# THE USE OF MOLECULAR MARKERS TO DETECT HYBRIDIZATION IN INTROGRESSION ZONES

Y.A. El-Kassaby [1,2/] and J.E. Carlson [2,3/]

Abstract.--Operational spruce [Sitka *(Picea sitchensis),* white *(P.glauca),* and Engelmann *(P. engelmannii)]* seedlots collected from suspected zones of introgression often contain pure, mixed or hybrid seed of these three species. The contrasting seedling growing cultural requirements between Sitka and interior (white and Engelmann) spruce pose a seedling production problem and seedling quality is substantially affected. On the other hand, hybrids or mixes between interior spruce do not cause any operational problems due to their similar cultural requirements. The successful use of molecular markers [chloroplast (cpDNA) and mitochondrial DNA (mtDNA)] is illustrated and a comparison between molecular classification and operationally grown seedlots is presented. The production of species-specific unique mtDNA and cpDNA probes are used to determine their maternal and paternal inheritance, respectively. Finally, a protocol for molecular karyotyping is presented as an aid to determine the degree of hybridity at the nuclear genome level in parent trees of the interior spruce breeding program to separate interspecific superiority from that of intraspecific superiority.

Keywords: *Picea sitchensis, P. glauca, P. engelmannii,* cpDNA and mtDNA RFLPs, paternal and maternal inheritance, introgression.

## INTRODUCTION

A total of 115 million spruce seedlings (Sitka *[Picea sitchensis* (Bong.) Carr.], white *[P. glauca* (Moench) Voss], Engelmann *[P. engelmannii* (Perry)], and their hybrids) are being produced annually in British Columbia nurseries for reforestation programs (Table 1). Engelmann and white spruce and their hybrid (spruce hybrids; Sxi),), collectively called "interior spruce" (Kiss 1976), alone reached an average planting of 105 million seedlings over the past four years (Table 1).

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Table 1. Number of spruce seedlings requested by sowing year (1988-1991) (in thousands).1/

| Species | 1988 | 1989 | 1990 | 1991 | Average |
|---|---|---|---|---|---|
| White spruce (Sw) | 30,510 | 35,705 | 33,199 | 18,766 | 29,545 |
| Engelmann spruce (Se) | 39,218 | 39,830 | 35,364 | 31,622 | 36,509 |
| Sitka spruce (Ss) | 2,727 | 2,364 | 1,912 | 1,657 | 2,165 |
| Sitka hybrids (Sxc)P | 3,587 | 5,811 | 3,629 | 3,166 | 4,048 |
| Spruce hybrids (Sxi)3/ | 39,544 | 55,967 | 37,496 | 33,418 | 41,606 |
| Total | 115,586 | 139,677 | 111,600 | 88,629 | 455,492 |
| Sitka hybrids (%) | 3.1 | 4.2 | 3.3 | 3.6 | 3.6 |

1/ Personal communication (Mr. M. Pelchat, British Columbia Ministry of Forests, Silviculture Branch, Victoria, B.C., May 1991).

2/ Sitka hybrids; hybrids between Sitka spruce and any of the interior spruces (Si) (white or Engelmann). Spruce hybrids; hybrids between white and Engelmann spruces.

The natural ranges of the Sitka, white, and Engelmann spruce overlap and several sympatric populations are present (Roche 1969; Krajina *et al.* 1982) (Fig. 1). The presence of these species in sympatric populations and the apparent lack of reproductive barriers to hybridization have led to the creation of several introgression zones (Little 1953; Daubenmire 1968; Roche 1969). Reforestation with spruce in the areas of Sitka by "interior spruce" hybridization is common, and operational natural stand seed collections provide the majority of seed required for seedling production.

Due to the typically sporadic nature of cone production in the wild, the cone bearing habit of the species (i.e., cones being produced on the upper crown), and the narrow biological window for cone collection (i.e., time between cone maturation and seed shed), operational cone collections are being carried out by helicopters. The use of aerial cone-rakes to strip the cone crop from crop trees and their deposit on collection sites represents the major cone collection method practiced. In introgression zones, cone collections and subsequent seed crops may represent one species, a mixture of two or more species, or hybrids with various degrees of introgression.

Nursery cultural regimes required for the production of acceptable planting stock of Sitka and "interior spruce" in container nurseries are well documented (Brix 1972; Arnott 1974, 1979). Attempts to grow the two species in a common environment results in unacceptable stock quality. "Interior spruce" seedlings grown under the Sitka spruce cultural regime (i.e., without extended photoperiod) will terminate their leader growth

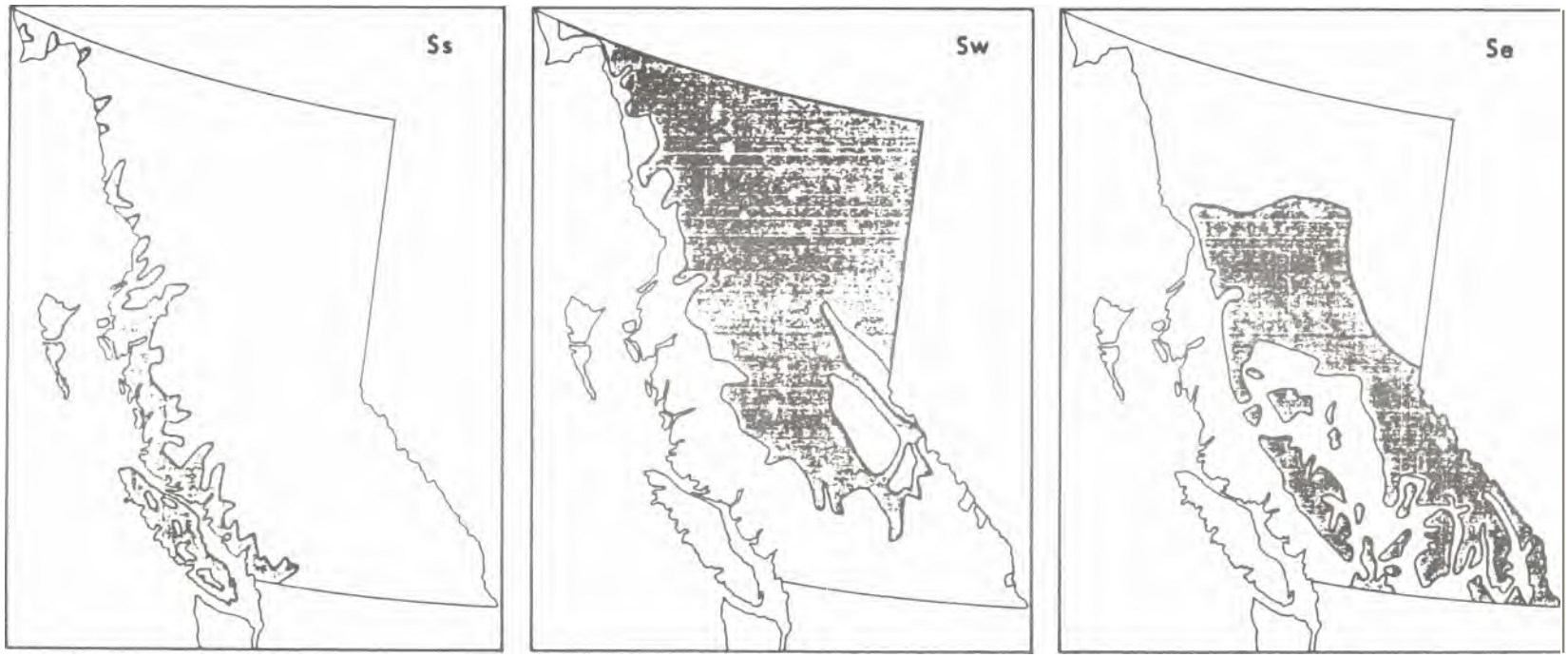



Figure 1. Map of British Columbia showing the natural range of Sitka spruce (Ss), white spruce (Sw), and Engelmann spruce (Se) (source; Krajina *et al.* 1982).

*(i.e.,* set bud) early, before reaching target height. Conversely, Sitka spruce grown under extended photoperiod produces unacceptably tall stock. Thus, the production of acceptable planting stock requires different light regimes for each species. Seed collected from introgression zones grown under any of the previously-mentioned light regimes produce stock of variable quality.

The distinction between "interior spruce" and their hybrid (Sxi) has no operational significance since both species and their hybrid can he successfully grown under the same cultural regime and are planted on similar sites. Sitka hybrids (Sxi) (i.e., hybrids between Sitka and either white or Engelmann spruce) or Sitka/interior spruce mixes pose an operational seedling production problem; although they only represent 4% of the total spruce produced annually, a total of 3-6 million seedlings are still needed for reforestation (Table 1). The development of a reliable and cost-effective means of screening these seedlots would allow hybrids or mixed seedlots to be grown under appropriate cultural conditions for the predominant species resulting in saving most of the loss experienced during the seedling production phase.

This paper describes the use of species-specific molecular cytoplasmic organelle markers as a species classification method and its evaluation on an operational level. In addition, a protocol for molecular karyotyping is presented as an aid to determine the degree of hybridity at the nuclear genome level.

## PROJECT SUMMARIES

### Chloroplast DNA (cpDNA)

This section describes the methods used to identify Sitka, white, and Engelmann spruce and their hybrids/mixes using cpDNA. The extraction protocol of cpDNA from conifers (White 1986) and the first demonstration of cpDNA paternal inheritance in Douglas-fir *[Pseudotsuga menziesii* (Mirb.) Franco] (Neale *et al.* 1986) were instrumental to the development and the conceptual framework for this method. El-Kassaby *et al.* (1988) and Szmidt *et al.* (1988) presented the first evidence of species-specific cpDNA restriction patterns that were successful in identifying Sitka, white and Engelmann spruce from each other. Three restriction enzymes *(Barn-HI, Bcl-I,* and *Xba-I)* were used (Fig. 2). *Barn-HI* differentiated Sitka from "interior spruce," *Bcl-I* differentiated white from Engelmann spruce, and *Xba-I* confirmed results obtained from *Barn-HI* and *Bcl-I* (Fig. 2).

The identification of seedlots (pure or mixed) was based on three assumptions: 1) the mode of inheritance of cpDNA is paternal in spruce; 2) pure seedlots (Sitka, white, and Engelmann) are identified with certainty (i.e., should show banding patterns identical to reference species), and 3) mixed/hybrid seedlots should show a mixture of

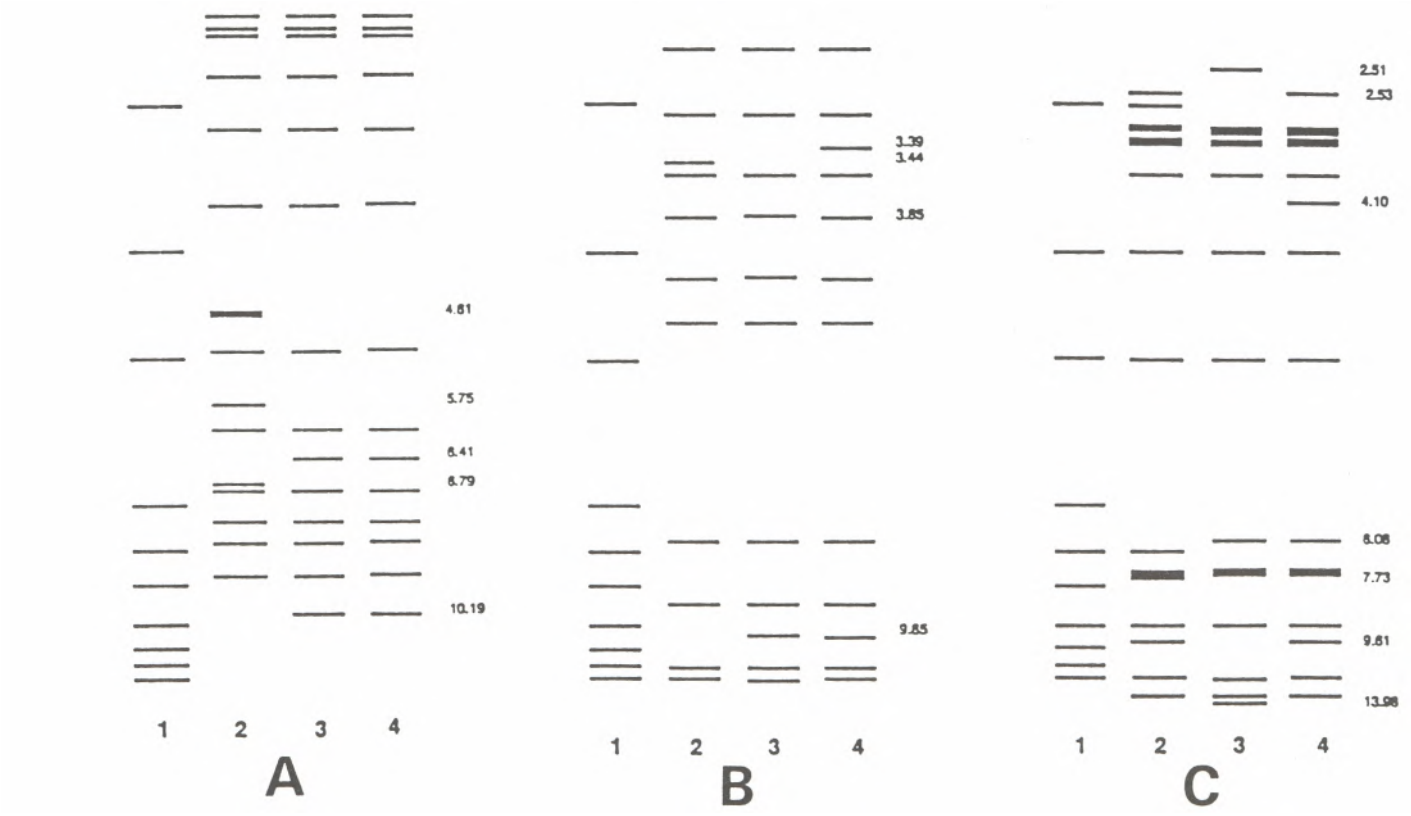



Figure 2. Schematic drawing illustrating cpDNA restriction patterns from three spruce species generated by: (A) *Bam-HI*, (B) *Bcl-I*, and (C) *Xba-I*. Lane 1: 1kb ladder (BRL); Lane 2: Sitka spruce; Land 3: white spruce; Lane 4: Engelmann spruce (source; El-Kassaby *et al.* 1988).

restriction fragments that are unique to each species concurrently, due to the presence of more than one species' pollen in the introgression zone. The concurrent appearance of two or more species' unique restriction fragments is expected when cpDNA is extracted from a bulk sample of needles obtained from 40-60 seedlings that originated from a seedlot collected from introgression zones.

A paternal mode of inheritance of cpDNA in conifers has been demonstrated for several species (Neale *et al.* 1986, 1989; Szmidt *et al.* 1987, 1988; Wagner *et al.* 1987, 1989; Neale and Swederoff 1989; Stine *et al.* 1989; Stine and Keathley 1991; White 1990; Sutton *et al.* 1991b), however, occasional biparental inheritance was detected in some cases (Szmidt *et al.* 1987; Govindaraju *et al.* 1988; White 1991; Sutton *et al.* 1991a). Although cpDNA is regarded as highly conservative (Palmer 1987), intra-specific variation has been reported for several species (Wagner *et al* 1987; Govindaraju *et al.* 1989; White 1990; Ali *et al.* 1991). However, the chance of observing all unique fragments collectively as intrapopulational variation in one sample was considered to be highly unlikely (El-Kassaby *et al.* 1988; Szmidt *et al.* 1988).

This approach was tested by Szmidt *et al.* (1988) on five seedlots and an operational scale screening was carried out by El-Kassaby *et al* (1988) on 20 seedlots. Although this method was effective in correctly classifying unknown seedlots (Table 2), it required the tedious isolation of purified cpDNA. A simplified approach, based on a rapid Southern plot hybridization protocol with a species-specific DNA probe that yielded quantitative determination of one species in a mixed seedlot was developed by Sutton *et al.* (1991a).

Detailed descriptions of identification and cloning of chloroplast probes for distinguishing Sitka and interior spruce are documented in Sutton *et al.* (1991a). Briefly, *Barn-HI* digested cpDNA from mature individuals revealed a 10.5 kb (kilobases) fragment unique to white spruce and fragments of 4.3 and 5.5 kb unique to Sitka spruce. Probes were selected based on their ability to distinguish each species, as well as the lack of hybridization with the nuclear fractions. Two probes (pSS4 and pSS6) were selected and results showed that either one could be used. The smaller probe (pSS4) was used in the routine screening.

cpDNA Seedlot Classification

Needle samples (0.5 g) from 200, two-week-old germinants were pooled and total DNA was isolated for 13 operational seedlots (Fig. 3a). When mixed/hybrid seedlots were identified, the hybridizing bands were scanned using a densitometer with area integration to determine the percent contribution of the two species (see Sutton *et al.* 1991a for details). Density is compared to a standard curve that was generated from a series of mixtures of white and Sitka spruce total DNA standards (Fig. 4). The detection limit of the procedure allows less than 5% of either Sitka or interior spruce to be

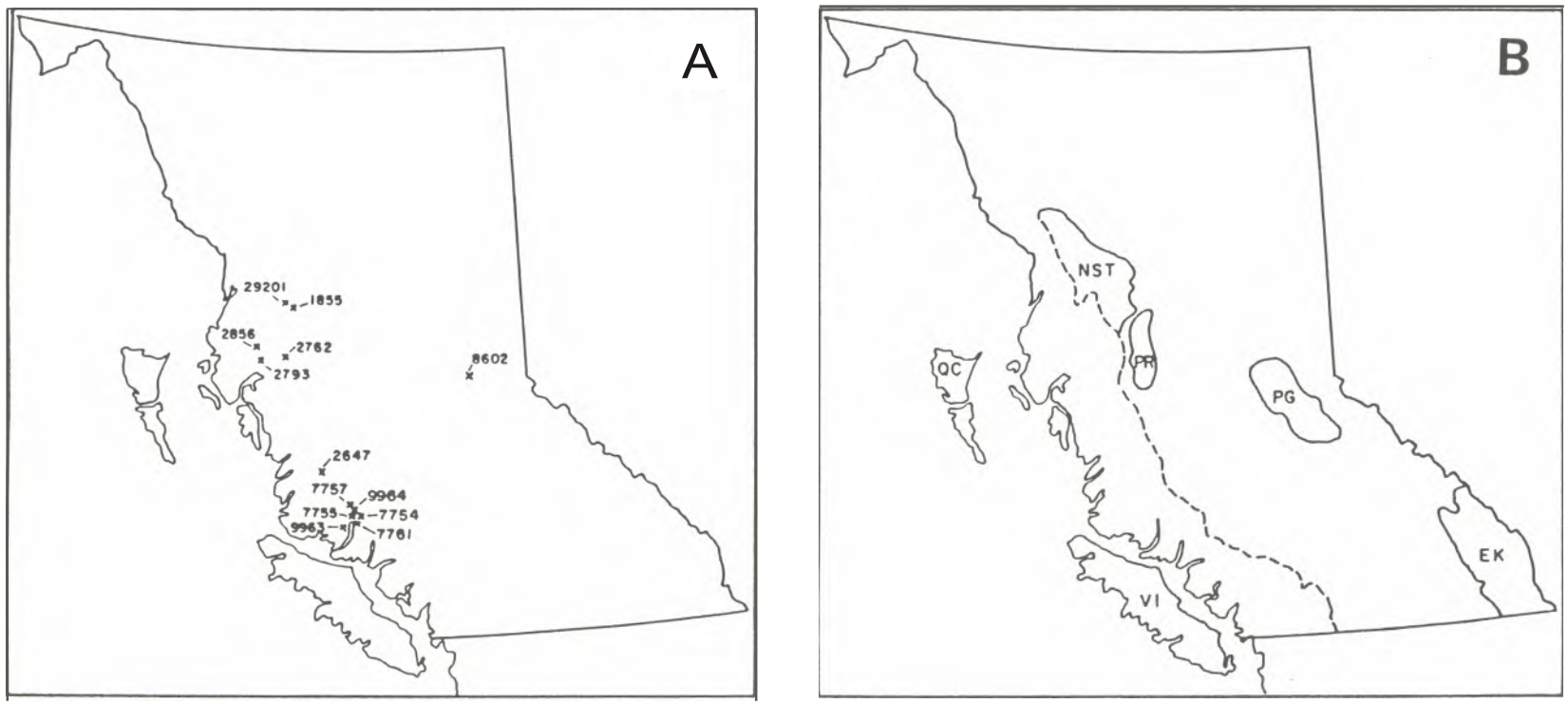



Figure 3. (A) Map of British Columbia showing the locations of the 13 spruce seedlots classified. (B) Map of British Columbia showing the locations of trees used in the inheritance study and tested trees (EK, East Kootenay; PG, Prince George; VI, Vancouver Island; QC, Queen Charlotte Islands; PR, Prince Rupert, Smithers selection unit; NST, Nass Skeena Transition) (source; El-Kassaby ***et al.*** 1988, Sutton ***et al.*** 1991a and b).

detected [Engelmann spruce quantification yielded similar results to white spruce (Sutton *et al* 1991a)]. In addition, the classification of these 13 seedlots based on diagnostic probes on total DNA is compared to the restriction fragment banding pattern of purified cpDNA along with their nursery performance (Table 2). Generally, both methods gave similar results, however, the use of diagnostic probes eliminated the tedious work required for isolating pure cpDNA samples and allowed the quantitative determination of each species in the mix.

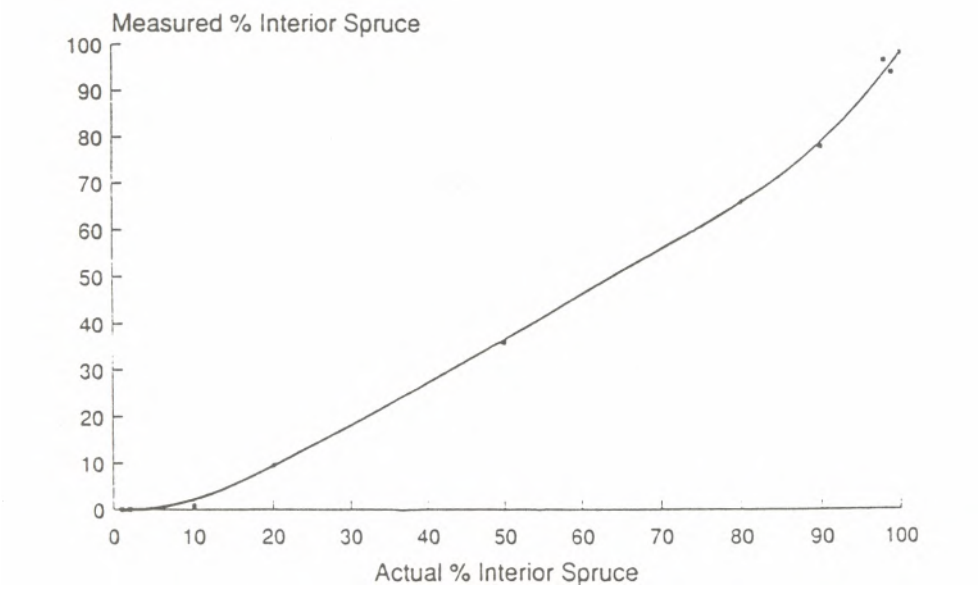



Figure 4. Standard curve for determination of cpDNA content of interior and Sitka spruce (source; Sutton *et al*, 1991a).

Mitochondria' DNA (mtDNA)

The previous section demonstrated the utility of cpDNA genome as a pollen donor marker in interspecific mating events. If the mtDNA genome is maternally inherited in spruce, then a combination of probes that are capable of distinguishing both the maternal (Le., mtDNA) and the paternal (i.e., cpDNA) parents of an individual would be of great use as a first step in determining the pedigree of that individual.

The mode of inheritance of mtDNA in conifers has been studied for several species representing three families. Loblolly pine *(Pinus taeda* L.) (Neale and Sederoff 1989), spruce (Sitka, white and Engelmann) (Sutton *et aL* 1991b), and Douglas-fir (D. B. Neale, personal communication, May 1991) of the Pinaceae exhibited maternal inheritance while coastal redwood *(Sequoia sempervirens* D. Don Endl.) of the Taxodiaceae (Neale *et aL* 1989) and incense-cedar *[Calocedrus decurrens* (Torr.) Florin] of the Cupressaceae (Neale *et aL* 1991) showed paternal inheritance.

Table 2. Comparison between methods used to classify spruce seedlots based on cpDNA analyses and nursery trial.

| Seedlot Registration | | Classification | | | |
|---|---|---|---|---|---|
| | | El-Kassaby1/ | Sutton2/ | | Woods 3/ |
| 1855 | | Sw | Sxc | (40) | Sst |
| 2647 | Ss | Ss | Sxc | (<5) | Ss |
| 2762 | Sxc | Ss/Sw/Se | Sxc | (50) | Sst |
| 2793 | | Ss | Ss | (--) | Ss |
| 2856 | Sxc | Ss | Ss | (--) | Ss |
| 7754 | Sxc, | Ss | Ss | (--) | Ss |
| 7755 | | Ss | Ss | (--) | Ss |
| 7757 | Sxc, | Ss | $Sx_c$ | (<5) | Ss |
| 7761 | | Ss | Ss | (--) | Ss |
| 8602 | Se | Sw/Se | S, | (100) | Si |
| 9963 | Se | Ss | Ss | (--) | Ss |
| 9964 | Se | Ss | Sxc | (<5) | Ss |
| 29201 | | Ss/Sw/Se | Sxc | (60) | Sst |

El-Kassaby *et al.* (1988); classification of spruce seedlots based on purified cpDNA.

Sutton *et al.* (1991a); classification of spruce seedlots based on hybridization to DNA probe. Numbers in parentheses represent the percentage of interior spruce as determined by densitometry analysis.

2/ Woods (1988); recommended growing regime based on nursery comparison of height, caliper, root and shoot dry weights, and days to bud set.

t Seedlots classified as mix/hybrid.

The methods of isolating mtDNA and the preparation of probes that are capable of distinguishing among the three spruce species (Sitka, white and Engelmann) are described in Sutton *et al.* (1991b). In their study, a polymorphism which distinguished white spruce from both Sitka and Engelmann spruce was found by using *Hpa-I* digestion and probing with pWSm1. In this case, a 15 kb fragment was found to be unique to white spruce while a 20kb fragment was unique to both Sitka and Engelmann spruce. Additionally, hybridization with pWSm2 probe in combination with *Sma-I* digestion provided unique polymorphisms that can distinguish all three species as follows: Engelmann spruce contained a 23 kb band, white spruce was characterized by a 8.1 kb band, and Sitka spruce individuals were of two types, 21 kb or 31 kb. The use of these probes in the identification of spruce individuals is presented below.

Paternal Inheritance of cpDNA and Maternal Inheritance of mtDNA in Spruce

Sutton *et al.* (1991b) conducted some reciprocal interspecific crosses demonstrating the mode of inheritance of both cpDNA and mtDNA using the previously described probes. In their study, all investigated progenies conformed to expectations, providing further support for previous observations on Pinaceae. Sutton *et al.* conducted crosses between individuals that were selected to represent a "typical" species. White spruce trees were obtained from low-elevation areas in the Prince George region, Engelmann spruce from high-elevation areas in the East Kootenay region, and Sitka spruce from low-elevation areas of Vancouver Island and the Queen Charlotte Islands (Fig. 3b). A total of 27 progenies were investigated for cpDNA while 32 were studied for mtDNA (Sutton *et al.* 1991b) (Table 3).

Table 3. Results of cpDNA and mtDNA inheritance in some reciprocal interspecific crosses (source; Sutton *et al.* 1991b).

| Cross (female x male) | Probe2/ | Organelle | Inheritance | Sample Size |
|---|---|---|---|---|
| Ss x Se | pSS4 | cp | paternal | 6 |
| | pWSm2 | mt | maternal | 6 |
| Se x Ss | pSS4 | cp | paternal | 7 |
| | pWSm2 | mt | maternal | 5 |
| Ss x Sw | pSS4 | cp | paternal | 7 |
| | pWSml | mt | maternal | 7 |
| Sw x Ss | pSS4 | cp | paternal | 7 |
| | pWSm1 | mt | maternal | 7 |
| Se x Sw | pWSm2 | mt | maternal | 7 |

/ See Table 1 for explanation.
2/ See Sutton *et al.* 1991a and b for description.

Identification of Parentage of Trees from Introgression Zones

Sutton *et al.* (1991b) used both the mitochondrial and chloroplast probes to determine the parentage of 14 individual trees selected from Prince Rupert region (PR) (Fig. 3b). This region is located east of the recognized Nass Skeena Transition (NST) introgression zone (Roche 1969). It is noteworthy to mention that this area is in the eastern watershed of the Coast Mountains where interior spruce is expected to he the dominant species. The maternal and parental lineage of these individuals are summarized in Table 4. Only one individual (#86) produced unique bands of interior spruce for both cpDNA and mtDNA (Table 4). Of the remaining 13 individuals, all but one (#14) produced a cpDNA banding pattern that is typical of interior spruce

(Table 4). This was due to the high frequency of interior spruce pollen present in this region's pollen pool. However, the mtDNA banding pattern of these 13 individuals was dominated by the Sitka spruce type (12 out of 13) and only one tree produced a pattern that is similar to Engelmann spruce (#14). These results demonstrate that the introgression zone is larger than expected and that Sitka spruce is being maintained mainly as a seed donor.

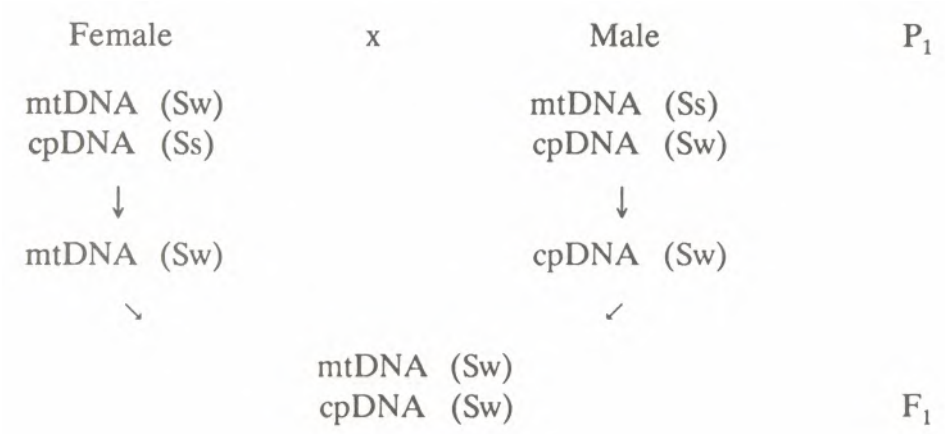
Table 4. Parentage identification for 14 individual trees selected from the Prince Rupert, B.C. region (source; Sutton *et al.* 1991b).

| Tested Tree Number | Unique Bands (in kb)1/ | | Species Designation 2/ | |
|---|---|---|---|---|
| | probe mtDNA pWSm2 | probe cpDNA pSS4 | mt | cp |
| 1 | 21 | 10.5 | Ss | Si |
| 2 | 21 | 10.5 | Ss | Si |
| 4 | 21 | 10.5 | Ss | Si |
| 5 | 21 | 10.5 | Ss | Si |
| 14 | 23 | 4.3 | Se | Ss |
| 19 | 21 | 10.5 | Ss | Si |
| 20 | 21 | 10.5 | Ss | Si |
| 33 | 21 | 10.5 | Ss | Si |
| 75 | 21 | 10.5 | Ss | Si |
| 78 | 21 | 10.5 | Ss | Si |
| 85 | 21 | 10.5 | Ss | Si |
| 86 | 23 | 10.5 | Se | Si |
| 109 | 21 | 10.5 | Ss | Si |
| 110 | 21 | 10.5 | Ss | Si |

1/ From Sutton *et al.* (1991a and b).
2/ See Table 1 for explanation.

Limitations of Parentage Identification Based on Cytoplasmic Genomes

While cpDNA and mtDNA analyses are valid first steps in evaluating seedlots or individuals, they are limited to identifying only the cytoplasmic genomes and immediate pollen/seed parents (El-Kassaby *et al.* 1988; Szmidt *et al.* 1988; Sigurgeirsson *et al.* 1990, 1991; Stine and Keathley 1990; Sutton *et al.* 1991a and b). CpDNA and mtDNA patterns will not reveal if the parents were hybrids or if they were pure species. Incorrect classification could occur if both parents were hybrids and true hybrid progeny will go undetected. This situation is illustrated in the following hypothetical cross:




In this example, the progeny is classified as a "pure" white spruce based on the cytoplasmic genomes, however, in reality it is a cross of two hybrids and at the nuclear level is hybrid in nature.

At present, the majority of spruce seed used in reforestation are from natural stand collections. This situation will eventually change when seed orchards reach production phase. These seed orchards will also undergo genetic upgrading (i.e., roguing) based on progeny test information. Some of the progeny test families could have been the product of interspecific crosses and might exhibit phenotypic superiority due to hybrid vigour and not their actual intraspecific variation. Therefore, the determination of the exact degree of introgression in the breeding population is of importance and the DNA content at the nuclear genome level is warranted.

Traditionally, following introgression, the detection of chromosomes or chromosome segments from one species into another or one breeding population into another has relied upon cytogenetics. When detailed karyotypes are available, individual chromosomes can be identified and the movement of large blocks of chromosomal material can be mapped using various cytological staining techniques. However, no satisfactory method for karyotyping members of the genus *Picea* are available. We have been able to use methods developed by Kiss (1973) to visualize all of the spruce chromosomes in root tip squashes. However, no cytological staining technique provides detailed or unambiguous chromosome identification in *Picea* spp.

However, molecular cytogenetics provides a powerful approach to the identification and detailing of chromosome structure. The field of molecular cytogenetics has developed around the technique of *in situ* hybridization. *In situ* hybridization is a physical genome mapping technique developed by Gall and Pardue (1969) in which labelled DNA fragments are hybridized to cytological preparations of chromosomes. Individual DNAs are mapped to specific chromosome regions, usually heterochromatic regions, to obtain a hybridization pattern that is species specific. We are in the process of developing such a molecular karyotype for the Engelmann, Sitka, and white spruce species. With this molecular karyotype information, we will be able to

determine the origin of each chromosome in natural spruce hybrids. We also anticipate being able to follow the introgression of much smaller chromosome segments than can be followed with cytological staining techniques.

Our physical mapping capability derives from the more recent development of the Fluorescence *In Situ* Hybridization (FISH) technique and the use of confocal microscopy. With FISH (Lichter *et al.* 1990) and confocal imaging (Albertson 1991), resolution can be obtained at the 10 Mbp range for metaphase chromosomes and the 50 to 1000 kbp range for interphase nuclei (Trask *et al.* 1991). With FISH, the DNA probe is labelled with biotin either directly or by DNA polymerase incorporation. The hybridization signal is detected either with an anti-biotin fluorochrome conjugated antibody or fluorochrome conjugated streptavidin, followed by signal amplification if necessary (Fig. 5). Confocal microscopy in particular has an advantage for *in situ* hybridization with conifer root tips which yield thick squashes making it often difficult to observe all chromosomes in a spread. With the BioRAD MRC-600 confocal microscope, individual optical sections are taken through the preparation in each focal plane. All of the chromosomes are then overlaid in one image. The BioRad MRC-600 confocal microscope image analysis software also includes cut and paste routines for aligning the chromosomes and measurement routines for quantifying banding patterns along each chromosome. With this capability, we can locate and identify the source of individual chromosome or chromosome segments in a suspected hybrid individual or seedlot.

## ACKNOWLEDGEMENTS

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# Fluorescence In Situ Hybridization

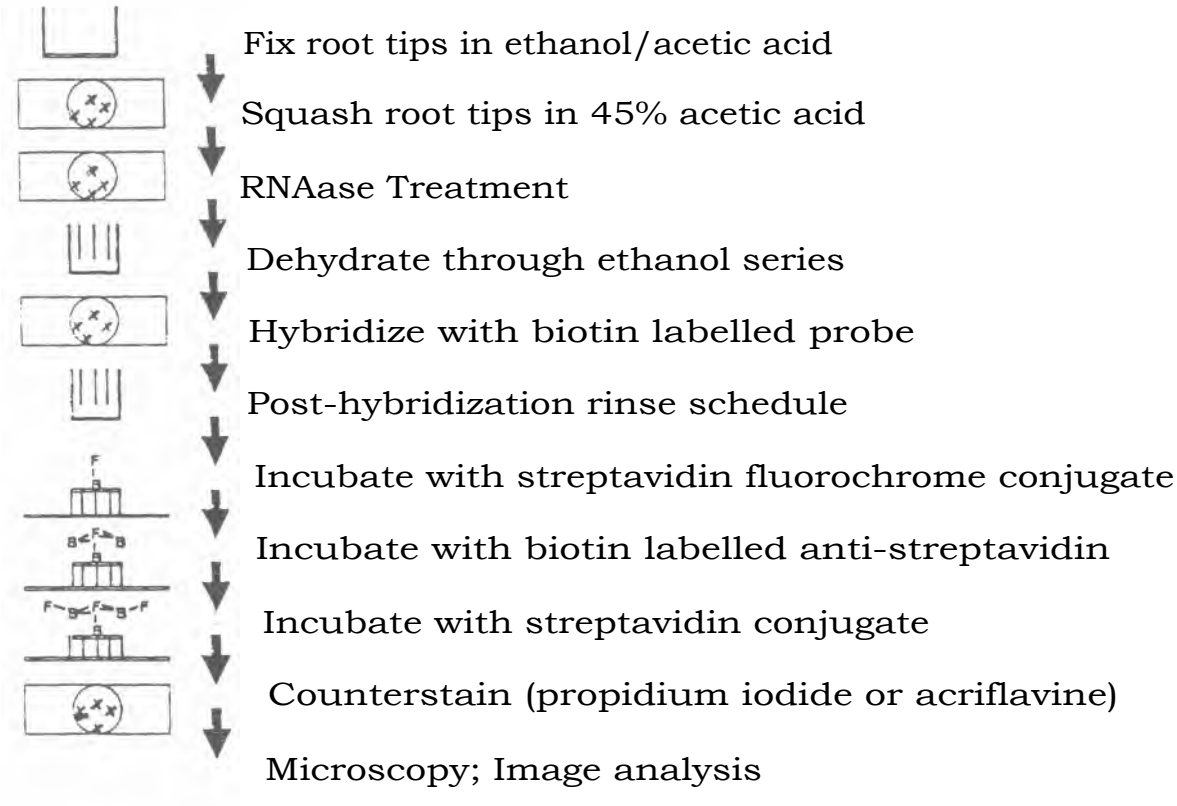


Figure 5. Protocol for fluorescence *in situ* hybridization (FISH) to conifer root tip chromosomes (source; Brown, G.R., and J.E. Carlson, unpublished).

## LITERATURE CITED


Albertson, D. 1991. Non-isotopic *in situ* hybridization. BioRad Laboratories. Application Note: 02.

Ali, I.F., **D.B.** Neale, and K.A. Marshall. 1991. Chloroplast DNA restriction fragment length polymorphism in *Sequoia sempervirens* D. Don Endl., *Pseudotsuga menziesii* (Mirb.) Franco, *Calocedrus decurrens* (Torr.), and *Pinus taeda* L. Theor. Appl. Genet. 81:83-89.

Arnott, J.T. 1974. Growth response of white and Engelmann spruce provenances to extended photoperiod using continuous and intermittent light. Can. J. For. Res. 14:69-75.

Arnott, J.T. 1979. Effect of light intensity during extended photoperiod on growth of amabilis fir, mountain hemlock, and white and engelmann spruce seedlings. Can. **J.** For. Res. 19:82-89.

Brix, H. 1972. Growth response of Sitka spruce and white spruce seedlings to temperature and light intensity. Can. For. Serv., Pac. For. Res. Cent., Info. Rep. BC-X-74.

Doubenmire, R. 1968. Some geographic variations in *Picea sitchensis* and their ecological interpretation. Can. J. Bot. 46:787-798.

El-Kassaby, Y.A., A. Sigurgeirsson, A.E. Szmidt. 1988. The use of restriction analysis of chloroplast DNA in classifying hybrid spruce seedlots. P. 67-88 *in* Molecular Genetics of Forest Trees, Proc. of the Frans Kempe Symposium, Umeå , Sweden, J.-E. Hällgren (ed.).

Gall, J.G. and M.L. Pardue. 1969. Formation and detection of RNA-DNA hybrid molecultes in cytological preparations. Proc. Natl. Acad. Sci. (USA) 63:378-382.

Govindaraju, D.R., **B.P.** Dancik, and D.B. Wagner. 1989. Novel chloroplast DNA polymorphism in a sympatric region of two pines. J. Evol. Biol. 2:49-59.

Kiss, G. 1973. Preliminary karyotype analyses in *Picea.* British Columbia Ministry of Forests Report E.P. 670.98.

Kiss, G. 1976. Plus-tree selection in British Columbia. P. 24-31 *in* Proc. 15th Can. Tree Imp. Assoc., Part 1, E.K. Morgenstern (ed.).

Krajina. V.J., K. Klinka, and J. Worrall. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. J&M Publishing, Vancouver, B.C.

Lichter, P., C.C. Tang, K. Call, G. Hermanson, G.A. Evans, D. Housman and D.C. Ward. 1990. High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. Science 247:64-69.

Little, E.L. 1953. A natural hybrid species in Alaska. J. For. 41:745-746.

Neale, D.B., N.C. Wheeler, and R.W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152-1154.

Neale, D.B. and R.R. Sederoff. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77:212-216.

Neale, D.B., K.A. Marshall, And R.R. Sederoff. 1989. Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don. Endl. Proc. Natl. Acad. Sci. (USA) 86:9347-9349.

Neale, D.B., K.A. Marshall, and D.E. Harry. 1991. Inheritance of chloroplast and mitochondrial DNA in incense-cedar *[Calocedrus decurrens* (Torr.) Florin]. Can. J. For. Res. (in press).

Palmer, J.D. 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am. Nat. 130:S6-S29.

Roche, L. 1969. Introgressive hybridization in the spruce species of British Columbia. P. 249-270 *in* Proc. 11th Comm. For. Tree Breeding in Canada, C.W. Yeatman (ed.).

Sigurgeirsson, A., A.E. Szmidt, and J.N. Alden. 1990. A molecular study of interspecific hybridization in the spruce complex of Alaska. P. 6.14-6.23 *in* Joint Meeting of Western For. Genet. Assoc. and IUFRO Work. Parties - S2-02-05, 06, 12 and 14, Olympia, Washington.

Sigurgeirsson, A., A.E. Szmidt, and B. Korpinska. 1991. Alaskan *Picea sitchensis* populations infiltrated with *Picea glauca* genes: A study using DNA markers. P. 197-207 *in* Biochemical markers in the population genetics of forest trees, Fineschi, S., M.E. Malvolti, F. Cannata, and H.H. Mattemer (eds). SPB Academic Publishing b$^{y}$, The Haugue, The Netherlands.

Stine, M., B.B. Sears, and D.E. Keathley. 1989. Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. Theor. Appl. Genet. 78:768-774.

Stine, M., and D.E. Keathly. 1990. Paternal inheritance of plastids in Engelmann spruce x blue spruce hybrids. J. Hered. 81:443-446.

Sutton, B.C.S., D.J. Flanagan, and Y.A. El-Kassaby. 1991a. A simple and rapid method for estimating representation of species in spruce seedlots using chloroplast DNA restriction fragment length polymorphism. Silvae Genet. (in press).

Sutton, B.C.S., D.J. Flanagan, J.R. Gawley, C.H. Newton, D.T. Lester, and Y.A. El-Kassaby. 1991b. Inheritance of chloroplast and mitochondrial DNA in *Picea* and composition of hybrids from introgression zones. Theor. Appl. Genet. (in press).

Szmidt, A.E., T. Alden, and J.-E. Hallgren. 1987. Paternal inheritance of chloroplast DNA in *Larix*. Plant Mol. Biol. 9:59-64.

Szmidt, A.E., Y.A. El-Kassaby, A. Sigurgeirsson, T. Alden, D. Lindgren, and J.-E. Hallgren. 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.

Trask, B., A. Fertitta, M. Christensen, B. Branndiff, L. Gordon, A. Copeland, H. Massa, K. Tynan and A. Carrano. 1991. Two-color fluorescence *in situ* hybridization mapping of chromosome 19. Cold Spring Harbor Laboratory Conference in Genome Mapping and Sequencing, Cold Spring Harbor, New York. (Abstracts). P. 209.

Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroof, S.M. Williams, B.P. Dancik, and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pine and their hybrids. Proc. Natl. Acad. Sci. (USA) 84:2097-2100.

Wagner, D.B., D.R. Govindaraju, C.W. Yeatman, and J.A. Pitel. 1989. Paternal chloroplast DNA inheritance in a diallel cross of jack pine *(Pinus banksiana* Lamb.) J. Hered. 80:483-485.

White, E.E. 1986. A method for extraction of chloroplast DNA from conifers. Plant. Mol. Biol. Rep. 4:98-101.

White, E.E. 1990. Chloroplast DNA in Pinus monticola. 2. Survey of within-species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. 74:251-255.

Woods, J.H. 1988. Sitka-interior hybrid spruce seedlot trials. Unpublished Report. B.C. For. Serv., Res. Branch.