Five Major Nuclear Ribosomal Repeats Represent a Large and Variable Fraction of the Genomic DNA of *Picea rubens* and *P. mariana*[1]

Michael S. Bobola, Dirk E. Smith,† and Anita S. Klein*,†*

*Department of Biochemistry and †Program in Genetics, University of New Hampshire

The nuclear ribosomal repeats for the 18S, 5.8S, and 26S RNAs of two closely related *Picea* (spruce) species were characterized by restriction mapping and Southern blot hybridization. Restriction polymorphisms were identified in the IGS and ITS sequences; however, no polymorphism was species specific. As many as five different rDNA repeat units were observed in individual genomes. The repeat size for these gymnosperms ranged from a minimum of 32 kbp to >40 kbp, two- to threefold larger than the typical angiosperm rDNA unit. Slot-blot hybridizations were used to determine the nuclear rDNA copy concentration. Among *P. rubens* individuals threefold variation was observed in the rDNA copy concentration, and among *P. mariana* individuals such variation was as much as sixfold. At a size >32 kbp and at a concentration averaging $1.2–1.3 \times 10^4$ copies/pg, the rDNA constitutes ~4% of the total genome. Regression analysis revealed a significant relationship between copy concentration of the rDNA repeat unit in *P. rubens* and geographic origins. Differences in the rDNA content in *Picea* could contribute to the variation, in overall genome size, that has been observed within conifer species.

Introduction

Red spruce (*Picea rubens* Sarg.) is predominantly a New England species whose range extends from high elevations in the mountains of North Carolina to coastal Nova Scotia (Fowells 1965). Red-spruce stands have been in decline in the northeastern American forests (Scott et al. 1984; Vogelmann et al. 1985). White spruce, *Picea glauca* (Moench) Voss, and black spruce, *P. mariana* (Mill) B.S.P., are sympatric species with red spruce, yet, while red spruce has been declining, declines in populations of white and black spruce have not been reported. Black spruce appears to be more closely related to red spruce; the two species are thought to cross-hybridize in disturbed habitats (Manley 1972).

A better understanding of the genetic makeup and relationship of red- and black-spruce populations may aid in understanding the decline of red spruce. Results of isozyme studies suggest that red spruce is genetically less variable than black and white spruce (Eckert 1989). This low genetic diversity may be due either to the restricted range of red spruce or to genetic bottlenecks. The latter may have resulted from glaciation events or, more recently, from overlogging during colonial settlement. The narrow genetic base of red spruce may, in part, explain its susceptibility to environ-

1. Key words: ribosomal genes, *Picea rubens, Picea mariana,* spruce. Abbreviations: rDNA = ribosomal DNA; ITS = internal transcribed spacer; IGS = intergenic spacer; RFLP = restriction-fragment-length polymorphism; PCR = polymerase chain reaction.

Address for correspondence and reprints: Michael S. Bobola, Department of Biochemistry, University of New Hampshire, Durham, New Hampshire 03824-3544.

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mental stresses. Its occasional hybridization with black spruce (Gordon 1976) may afford opportunity for enhancing genetic variation.

The rDNA repeat has been useful in intra- and interspecific phylogenetic studies among some groups within the angiosperms (Saghai-Maroof et al 1984; Doylea and Beachy 1985; Sytsma and Schaal 1985; Zimmer et al 1988; King and Schaal 1989; Springer et al. 1989). The rDNA repeat unit of flowering plants consists of 18S, 5.8S, and 26S coding regions with two types of internal transcribed spacer (ITS), i.e., ITS 1 and ITS 2, and an intergenic spacer (IGS). The overall size range of the rDNA unit is 6–14 kbp, and individual units are arranged in tandem arrays. Among angiosperms, the number of the rDNA genes is 10^2–10^4 copies per genome. The rDNA genes may be present in one or more arrays, and these arrays may be found on different chromosomes. Appels and Honeycutt (1986) and Jorgensen and Cluster (1988) provide a more complete description of the structure and variation in the rDNA repeat unit.

Restriction analysis has been useful in revealing variation in the rDNA repeat unit of angiosperm species. Both restriction-site polymorphisms and repeat-length variation have been observed in several species of soybean (*Glycine* max) and its relatives; however, no variation in restriction sites or length was observed within an individual (Doyle and Beachy 1985). A phylogenetic tree for the *Lisianthius skinneri* (Gentianaceae) species complex was constructed using restriction-site polymorphisms in the rDNA repeat (Sytsma and Schaal 1985). Again, no variation was observed in the rDNA repeat units within an individual. Length variation was absent in the rDNA repeat unit of *Rudbeckia missourienses* (Englm.); however, restriction-site variation occurred among rDNA repeat units within all individuals studied (King and Schaal 1989). Both length and site variation have been observed within individuals of the genera *Zea* and *Tripsacum* (Zimmer et al. 1988). Restriction-site variation in the rDNA repeat unit was used to differentiate between sugarcane, maize, and sorghum, while space-length variation was used to differentiate some sorghum varieties and races (Springer et al. 1989).

Little is known about the rDNA repeat unit in gymnosperms. Cullis et al. (1988) characterized the rDNA repeat unit in *Pinus radiata* D. Don and compared it with that in angiosperms. The rDNA repeat unit size of *Pinus* was more than twice the size of the typical angiosperm repeat unit, ~27 kbp in length. The distribution of arrays in the nucleus was also found to differ between angiosperms and *Pinus radiata. Pinus radiata* has 10 major rDNA arrays and a number of minor arrays. The arrays do not appear to be localized on any particular chromosome or region of the nucleus.

Among the Pinaceae, genome size has been shown to vary considerably within species (Mergen and Thielges 1967; Miksche 1967, 1968, 1971; Dhir and Miksche 1974). The rDNA gene number was found to increase with latitude in Douglas fir [*Pseudotsuga menziesii* (Mifb.) Franco] (Strauss and Tsai 1988), corresponding with trends of increasing genome size. Several investigators have suggested that variation in the copy number of the rDNA repeat unit could account for a disproportionate part of the variation in genome size (Grime and Mowforth 1982; Strauss and Tsai 1988).

The primary goal of the present paper is to characterize the nuclear rDNA repeat unit in *Picea rubens* and *Picea mariana.* In addition, the rDNA copy concentration is analyzed, to test for significant differences in the nuclear rDNA copy concentration between red and black spruce and to examine the relationship between rDNA copy concentration and geographic origins of spruce populations.

Material and Methods

Foliage Collections

Red-spruce samples were collected from a provenance (seed source) test site located in Coleman State Forest, Stewartstown, N.H. The red-spruce site is maintained by the State of New Hampshire. Foliar tissue samples were collected from stands representing 12 provenances. There were five to seven trees sampled per provenance. Black-spruce samples were collected from a provenance (seed source) test site run by the U.S. Forest Service (USFS), at the Massabesic Experiment Station, Alfred, Me. Black-spruce samples represent 31 provenances of the eastern *Picea mariana* complex, with one to four individuals from each provenance.

Five to 10 g current-year needles were collected from each tree, 3–4 wk after bud break. Duplicate samples were collected from random individuals. Duplicate samples normally consisted of one sample taken from the lower branches and a second sample taken from near the crown. Samples were held in either dry ice or wet ice in the field and then were stored at −70°C.

DNA Extraction

DNA was extracted using the method described by Greenwood et al. (1989), with minor modifications. Intact needles were added directly to 150 ml ice-cold extraction buffer [10 mM Tris (pH 8.0), 5 mM ethylenediaminetetraacetate (EDTA), 350 mM D-sorbitol, 0.1% bovine serum albumin, 14 μM β-mercaptoethanol, and 10% polyethylene glycol (average molecular weight 8,000)]. The needles were homogenized in extraction buffer for 20–30 s by using a Brinkman Polytron. Ethanol was substituted for isopropanol for the first DNA precipitation. The RNase digestion step was found to be unnecessary. This extraction procedure normally yielded 1 mg DNA/6 g needles.

Electrophoresis was used to determine both RNA contamination of the DNA preparations and the overall quality of the extracted DNA. DNAs have been stable at 4°C for at least 1 year. DNA samples used for the quantitative analysis of rDNA copy number were further purified by an RNase digestion step and an isopropanol precipitation.

Restriction Digests and Gel Electrophoresis

DNA samples were digested with restriction endonucleases by using the conditions specified by the manufacturer (Boehringer Mannheim Biochemical; BRL Life Technologies, Inc.; New England Biolabs, Inc.; Promega Corp.; or United States Biochemical Corp.).

Restricted DNA fragments and radiolabeled lambda DNA size markers were separated by size on 0.5%–0.9% agarose gels, according to a method described by Sambrook et al. (1989, section 6, book 1). For fragment isolation, restricted DNAs were separated on low-melt, SeaPlaque GTG, or NuSieve GTG agarose purchased from FMC. Direct labeling of DNA was accomplished by separating DNAs on 0.8% SeaPlaque agarose gel (FMC Bioproducts) in NTBE buffer [0.3 μg EtBr/ml, 100 mM Tris-HCl (pH 8.0), 100 mM Boric acid, and 0.2 mM EDTA] and following an FMC protocol for in-gel labeling.

Southern Blots

Southern blots were made by transferring DNA fragments from agarose gels onto a nylon membrane (Bio-Rad Zeta-probe blotting membrane), according to a method

described by Sambrook et al. (1989, section 9.34–9.44, book 2). DNAs were fixed onto the membrane by ultraviolet irradiation (Khandjian 1986; Dowe et al. (1990). Southern blots prepared in this fashion could be probed, stripped, and reprobed as many as 10 times.

Probes and Hybridizations

Probes consisted of heterologous 18S and 26S rDNAs from soybean (*Glycine* max) rDNA and ITS 1 DNA, prepared by polymerase chain amplification (PCR), from a red-spruce template described below. The 18S and 26S rDNA soybean probes were obtained from Dr. Elizabeth Zimmer in the form of plasmid pXBr 1 and pGmr 1 (Zimmer et al. 1988). pGmr 1 contains the complete rDNA repeat unit of the soybean rDNA gene subcloned from a phage clone (Jackson and Lark 1982). pXBr 1 is a subclone of pGmr 1 and consists of a *Xba*I-to-*Bam*HI fragment of the 18S rDNA (Zimmer et al. 1988). The 18S probe consisted of a *Xba*I-to-*Bam*HI fragment of the 18S rDNA unit of soybean, and the 26S probe consisted of an *Eco*RV-to-*Bgl*II fragment of the soybean rDNA.

Two maize clones containing the mitochondrial 18S-5S rDNA (Chao et al. 1984) and the 26S rDNA unit (Dale et al. 1984) were obtained from Dr. C. S. Levings III. A petunia clone containing the chloroplast rDNA was obtained from Dr. David Neale (Palmer et al. 1983).

DNA fragments were labeled using a Boehringer Mannheim molecular biology random-primed DNA-labeling kit and α-^{32}P-labeled dATP and/or TTP (3,000 Ci/mmol) [New England Nuclear (Dupont)].

Prehybridizations and hybridizations were performed according to a method described by Sambrook et al. (1989, section 9.47–9.51, book 2), with minor modifications. Prehybridization solutions contained 50% deionized formamide, 4% SSPE (1% SSPE = 0.15 M sodium cloride, 0.01 M sodium phosphate monobasic, and 0.001 M EDTA, pH 7.4), 1% sodium dodecyl sulfate (SDS), 0.25% BLOTTO (5% nonfat dried milk dissolved in water containing 0.02% sodium azide) and 0.3 mg blocking DNA (herring sperm DNA/ml). Hybridization solutions were similar to the prehybridization solution, except that dextran sulfate (final concentration 2.5%) and probe were added to the hybridization reaction. Both prehybridizations and hybridizations were performed in a batch mode, in Nalgene plastic boxes. Blots were prehybridized for $\geq$2 h at 42°C and were hybridized for $\geq$20 h at 42°C. Blots were then washed as follows: 1 × SSC (0.15 M sodium cloride and 0.015 M sodium citrate, pH 7.0) at room temperature for 10 min; 0.5 × SSC, 0.5% SDS at 65°C for 15 min; 0.2 × SSC, 0.2% SDS at 65°C for 30 min; and 0.15 × SSC at 65°C for 20 min.

Blots were wrapped in saran wrap and were exposed, to Cronex film (Dupont) with a Cronex Quanta III intensifying screen (Dupont) at −70°C, for variable times, to produce autoradiograms. Probe was reduced to 5.0×10^5 dpm per blot for slot-blot procedures, and autoradiogram exposures were limited to 15 min–2 h to insure that exposures were within the linear range of the X-ray film.

Amplification of ITS 1

Primers were designed within conserved coding regions of the 18S and 5.8S ribosomal genes that flank ITS 1. As no gymnosperm sequences were available at the time we started this project, GenBank and EMBL data bases were used to search for conserved sequences in these genes, among angiosperms. Both primers are 20-bp oligos and were synthesized by Operon Technologies, Inc. Their sequences are as follows:

DES18S2 (5′TTCAACGAGGAATTCCTAGT 3′) and DES5.8S (5′TACGTTCT-TCATCGATGCGA 3′). DES18S2 is located 237 bp upstream from the 3′ end of the 18S coding gene (soybean) and contains an *Eco*RI restriction site; its T_M is 56°C. DES5.8S is 30 bp from the 5′ end of the 5.8S gene and has a T_M of 58°C.

Extensive data-base searches found <50% identity between the conserved PCR primers and mitochondrial, chloroplast, or fungal rDNA sequences. This suggests that the primers were specific for nuclear ribosomal genes and would not amplify contaminating fungal DNA. To eliminate the possibility of amplifying other contaminating templates, pre-PCR reactions were ultraviolet-treated for 8 min at 340 nm, according to a method described by Sarkar and Sommer (1990).

Polymerase chain amplification was performed in a 50-µl reaction containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 µM each dNTP, each primer at 3 µM, 50 ng total genomic DNA, and 3 units AmpliTaq DNA Polymerase (Perkin Elmer—Cetus). Each reaction was overlaid with 50 µl mineral oil to reduce evaporation. The PCR profile consisted of denaturation at 93°C for 1 min, annealing at 53°C for 1½ min, and elongation at 72°C for 2 min. The cycle was repeated 30 times in a Coy model 50 temperature cycler. Reaction mixtures were extracted with 200 µl chloroform and electrophoresed. The desired band was excised from the gel and subsequently was either random-prime labeled for hybridization or cloned as described below.

Cloning and Sequencing of ITS 1 PCR Products

A 445-bp polymerase-chain-reaction product from red spruce was purified from low-melting-temperature agarose and was cloned into a pCR™ 1000 plasmid by using the TA Cloning™ system from Invitrogen Corp., by following the manufacturer's protocol. The cloned product was then sequenced using the Sequenase version 2.0 DNA sequencing kit (United States Biochemical).

Quantitative Analysis of rDNA Copy Number

Slot blots were used to determine the nuclear rDNA copy concentration. Slot blots were produced using a Schleicher & Schuell Minifold II slot-blot system. Blots were produced by following the directions provided by the manufacturer. The plasmid pXBr 1 was used as a standard. One microgram of genomic DNA was loaded into each slot. The 18S fragment cloned into pXBr 1 was used as a probe for the slot blots.

Densitometry

Data were collected for the slot-blot autoradiograms by measuring the band intensity. The intensities of these bands, for each individual, were determined by reading down the autoradiogram by using a GS 300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments). The relative intensities were determined using a GS 350 data-reduction system (Hoefer Scientific Instruments) on a Zenith data system computer linked directly to the densitometer.

Slot-blot data were collected for all DNA samples, representing 100 black spruce, 58 red spruce, and duplicate samples. Duplicate samples were run in all tests, to determine the variation due to experimental procedures. Individual DNA samples were also run in duplicate or triplicate on different slot blots, to determine the variation between blots.

Statistical Analysis

Standard multiple-regression analysis was used to examine relationships between copy number and geographic origins. The analysis was carried out using an SAS Institute (1982) version 6.06 statistical package (SAS Institute, Cary, N.C.) on a Digital VAX 5800 computer.

Results and Discussion

Structure of Nuclear rDNA Repeat Unit in *Picea*

A composite restriction map of the nuclear rDNA repeat unit (fig. 1) in *Picea* was generated using heterologous soybean rDNA probes and a PCR-amplified spruce ITS 1 DNA fragment. Coding regions from the soybean rDNA repeat and from the 18S and 26S rDNAs hybridized at high stringency (0.15 × SSC; 30 min at 65°C). Hybridizations, using coding regions as probes, produced autoradiograms with a high signal-to-noise ratio (fig. 2). Noncoding sequences, the soybean IGS and the ITS 1 and 2 regions, were not sufficiently conserved between angiosperm and gymnosperm to allow the cloned soybean IGS or the ITS regions to be used as probes for mapping the gymnosperm rDNA repeat (data not shown). Chloroplast and mitochondrial rDNA clones (from petunia and maize, respectively) annealed to restriction fragments of sizes different than that of the nuclear rDNAs (data not shown).

Eleven restriction endonucleases were used to map the *Picea* rDNA repeat. The positions and distances between the restriction sites within the 18S and 26S regions were identical between *Picea rubens* and *Picea mariana*. Several restriction-fragment-length polymorphisms (RFLPs) were detected in the IGS and ITS regions; these are denoted by lowercase symbols in figure 1. There are several forms of the rDNA unit within a given individual. For example, a given restriction-enzyme/probe combination sometimes revealed both shorter and longer forms of the restriction fragment, indicating that the rDNA repeat units within an individual are polymorphic with respect to the specific restriction site. Figure 2 represents a *Hin*dIII digest of genomic DNAs hybridized with the 18S probe. In individual 4986-1 three bands appear (1.6 kbp, 2.6 kbp, and 3.4 kbp, respectively). The 3.4-kbp band represents rDNA units lacking both of the polymorphic *Hin*dIII sites.

Repeat Size

The size of the spruce rDNA repeat unit was estimated from Southern blots of *Eco*RV- or *Bgl*II-digested DNA by using the 18S probe. The fragments observed using

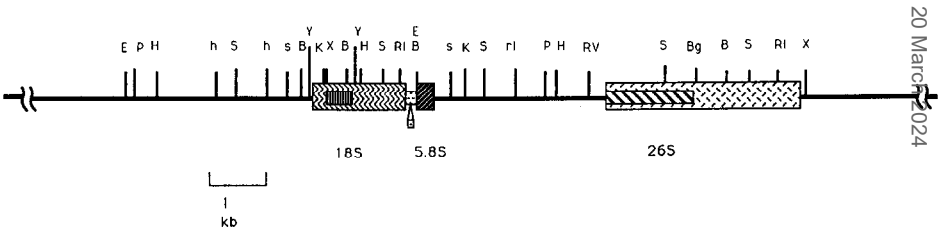

FIG. 1.—Composite restriction map of nuclear rDNA repeat of *Picea rubens* and *Picea mariana*. Total genomic DNAs were restricted, separated by size by using agarose gel electrophoresis, and transferred via capillary action to nylon membranes. Radiolabeled restricted lambda DNA were included on all the gels as internal size standards. Blots were hybridized with soybean rDNA [18S (▥) and 26S (▨)] or spruce ITS 1 (▩) probes. Polymorphic restriction sites are shown as lowercase letters. B = *Bam*HI; Bg = *Bgl*II; E = *Bst*EIII; H = *Hin*dIII; K = *Kpn*I; P = *Pst*I; RI = *Eco*RI; RV = *Eco*RV; S = *Sac*I; X = *Xba*I; and Y = *Bst*yI.

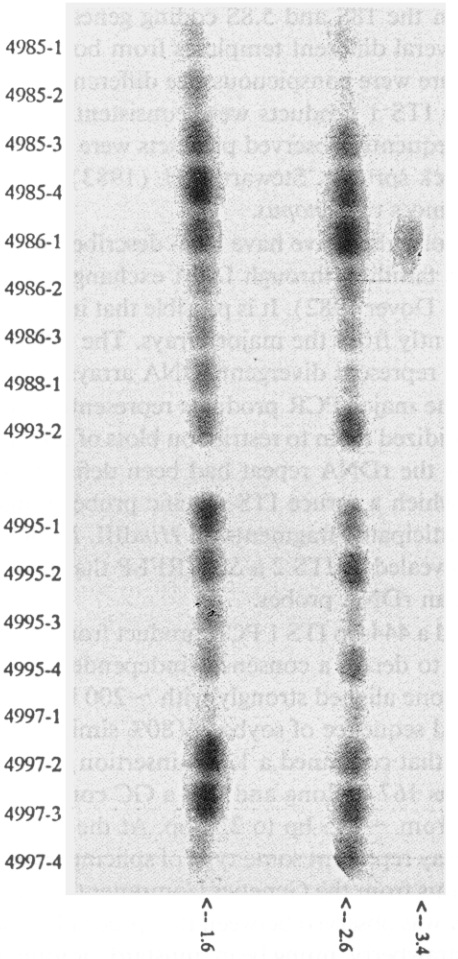

FIG. 2.—Polymorphic restriction site in *Picea mariana* rDNA. DNAs from 17 different trees were cut with *Hin*dIII, separated electrophoretically, and blotted onto nylon membrane. The 18S soybean rDNA probe hybridizes, at high stringency, to two or three fragments (1.6 kbp, 2.6 kbp, and 3.4 kbp, respectively) in each sample. The 1.6-kbp band corresponds to the presence of a polymorphic *Hin*dIII site in the IGS region of the rDNA repeat unit; the 2.6-kbp band corresponds to the absence of the polymorphic *Hin*dIII site; and the 3.4-kbp band corresponds to the absence of both polymorphic *Hin*dIII sites. Note that the relative intensities of the bands differ between the samples. The sample identification number is located below each lane.

this combination of probe and either enzyme varied between 32 kbp and >40 kbp in size. Reprobing blots with the 26S probe generated bands the same size, suggesting that only one *Eco*RV and only one *Bgl*II recognition site are located within a repeat unit. The sizes of the *Picea* rDNA repeat units are more than twice that observed in angiosperms but are similar to the 27-kbp rDNA repeat size reported in *Pinus radiata* D. Don (Cullis et al. 1988).

Characterization of *Picea* ITS 1

The ITS regions are known to be considerably more variable than the coding genes of the rDNA repeat. To achieve finer resolution mapping of this region, an ITS

1 (i.e., the ITS between the 18S and 5.8S coding genes) probe was generated using PCR amplification. Several different templates from both species were used in standardized reactions. There were conspicuous size differences among ITS amplification products. Multiple-size ITS 1 products were consistently produced from individual templates. The most frequently observed products were ~444 bp (in the red spruce) and 425 bp (in the black spruce). Stewart et al. (1983) also observed heterogeneity in the size of ITS sequences of *Xenopus*.

Mechanisms of molecular drive have been described whereby there is concerted evolution of multigene families, through DNA exchanges within and between chromosomes (see review in Dover 1982). It is possible that individual arrays or "orphans" may diverge independently from the major arrays. The different size classes of ITS 1 amplified by PCR may represent divergent rDNA arrays.

To confirm that the major PCR products represented spruce ITS DNA, we isolated, labeled, and hybridized them to restriction blots of total genomic DNA. Because the restriction map for the rDNA repeat had been defined, we were able to predict the fragment sizes to which a spruce ITS-specific probe should hybridize. The PCR probes hybridized to anticipated fragments on *Hin*dIII, *Eco*RI, and *Sac*I digests (data not shown) and also revealed in ITS 2 a *Sac*I RFLP that had not been detected with the heterologous soybean rDNA probes.

We have sequenced a 444-bp ITS 1 PCR product from red spruce; three individual clones were sequenced to derive a consensus independent of polymerase errors (fig. 3). The 5′ end of the clone aligned strongly with ~200 bases at the 3′ end of the 18S gene from the published sequence of soybean (80% similarity), although there was a block of low similarity that contained a 12-bp insertion/deletion. The sequence corresponding to ITS 1 was 167 bp long and had a GC content of 49%. Sizes for ITS 1 in angiosperms range from ~195 bp to 225 bp. At the ITS-5.8S junction there is a poly A-T tract which may represent some type of splicing signal. When BESTFIT and GAP alignment programs from the Genetics Computer Group (Devereux et al. 1984) were used, no similarity was observed between the spruce ITS 1 sequence and published ITS 1 sequences from strawberry, mung bean, mustard, or tomato. Alignments between

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  1  AAGCGTACGTCATCAGCGTGCGTTGATTACGTCCCTGCCCTTTGTACACA  50
 51  CCGCCCGTCGCTACTACCGATTGAATGGCTCAGTGAGGCCTCCGGACTGG 100
101  CCCAGGGAGGTCGGCAACACCAGGAAAGTTGGTCAAACTTGGTCATTTAG 150
151  AGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGAT 200
201  CATTAAAGAGTAAGGTATTCGTAGCCCGACCTCCAACCCTTTGTTGTTAT 250
251  AACTACCTCGTTGCTTTGCGGGACCGTCGGTCTCGAGCTGCTGGTCTTAG 300
301  GCCCGGCAAGCGCCGCCAGAGTCTACTCAAACTCTTGTTTTAACCGGTCG 350
351  TCTGAGTTAAACTTTTAATTAAATTAAAACTTTCAACAACGGATCTCTTG 400
401  GTTC  404
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FIG. 3.—Sequence of *Picea rubens* ribosomal PCR clone: bases 1–205, 3′ end of 18S gene; bases 206–373, first ITS (underlined); bases 374–404, 5′ end of 5.8S gene.

these angiosperms were all <60% identical, exemplifying the significant divergence within this region.

Intraindividual Variation in rDNA Repeat Unit

Restriction analysis was extended to provenances of red and black spruce. Red spruce was sampled from a range-wide provenance test. The black-spruce provenance test represents the Eastern complex of *Picea mariana.* No single polymorphic restriction site was exclusive to one species (data not shown), but the relative proportions of restriction sites varied between individuals (fig. 2).

Bands generated using the 18S probe on *Eco*RI-digested DNA varied between 22 kbp and 30 (±2) kbp in size. Five major rDNA fragments, representing different rDNA units, could be distinguished: 23 kbp, 24 kbp, 25 kbp, 27 kbp, and 28 kbp. Additional experiments indicated that the multiple fragments were not due to incomplete digestion. *Eco*RI digests probed with the 26S probe yielded distinct bands of 4.35 kbp and 6.5 kbp, suggesting that as many as five different nuclear rDNA repeats exist within the picea genome. In *Pinus radiata,* 10 major rDNA arrays were detected, along with a number of minor arrays, by in situ hybridization (Cullis et al. 1988). The different major polymorphic repeat units detected in *Picea* may correspond to different major arrays in the genome.

Slot-blot analysis was performed on all red- and black-spruce individuals from the provenance test sites, to determine the copy number of the rDNA repeat unit (fig. 4). The standard slots contained pXBr 1 corresponding to 770–3,850 copies/pg spruce DNA. The mean rDNA copy concentration observed in red spruce was 1,320 (±290) copies/pg, with nearly a threefold range (750–2,220 copies/pg), and that for black spruce was 1,250 (±370) copies/pg, with a sixfold range (430–2,650 copies/pg). Strauss and Tsai (1988) observed as much as 12-fold variation in the relative rDNA content of Douglas fir [*Pseudotusuga menziesii* (Mirb.) Franco] provenances. The rDNA copy concentration was not significantly different between red and black spruce, on the basis of an *F*-ratio test ($F = 3.35$; $P = 0.069$); therefore, the rDNA copy concentration cannot be used to accurately discriminate between the two species.

Relationship between Geographic Origins and Nuclear rDNA Concentration

No significant statistical relationship was detected between rDNA copy concentration and geographic origin of the *Picea mariana* provenances. In *Picea rubens,* however, a significant ($P = 0.002$) relationship was detected between rDNA copy concentration and geographic origins (latitude, longitude, latitude by longitude, and latitude squared) (table 1) when a standard multiple-regression analysis was used. Longitude squared, elevation, and elevation squared did not display a significant relationship with copy concentration and were therefore eliminated from the analysis. The red-spruce provenance test site used in the present study was not set up for examining variation over elevational gradients. Geographic origins explained 21% of the variance in rDNA copy concentration. This implies that, within *Picea rubens,* rDNA copy number differentiates across the geographic range of the species. Since only a fraction of the variations in rDNA content was accounted for by geographic origin, there must be other factors influencing the rDNA content of the spruce genome.

A weak relationship was observed between the latitude and elevation of Douglas fir provenances and relative rDNA copy number (Strauss and Tsai 1988), with relative rDNA copy number increasing with latitude. Grime and Mowforth (1982) and Price

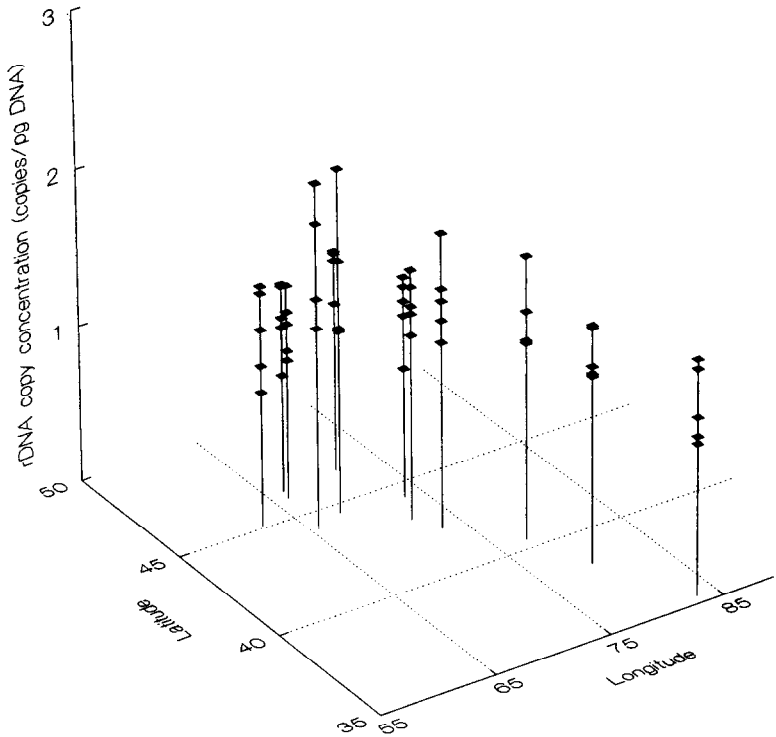

FIG. 4.—Three-dimensional plot depicting rDNA copy concentration for *Picea rubens* (*facing page*) and (b) *Picea mariana* (*above*), over geographic origins. Lines connect the data points (copy concentration) with the *x-y* plane (geographic origins).

(1988) suggest that, in plant species, increases in genome size, correlate with the climate at which the organism originates, with colder climates giving rise to larger genomes. Grime and Mowforth (1982) also suggest that the increases in genome size may be due to amplification of genes involved in protein synthesis.

The total genomic DNA content for several species of conifers has been shown to vary with geographic location, and these differences are not thought to be due to simple changes in ploidy (Mergen and Thielges 1967; Miksche 1967, 1968, 1971; Dhir and Miksche 1974). Both the relatively high rDNA content of the *Picea* genomes and the substantial range in rDNA content could contribute to the overall intraspecies variation in conifer genome sizes.

Sequence Availability

The ITS 1 sequence reported in the present paper appears in the EMBL, GenBank, and DDBJ Nucleotide Sequence Databases under accession number M63721.

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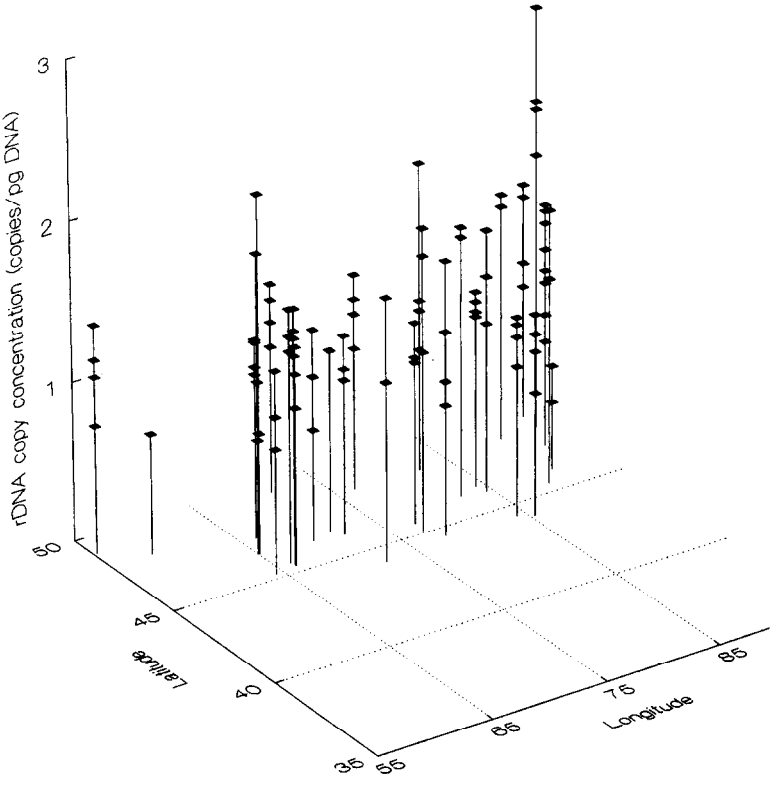

FIG. 4 (*Continued*)

from the Massabesic Experiment Station was granted by Dr. Peter Garrett (USFS), Durham, N.H. Dr. Peter Brym and John Carlson of University Computing and Information Services instructed us on the use of the SAS package. Denis Guenette assisted with DNA isolations. This work was supported in part by grants from the UNH

Table 1
Regression Analysis of Copy Number on Geographic Origins of Provenances

Parameter	Degrees of Freedom	Parameter Estimate[a]	Standard Error	*t*-Test[b] for Hypothesis	$P > \|T\|$
Intercept	1	189.57	71.53	2.65	0.0106
Latitude	1	−5.81	2.33	−2.49	0.0158
Longitude	1	−1.56	0.54	−2.86	0.0060
Latitude by longitude	1	0.03	0.01	2.84	0.0063
Latitude squared	1	0.04	0.02	2.21	0.0318

NOTE.—$R^2 = 0.27$; Adj-$R^2 = 0.21$; $P = 0.002$. R^2 is a measure of the proportion of the variation, in the dependent variable, explained by the model. Adj-R^2 corrects R^2 to more closely reflect the goodness of fit of the model in a natural population. Adj-R^2 takes both the sample size and the number of independent variables into consideration.

[a] Based on standard multiple regression analysis and can be applied to raw data to obtain predicted rDNA copy concentration.

[b] Measures strength of linear relationship between dependent and independent variables.

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