, and *rbc*L sequence in their cpDNA. Moreover, *A. veitchii* had little intra-specific variation in mtDNA. In this report, we also detected variation of mtDNA in Saghalin fir by use of RFLP. The mtDNA variation and sequence results might reflect its distribution during the last glacial period, and the relationship between these species. There may have been refugia of this species in northeast Hokkaido during the last glacial age (OKADA 1983). In that case, the western populations might have lost their mtDNA variation during their migration westward after the last glacial period. Further investigation, covering the natural distribution of the species more completely, might elucidate more thoroughly the inter- and intraspecific phylogeography.

Maternal inheritance of mtDNA and paternal inheritance of cpDNA

Although transmission of mitochondrial DNA in angiosperms is predominantly maternal, ultrastructural studies by electron microscope have shown that different species of gymnosperms have differing modes of transmission. Fertilization mechanisms and male gamete characteristics consistent with paternal transmission of organelles, both chloroplasts and mitochondria, are found in Cupressaceae and Taxodiaceae, but

Table 2. Population genetic parameters of RFLP markers with contrasting modes of inheritance.

Markers	H_S [a]	H_T	G_{ST}	θ (s.d.)[b]
*cox*II (mitochondria)	0.092 (0.096)	0.324 (0.331)	0.715 (0.710)	0.609*** (0.016)
*atp*A (chloroplast)	0.491 (0.517)	0.493 (0.493)	0.003 (–0.049)	–0.028*** (0.017)

a) PONS & PETIT (1995) estimates were shown in parentheses for H_S, H_T and G_{ST}.

b) θ is Wright's fixation index (WRIGHT 1965; WEIR 1990). Test of significance of θ by permuting variants within the total population with 1000 permutations *** $p < 0.001$.

maternal mitochondrial inheritance and paternal chloroplast inheritance in progeny have been demonstrated in Pinaceae, Cephalotaxaceae, Podocarpaceae, and Taxaceae (CHESNOY & THOMAS 1971). RFLP analysis has supported the results of these ultrastructural studies (NEALE *et al.* 1986; SZMIDT *et al.* 1987; NEALE & SEDEROFF 1989; STINE *et al.* 1989; NEALE *et al.* 1991; SUTTON *et al.* 1991;WAGNER *et al.* 1991; DEVERNO *et al.* 1993; ZIEGENHAGEN *et al.* 1995; KONDO *et al.* 1998). In the study presented here, the RFLP technique was used to monitor inheritance of organelle DNAs in reciprocal crosses of Saghalin fir. The maternal inheritance of the *cox*II sequence (Fig. 2a) suggests that mtDNA is maternally inherited in Saghalin fir, as well as in other Pinaceae species. In contrast to the maternal inheritance of mtDNA, the mode of inheritance of chloroplast DNA seems to be paternal (Fig. 2b). ENNOS (1994) defined the method in which estimate of relative rate of pollen and migration among population was caluculated using F_{ST}. The derived value caluculated from frequencies of variant 11.7 kb mitochondria variant and 6.6 kb chloroplast variant was 11.96. This value was supports that pollen migration rate are greater than seed migration rate in Saghalin fir. Consequently, the different rate of gene flow through seed and pollen dispersal reflected in the differentiation of each genome (Table 2). Average heterozygosity (F_{IT} = 0.105), corresponding to genetic diversity, has been estimated by isozyme analysis of this fir (NAGASAKA *et al.* 1997). The difference in genetic diversities between mtDNA and genomic DNA might be due to the frequent recombination and rearrangements characteristic of the mitochondrial genome (SEDEROFF 1987; PALMER 1992) and the higher rate of migration of nuclear genome.

In this inheritance study, however, progenies of two crosses were analyzed. The number of offsprings examined of each cross was 14. Because only a limited number of crossed and offsprings were analyzed, paternal leakage of mtDNA and maternal leakage of cpDNA can not be excluded. Paternal mtDNA leakage in *Pinus* have reported (WAGNER *et al.* 1991). A simple binomial model of organelle inheritance to determine the power of the experiment to distinguish between the hypothesis of strict maternal inheritance and the hypothesis involving the presence of both organelle types within a progeny has been presented (MILLIGAN 1992). Even if no progeny containing paternally derived organelles are found in 14 offsprings as this study, it is impossible to assure that the degree of paternal transmission is not more than 28% ($P < 0.01$). The possibility of biparental transmission cause recombination among different organelle genotypes, and may affect the patterns of diversity observed in organelle DNAs (CHAPMAN *et al.* 1982; BIRKY *et al.* 1989; BIRKY 1991). Therefore, inferences concerning the genetic parameters presented in this paper must be approached with care.

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REFERENCES

BIRKY, C. W. 1991: Evolution and population genetics of organelle genes: mechanisms and models. *In*: Evolution at the molecular level. (eds. R. K. Selander, A. G. Clark & T. S. Whittam). pp. 112–134. Sinauer, Sunderland, MA.

BIRKY, C. W., FUERST, P. & MARUYAMA, T. 1989: Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics* **121**: 613–627.

BONEN, L., BOER, P. H. & GRAY, M. W. 1984: The wheat cytochrome oxidase subunit II gene has an intron insert and three radical amino acid changes relative to maize. *EMBO J.* **3**:2531–2536.

CHAPMAN, R. W., STEPHENS, J. C., LANSMAN, R. A. & AVISE, J. C. 1982: Models of mitochondrial DNA transmission genetics and evolution in higher eucaryotes. *Genet. Res. Camb.* **40**: 41–57.

CHESNOY, L. & THOMAS, M. J. 1971: Electron microscopy studies on gametogenesis and fertilization in Gymnosperms. *Phytomorphology* **21**: 50–63.

DEVERNO, L. L., CHAREST, P. J. & BONEN, L. 1993: Inheritance of mitochondrial DNA in the conifer *Larix*. *Theor. Appl. Genet.* **86**: 383–388.

DONG, J. & WAGNER, D. B. 1993: Taxonomic and population differentiation of mitochondrial diversity in *Pinus banksiana* and *Pinus contorta*. *Theor. Appl. Genet.* **86**: 573–578.

EIGA, S. 1984: [Ecogenetical study on the freezing resistance of Saghalin fir (*Abies sachalinensis* Mast.) in Hokkaido]. *Bull. For. Tree Breed. Inst.* **2**: 61–107 [In Japanese].

ENNOS, R. A. 1994: Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* **72**: 250–259.

FARJON, A. 1990: Pinaceae. *In*: Drawing and descriptions of the genera. pp. 330. Koelzt, Königstein, Germany.

FOX, T. D. & LEAVER, C. J. 1981: The zea mays mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. *Cell* **26**: 315–323.

GOUDET, J. 1995: FSTAT. Version 1.2. A computer program to calculate F- statistics. *J. Hered.* **86**: 485–486.

GRABAU, E., HAVLIK, M. & GESTELAND, R. 1988: Chimeric organization of two genes for the soybean mitochondrial ATPase subunit 6. *Curr. Genet.* **13**: 83–89.

HATAKEYAMA, S. 1981: [Genetical and breeding studies on the regional differences of interprovenance variation in *Abies sachalinensis* Mast.]. *Bull. Hokkaido For. Exp. Station* **19**: 1–91. [In Japanese].

HIESEL, R. & BRENNICKE, A. 1983. Cytochrome oxidase subunit II gene in mitochondria of *Oenothera* has no intron. *EMBO J.* **2**: 2173–2178.

JAPAN FOREST TECHNICAL ASSOCIATION 1966: Typical Forests in Japan, Chikyu Shuppan, Tokyo, 193 pp.

KAO, T.-H., MOON, E. & WU, R. 1984. Cytochrome oxidase subunit II gene of rice has an insertion sequence within the intron. *Nucleic Acids Res.* **12**: 7305–7315.

KONDO, T., TSUMURA, Y., KAWAHARA, T. & OKAMURA, M. 1998. Paternal inheritance of chloroplast and mitochondrial DNA in interspecific hybrids of *Chamaecyparis* spp. *Breeding Science* **48**: 177–179.

KUBO, T., MIKAMI, T. & KINOSHITA, T. 1993. The sugar beet mitochondrial genome contains an ORF sharing sequence homology with the gene for the 30 kDa subunit of bovine mitochondrial complex I. *Mol. Gen. Genet.* **241**: 479–482.

MILLIGAN, B. G. 1992. Is organelle DNA strictly maternally inherited? Power analysis of a binomial distribution. *Amer. J. Bot.* **79**: 1325–1328.

MOON, E., KAO, T.-H. & WU, R. 1985. Pea cytochrome oxidase subunit II gene has no intron and generates two mRNA transcripts with different 5'-termini. *Nucleic Acids Res.* **13**: 3195–3212.

MORIKAMI, A. & NAKAMURA, K. 1987. The pea mitochondrial ATPase subunit 9 gene is located upstream of the ATPase α-subunit gene. *Nucleic Acids Res.* **15**: 4692.

MURRAY, M. G. & TOMPSON, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **8**: 4321–4325.

NAGASAKA, K., WANG, Z. M. & TANAKA, K. 1997. Genetic variation among natural *Abies sachalinensis* populations in relation to environmental gradients in Hokkaido, Japan. *For. Genet.* **4**: 43–50.

NEALE, D. B., WHEELER, N. C. & ALLARD, R. W. 1986: Paternal inheritance of chloroplast DNA in Douglas-fir. *Can. J. For. Res.* **16**: 1152–1154.

NEALE, D. B. & SEDEROFF, R. R. 1989: Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. *Theor. Appl. Genet.* **77**: 212–216.

NEALE, D. B., MARSHALL, K. A. & HARRY, D. E. 1991: Inheritance of chloroplast and mitochondrial DNA in incense-cedar (*Calocedrus decurrens*). *Can. J. For. Res.* **21**: 717–720.

NEI, M. 1978: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.

OKADA, S. 1983: [On the variation in Sakhalin fir (*Abies sachalinensis* Mast.) from different areas of Hokkaido]. *Bull. Forest Tree Breed. Inst.* **1**: 15–92. [In Japanese].

OKADA, S., MUKAIDE, H. & SAKAI, A. 1973: Genetic variation in Saghalin fir from different areas of Hokkaido. *Silvae Genetica* **22**: 24–29.

PALMER, J. D. 1992: Mitochondrial DNA in plant systematics: application and limitations. *In*: Molecular systematics of plants. (eds. P. S. Soltis, D. E. Soltis & J. J.Doyle). pp. 36–49. Chapman and Hall, New York.

PONS, O. & PETIT, R. J. 1995: Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. *Theor. Appl. Genet.* **90**: 462–470.

PRUITT, K. D. & HANSON, M. R. 1989: Cytochrome oxidase subunit II sequences in *Petunia* mitochondria: two intron-containing genes and an intron-less pseudogene associated with cytoplasmic male sterility. *Curr. Genet.* **16**: 281–291.

SEDEROFF, R. R. 1987: Molecular mechanisms of mitochondrial-genome evolution in higher plant. *Am. Nat.* **130**: s30–s45.

SENDA, M., HARADA, T., MIKAMI, T., SUGIURA, M. & KINOSHITA, T. 1991: Genomic organization and sequence analysis of the cytochrome oxidase subunit II gene from normal and male- sterile mitochondria in sugar beet. *Curr. Genet.* **19**: 175–181.

SENDA, M., MIKAMI, T. & KINOSHITA, T. 1993: The sugar-beet mitochondrial gene for the ATPase alpha-subunit: sequence, transcription and rearrangements in cytoplasmic male-sterile plants. *Curr. Genet.* **24**:164–170.

SOKAL, R. R. & ROHLF, F. J. 1995: Biometry, W.H. Freeman And Company, New York, 887 pp.

SOUTHERN, E. M. 1975: Detection of specific sequences among DNA fragments separated by electrophoresis. *J. Mol. Biol.* **98**: 503–517.

STINE, M., SEARS, B. B. & KEATHLEY, D. E. 1989: Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. *Theor. Appl. Genet.* **78**: 768–774.

STRAUSS, S. H., HONG, Y.-P. & HIPKINS, V. D. 1993: High levels of population differentiation for mitochondrial DNA haplotypes in *Pinus radiata*, *muricata*, and *attenuata*. *Theor. Appl. Genet.* **86**: 605–611.

SUTTON, B. C. S., FLANAGAN, D. J., GAWLEY, J. R., NEWTON, C. H., LESTER, D. T. & EL-KASSABY, Y. A. 1991: Inheritance of chloroplast and mitochondrial DNA in *Picea* and composition of hybrids from introgression zones. *Theor. Appl. Genet.* **82**: 242–248.

SZMIDT, A. E., ALDÉN, T. & HÄLLGREN, J.-E. 1987: Paternal

inheritance of chloroplast DNA in *Larix*. *Plant Mol. Biol.* **9**: 59–64.

SZMIDT, A. E., EL-KASSABY, Y. A, SIGURGEIRSSON, A., ALDÉN, T., LINDGREN, D. & HÄLLGREN, J.-E. 1988: Classifying seedlots of *Picea sitchensis* and *P. glauca* in zones of introgression using restriction analysis of chloroplast DNA. *Theor. Appl. Genet.* **76**: 841–845.

TSUMURA, Y. & SUYAMA, Y. 1998: Differentiation of mitochondrial DNA polymorphisms in populations of five Japanese *Abies* species. *Evolution* **52**: 1031–1042.

TURANO, F. J., DEBONTE, L. R., WILSON, K. G. & MATTHEWS, B. F. 1987: Cytochrome oxidase subunit II gene from carrot contains an intron. *Plant Physiol.* **84**: 1074 –1079.

WAGNER, D. B., FURNIER, G. R., SAGHAI-MAROOF, M. A. WILLIAMS, S. M., DANCIK, B. P. & ALLARD, R. W. 1987: Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proc. Natl. Acad. Sci. USA* **84**: 2097–2100.

WAGNER, D. B., DONG, J. CARLSON, M. R. & YANCHUK, A. D. 1991: Paternal leakage of mitochondrial DNA in *Pinus*. *Theor. Appl. Genet.* **82**: 510–514.

WEIR, B. S. 1990: Genetic data analysis: methods for discrete population genetics data, Sinauer, Sunderland, MA.

WRIGHT, S. 1965: The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**: 395–420.

YAMAZAKI, T. 1995: Pinaceae. *In*: Flora of Japan. Volume I. Pteridophyta and Gymnospermae. (eds. K. Iwatsuki, T. Yamazaki, D. E. Boufford, & H. Ohba). pp. 266–277. Kodansha, Tokyo.

ZIEGENHAGEN, B., KORMUŤÁK, A., SCHAUERTE, M. & SCHOLZ, F. 1995: Restriction site polymorphism in chloroplast DNA of silver fir (*Abies alba* Mill.). *For. Genet.* **2**: 99–107.