NOTE

Stability of nuclear DNA content among divergent and isolated populations of Fraser fir

L.D. Auckland, J.S. Johnston, H.J. Price, and F.E. Bridgwater

Abstract: Fraser fir (*Abies fraseri* (Pursh) Poir.) is an endemic species consisting of six major disjunct populations in the Appalachian Mountains, U.S.A. Nuclear DNA content was measured with laser flow cytometry to determine if genome size differences could be detected among the disjunct populations of Fraser fir and its close relatives, balsam fir (*Abies balsamea* (L.) Mill.) and Canaan fir (*A. balsamea* var. *phanerolepsis* Fern.). The mean DNA content for Fraser fir was 17.2 pg/C, which was similar to the two related fir species. There were no significant differences among disjunct Fraser-fir populations. Mean DNA content differences for fir species in the southern Appalachian Mountains were similar even with speciation events (7000 B.P.) and subsequent population isolation. In the absence of polyploidy or large chromosomal rearrangements, genome size changes in conifers occur on a broad evolutionary time scale.

Key words: conifers, gymnosperm, C-values, nuclear genome, *Abies fraseri*.

Résumé : Le sapin de Fraser (*Abies fraseri* (Pursh) Poir.) est une espèce endémique comportant six populations disjointes dans la chaîne montagneuse des Appalaches, aux Etats-Unis. Afin de déterminer si on peut déceler des différences dans la dimension du génome entre les populations disjointes du sapin de Fraser et ses proches parents, soient le sapin baumier (*Abies balsamea* (L.) Mill.) et le sapin de Canaan (*A. balsamea* var. *phanerolepsis* Fern.), les auteurs ont mesuré leurs contenus en acides nucléiques à l'aide de la cytométrie en flux au laser. La teneur moyenne en ADN chez le sapin de Fraser est de 17.2 pg/C, qui est similaire à celle des deux espèces de sapin voisines. Il n'y a pas de différence entre les populations disjointes du sapin de Fraser. Les différences de teneur moyenne en ADN chez les espbces de sapin du sud des montagnes appalachiennes sont semblables en dépit d'événements de spéciation (7000 A.P.) et l'isolation subséquente des populations. En absence de polyplofdie ou de larges réarrangements chromosomiques, les changements de dimension du génome chez les conifères s'effectue sur une large échelle temporelle d'évolution.

Mots clés : conifères, gymnosperme, valeur-C, génome nucléique, *Abies fraseri*

[Traduit par la Rédaction]

Introduction

Genetic divergence and speciation are frequently accompanied by changes in the amount of nuclear DNA (Price 1976). In this study, we tested the hypothesis that the nuclear DNA content of Fraser fir, *Abies fraseri* (Pursh) Poir., differs among its disjunct populations. These disjunct populations have characteristic needle morphology (Jett et al. 1993) and isozyme allele frequencies (Ross 1988). DNA content for three other *Abies* species show smaller genome sizes compared with *Pinus* spp., although the base chromosome number for the true firs and most other members of the Pinaceae is 12 (Ohri and Khoshoo 1986; Lin et al. 1988; Roth et al. 1997; Murray 1998).

Fraser fir is an endemic species found in the Appalachian Mountains extending from southwestern Virginia and western North Carolina to eastern Tennessee, at 35°15′N, 81°30′W to 36°45′N, 83°40′W. The species grows in isolated mountain stands at elevations ranging from 1372 to 2037 m above sea level. Fraser fir grows in a cold, moist, cool-temperate rain forest with mean annual precipitation of 1900-2450 mm, and with average summer temperatures of 16°C or lower (Beck 1990). Most natural stands occur at elevations above 1500 m above sea level. There are six major disjunct populations: Mount Rogers, Roan Mountain, Grand-

Received April 4, 2001. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on November 16, 2001.

L.D. Auckland. Department of Forest Science, Texas A&M University, TAMU 2135, College Station, TX 77843, U.S.A.
J.S. Johnston. Department of Entomology, Texas A&M University, College Station, TX 77843, U.S.A.
H.J. Price. Department of Soil & Crop Science, Texas A&M University, College Station, TX 77843, U.S.A.
F.E. Bridgwater.' USDA Forest Service, Forest Sciences Lab, Texas A&M University, College Station, TX 77843-2585, U.S.A.

'Corresponding author (e-mail: fbridgwater@fs.fed.us)

DOI: 10.1139/cjb-79-11-1375

father Mountain, and in the Black Mountains, the Balsam Mountains, and the Great Smoky Mountains (Fig. 1).

Balsam fir *(Abies balsamea* (L.) Mill.), Canaan fir *(Ahies balsamea* var. *phanerolepsis* Fern.), and Fraser fir probably arose from a single species (Jacobs et al. 1984). Morphological and monoterpene studies support their close relationship (Zavarin and Snajbeck 1972; Thor and Barnett 1974). Scattered Fraser-fir stands in the southern Appalachia are thought to be remnants of a once-continuous true-fir forest (Thor and Barnett 1974). The paleobotanical history of the region supports the existence of a larger continuous fir forest. After Pleistocene glaciation, boreal forests moved northward, and pockets of endemic Fraser-fir populations became isolated in the southern Appalachians ca. 7000 B.P. (Delacourt and Delacourt 1981). More recently, human disturbance, which began with heavy logging in the 1930s, has accelerated isolation of Fraser-fir populations. Logging was followed by a brief period of species expansion. At present, atmospheric pollutants and attack by an introduced exotic pest (the balsam woolly adelgid, *Adelges piceae* Ratzeburg) are again contributing to the species' decline. The balsam woolly adelgid attacks and kills mature trees after the onset of reproduction.

Intraspecific variation in true-fir DNA content has not been measured using consistent methods, such as laser flow cytometry (Galbraith et al. 1983), the preferred method of genome size determination for conifers (Wakamiya et al. 1993). *Abies balsamea* DNA content was reported to be 13.2 pg/C (Ohri and Khoshoo 1986) but it is based on root tips (2N) and Feulgen staining. Feulgen staining of conifer root-tip nuclei has historically been plagued with technical problems, which lead to unreliable estimates of DNA content. There are no previous reports of genome size for *A. fraseri,* nor a measurement of intraspecific variation among the true firs.

The objective of this study was to estimate nuclear DNA content among disjunct populations of Fraser fir and its close relatives, balsam fir and Canaan fir. Estimating nuclear DNA content is critical for genomics applications and for determining genome size changes with response to speciation events and subsequent population differentiation. In this study we tested the following hypotheses: *(i)* patterns of genome size variation parallel taxonomic relationship and *(ii)* intraspecific variation in DNA content occurs as a consequence of Fraser-fir population isolation.

Materials and methods

Seeds were sampled from a 1994 collection conducted jointly by North Carolina State University, the University of Tennessee, the North Carolina Division of Forestry, and the USDA Forest Service. Nuclear DNA content was determined for a small number of trees from each of the six major disjunct populations using laser flow cytometry. Haploid megagametophyte tissue was first excised and then processed using a protocol adapted from Price and Johnston (1996). The DNA content of the nuclei was determined for each sample using a Coulter Epic Elite flow cytometer (Coulter Electronics. Hialeah, Fla.) equipped with a Coherent water-cooled laser tuned at 514 nm (0.5 W). Each sample of Fraser-fir megagametophytes included pea (*Pisum sativum* cv. Minerva Maple) as a standard. A small piece of leaf was introduced into each sample when it was chopped in a 6 mL buffer solution consisting of 8.84 g sodium citrate, 4.2 g 3-[*N*-morpholino]propane–sulfonic acid, 4.26 mL of 4.9 M magnesium chloride, 1.0 mL of Triton X- 100, and 100 μL of 10 mg/mL RNAase A. Propidium iodide was added to a concentration of 50 ppm during maceration after first adjusting the buffer solution to pH 7.2. The macerated tissue was filtered through 50 μm nylon mesh and centrifuged at 1200 rpm for 3 min. Supernatant was then replaced with 700 mL of fresh buffer. The values for pea were first adjusted for replicate runs, then used to estimate Fraser-fir genome size. The genome size for diploid pea was determined to be 8.22 pg/2C (Joyner et al. 2001).

A two-step sampling scheme was used to determine optimum sampling. First, an individual gametophyte was sampled from each population, Mount Rogers, Richlands Balsam, Roan Mountain, Grandfather Mountain, Mount Mitchell, and the Smoky Mountains, in each of three replicate runs. The data from these preliminary runs indicated that the Smoky Mountain population had a smaller genome size than other populations. Due to the small number of individual parents (two to four per population) included in this preliminary work, an additional run was necessary.

For the second step, megagametophytes were bulked from different parent trees within each of the six populations and run as one additional replicate (Table 1). The individual and the bulked samples were selected from the lowest, middle, and highest elevations of each of the isolated mountain populations and were processed separately by elevation to determine whether there was an effect of elevation of genome size. For balsam fir and Canaan fir, a population was bulked.

Statistical analysis

Estimated values for genome size for Fraser fir, previously adjusted for run effects, were subjected to a weighted one-way analysis of variance using the model $\gamma_{ij} = \mu + a, + \alpha(\beta)_{i(j)}$, where γ was the mean DNA content for a sample, μ was the experimental mean, a, was population (i = 1–6), and β_{ij} was the jth sample in the ith population. Covariance analysis was performed using elevation as a covariate.

Results

Patterns of genome size variation did not parallel taxonomic relationship, and there was no intraspecific variation detected among isolated Fraser-fir populations. Nuclear DNA content for Fraser fir was slightly larger than its close *Abies* relatives, balsam and Canaan fir (Table 2), but these differences were not statistically different. Mean DNA content for Fraser fir was 17.2 pg/C with a coefficient of variation of 3.4%. The analysis of variance did not show a significant difference among the six populations examined at $\alpha \geq 0.10$ and elevation was not a significant covariate. DNA content of Fraser-fir populations did not reflect an adaptive response to environmental or elevational changes (Table 1).

Discussion

DNA content differences among Fraser fir, balsam fir, and Canaan fir were minimal. This is consistent with the observation that the true firs tend to have smaller DNA content than pines. This trend was noted by Murray (1998) for a wide range of genera within the Pinaceae. A similar pattern for other true firs in Eurasia has been previously reported: *Ahies alba* Mill. (16.6 pg/C) and *Ahies sibirica* Ledeb. (15.8 pg/C) (Lin et al. 1988; Roth et al. 1997). A previous estimate of genome size for *A. balsamea* (Ohri and Khoshoo 1986) had DNA content values much lower (4 pg/C or 25%)

Fig. 1. The natural range of Fraser fir in the southern Appalachians. Shaded areas indicate location of stands. Reprinted from Dull et al. (1988).

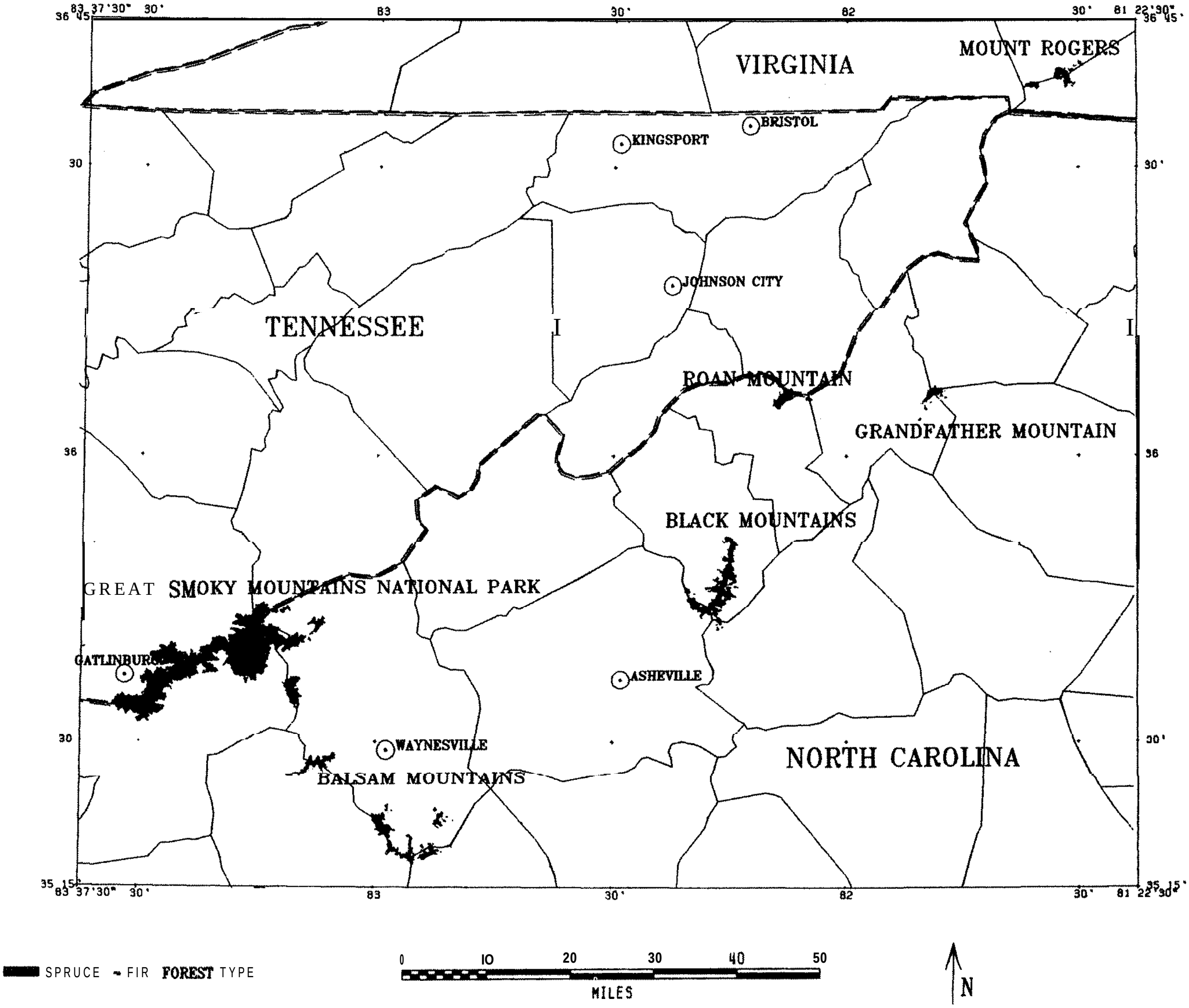

Table 1. Seed sources for Fraser-fir populations and their mean DNA content.

Population	Range in elevation (m)	Latitude (N)	Longitude (W)	Parent no.*	Mean DNA content (pg/C)
Mount Mitchell	1750–2175	35°45′	82°20′	14	17.5
Grandfather Mountain	1590–1970	36°05′	81°50′	14	17.3
Mount Rogers	1660–1810	36°40′	81°30′	7	17.2
Roan Mountain	1830-2075	36°05′	82°05′	8	17.2
Richlands Balsam	1650–2080	35°20′	82°55′	7	17.0
Smoky Mountains	1680-2240	35°40′	83°30′	6	16.9

"Number of parents refers to the number of haploid megagametophytes in the bulked samples.

than those reported in this study. The difference may bc due to the Feulgen method, the use of diploid tissue, or the use of' other provenances not sampled in this study.

Gradual changes in DNA content may be attributed to fluctuation within the highly repetitive DNA component of the genome (Gregory and Herbert 1999) rather than to changes in chromosome number or polyploidy. DNA content reflected neither very recent speciation events (7000 B.P.) nor even more recent population subdivision. In the absence of polyploidy or large chromosomal rearrangements, genome size changes in conifers may occur on a broad evolutionary time scale.

These results support the stability of intraspecific DNA content reported for other temperate conifers in North America (Wakamiya et al. 1993). Boreal and temperate North American conifers may have undergone more population reduction and movement as a consequence of glaciation so ancient modulation of DNA content may have been eliminated.

Table 2. Mean DNA content for Fraser fir, balsam fir, and Canaan fir.

Species	DNA content (pg/C)
Fraser fir	17.2
Balsam fir	17.0
Canaan fir	17.3

DNA content often varies greatly among congeneric species of both angiosperms and gymnosperms, but remains remarkably constant within most species (Greilhuber 1988). Since molecular mechanisms are known that can generate variation in DNA content (Kubis et al. 1988; Sanmiguel and Bennetzen 1998), the degree of genome size constancy in most plant species, including those of this study, is indeed surprising. It has been suggested that DNA content may normally be under the innate control of counting mechanisms that detect, quantify, and regulate genome size within quite tightly defined or preselected limits (Bennett et al. 2000). DNA content variation within a species would follow the activation of DNA sequence amplification and (or) deletion events, possibly due to environmental or genomic stress (Walbot and Cullis 1985). The occurrence and magnitude of destabilizing events apparently has not been sufficient to generate significant variation in DNA content within and among the species studied herein.

References

Beck, D.E. 1990. Fraser fir. *In* Silvics of North America. Vol. 1. *Technical coordinators* R.M. Burns and B.H. Honkala. USDA Agric. Handb. 654.

Bennett, M.D., Johnston, J.S., Hodnett, G.L., and Price, H.J. 2000. *Allium cepa* L. cultivars from four continents compared by flow cytometry show nuclear DNA constancy. Ann. Bot. 85: 35 1-357.

Delcourt, P.A., and Delcourt, H.R. 1981. Vegetation maps for eastern North America: 40,000 yr. B.P. to the present. *In* Geobotany. Vol. II. *Edited* by R.C. Romans. Plenum Publishing Corp., New York. pp. 123-I 65.

Dull, C.W., Ward, J.D., Brown, H.D., Ryan, G.W., Clerke, W.H., and Uhler, R.J. 1988. USDA For. Serv. South. Reg. Prot. Rep. R8 PR13.

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P., Firoozabady, E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science (Washington, D.C.), 220: 1049– 105 1.

Gregory, T.R., and Herbert, P.D.N. 1999. The modulation of DNA content: proximate causes and ultimate consequences. Genome Res. 9: 317-324.

Greilhuber, J. 1988. Intraspecific variation in genome size: a critical reassessment. Ann. Bot. **82**(Suppl. A): 27-35.

Jacobs, B.F., Werth, C.R., and Guttman, S.I. 1984. Genetic relationships in *Abies* (fir) of eastern United States: an electrophoretic study. Can. J. Bot. 62: 609-616.

Jett, J.B., McKeand, S.E., Liu, Y., and Huxster, W.T. 1993. Seed source variation for height and crown traits of Fraser fir Christmas trees. S. Jour. Appl. For. 17: 5-9.

Joyner, K.L., Wang, X.-R., Johnston, J.S., Price, H.J., and Williams, C.G. 2001. DNA content for Asian pines parallels New World relatives. Can. J. Bot. 79: 192-196.

Kubis, S., Schmidt, T., Heslop-Harrison, J.S. 1988. Repetitive DNA elements as a major component of plant genomes. Ann. Bot. **82**(Suppl. A): 45-55.

Lin, Y.Q., Bitonti, M.B., Ciolli, M., and Innocenti, A.M. 1988. Somatic mutations in *Pinus laricio*. A cytophotometric analysis of DNA and histone content in 2C meristematic nuclei. Caryologia, **41:** 137-142.

Murray, B.C. 1998. Nuclear DNA amounts in gymnosperms. Ann. Bot. 82: 3-15.

Ohri, D., and Khoshoo, T.N. 1986. Genome size in gymnosperms. Plant Sys. Evol. 153: 199-132.

Price, H.J. 1976. Evolution of DNA content in higher plants. Bot. Rev. 42: 27-52.

Price, H.J., and Johnston, J.S. 1996. Analysis of Plant DNA content by Feulgen microspectrophotometry and flow cytometry. *In* Methods of Genome Analysis in Plants. CRC Press, New York. pp. 115–132.

Ross, R.K. 1988. Patterns of allelic variation in natural populations of *Abies fraseri* (Pursh.) Poir. Ph.D. dissertation, Department of Forestry, North Carolina State University, Raleigh, N.C.

Roth, R., Ebert, I., and Schmidt, J. 1997. Trisomy associated with loss of maturation capacity in a long-term embryogenic culture of *Abies alba*. Theor. Appl. Genet. 95: 353–358.

Sanmiguel, P., and Bennetzen, J.L. 1988. Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. Ann. Bot. **82**(Suppl. A): 37–44.

Thor, E., and Barnett, P.E. 1974. Taxonomy of *Abies* in the southern Appalachians: variation in balsam monoterpenes and wood properties. For. Sci. 20: 32–40.

Wakamiya, I., Newton, R.J., Johnston, J.S., and Price, H.J. 1993. Genome size and environmental factors in the genus *Pinus*. American Jour. of Bot. 80: 1235–1241.

Walbot, V., and Cullis, C.A. 1985. Rapid genomic change in higher plants. Ann. Rev. Plant Physiol. 36: 367-396.

Zavarian E., and Snajbeck, K. 1972. Geographical variability of monoterpenes from *Abies balsamea* and *Abies fraseri*. Phytochemistry, **11:** 1407–1421.