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Phylogenetic and biogeographic implications of chloroplast DNA variation in Picea

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Purified chloroplast DNA (*cp*DNA) extracts from 31 species of *Picea* and two species of *Pinus* (*P. sylvestris* and *P. cembra*) were digested with eight restriction endonucleases, separated by electrophoresis and scored for restriction fragment length polymorphisms. The resulting data was analyzed phenetically and cladistically. The phenetic analysis indicated lower levels of *cp*DNA differentiation within *Picea* than within *Pinus* and lower levels of differentiation among Eurasian than among North-American *Picea* species. The cladistic analysis, using *Pinus sylvestris* as an outgroup, suggested monophyly for *Picea* and resolved several monophyletic groups among the 31 species of *Picea*. An assessment of biogeographic events, based on the cladogram, suggests that *Picea* originated in North-America and that the colonization of Eurasia occurred through separate, intercontinental migrations.

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Introduction

The phylogenetic structure of the genus *Picea* A. Dietr. (spruce) remains obscure and controversial, despite more than one century of intensive systematic effort (Schmidt-Vogt 1977; Aldén 1987; Rushforth 1987). The genus is considered morphologically uniform and discrete from other genera of the family Pinaceae. On this basis, monophyly of *Picea* is commonly accepted (Wright 1955; Prager et al. 1976; Schmidt-Vogt 1977; Price 1989). The genus is also considered more uniform in wood anatomy, growth and ecological preference than most large genera of temperate woody plants (Wright 1955). On the basis of morphological affinities, the subdivision of the genus into subgenera, sections and series has been proposed in a number of divergent classifications (e.g. Lacassagne 1934; Gaussen 1966; Colleau 1968; Sudo 1968; Bobrow 1970; Schmidt-Vogt 1977; Liu 1982; Schmidt 1989). In addition, artificial crossing experiments (e.g. Wright 1955; Mikkola 1969; Roulund 1971; Fowler 1983, 1987; Gordon 1984, 1990); chemical composition (e.g. von Schantz & Juvonen 1966; Corrigan et al. 1978) and allozyme variation (Wellendorf & Simonsen 1979) have been used to infer species interrelationships. The groupings derived do not, however, concur with one another nor with those based on morphological criteria.

The genus comprises 30–40 long-lived species, distributed in boreal and cool-montane regions of the northern hemisphere (Schmidt-Vogt 1977; Aldén 1987; Rushforth 1987). The majority (23) of the 34 species recognized by Aldén (1987) occurs in various parts of Asia, while nine are native to North America and two to Europe.

Comparative studies of restriction fragment length polymorphisms (RFLPs) of the chloroplast (*cp*) DNA

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Tab. 1. Species samples included in study.

Species	Distribution	Abbreviation	Source[1]	Voucher information
1. *Picea abies* (L.) Karst.[2]	Northern Eurasia	ABI	IFI	Up262
2. *P. asperata* Mast.	Central China	ASP	HA	V3189-61
3. *P. aurantiaca* Mast.[3]	Central China	AUR	HA	511-53
4. *P. bicolor* (maxim.) Mayr	Japan	BIC	HA	377-49
5. *P. brachytyla* (Franch.) Pritz.	Central China	BRA	WA	00.31.0281
6. *P. breweriana* S. Wats.	N.W. U.S.A.	BRE	HA	Plsk. 1944
7. *P. chihuahuana* Martinez	Mexico	CHI	HA	438-70
8. *P. engelmanni* (Parry) Engelm.	W. N-America	ENG	HA	383-71
9. *P. glauca* (Moench) Voss	North America	GLA	HA	798–53
10. *P. glehnii* (Fr. Schmidt) Mast.	East Asian islands	GLE	HA	75-64
11. *P. jezoensis* (Sieb. et Zucc.) Carr.	East Asian islands	JEZ	HA	104-34
12. *P. koraiensis* Nakai[4]	Korea/Manchuria	KOR	BP	n/a
13. *P. koyamai* Shirasawa	Japan	KOY	HA	A. Olsen 1948
14. *P. likiangensis* (French.) Pritz.	Central China	LIK	WA	00.31.0720
15. *P. mariana* (Mill.) B.S.P.	North America	MAR	IFI	Is 510
16. *P. maximowiczii* Regel ex Mast.	Japan	MAX	HA	168-65
17. *P. mexicana* Martinez	Mexico	MEX	HA	128-71
18. *P. meyeri* Rehd. et Wils.	Central China	MEY	FUB	n/a
19. *P. morrisonicola* Hayata	Taiwan	MOR	BP	8/157
20. *P. neoveitchii* Mast.	Central China	NEO	HA	194-57
21. *P. omorika* (Pancic) Purkyne	former Yugoslavia	OMO	HA	S4433–63
22. *P. orientalis* (L.) Link	Caucasus	ORI	CL	111–156
23. *P. polita* (Sieb. et Zucc.) Carr.	Japan	POL	HA	11014–1524
24. *P. pugens* Engelm.	W. U.S.A.	PUN	HA	392–71
25. *P. purpurea* Mast.[5]	Central China	PUR	HA	889-54
26. *P. rubens* Sarg.	E. North America	RUB	HA	117-68
27. *P. schrenkiana* Sarg.	Central Asia	SCH	HA	72-51
28. *P. sitchensis* (Bong.) Carr.	W. North America	SIT	BCFS	#4127
29. *P. smithiana* (Wall.) Boiss.	Central Asia	SMI	HA	200-71
30. *P. spinulosa* (Griff.) Henry	Himalayas	SPI	BP	8/158
31. *P. wilsonii* Mast.	Central China	WIL	HA	14-964
32. Genus *Pinus*, Subgenus *Strobus*: *P. cembra* L.	N. Asia, C. Europe	PNC	U	Sz-PNC-2
33. Genus *Pinus*, Subgenus *Pinus*: *P. sylvestris* L.	Europe, N. Asia	PNS	U	Sz-PNS-12

[1] Abbreviations: IFI: Institute for Forest Improvement, Sävar, Sweden; HA: Hørsholm Arboretum, Hørsholm, Denmark; BP: Bedgebury Pinetum, Bedgebury, Great Britain; WA: Westonbirt Arboretum, Tetbury, Great Britain; BCFS: British Columbia Forest Service (seedling grown from seedlot #4127). – [2] = *P. abies* ssp. *abies*. – [3] = *P. asperata* var. *aurantiaca* (Mast.) Boom. – [4] = *P. abies* var. *koraiensis* or *P. koyamai* var. *koraiensis*. – [5] = *P. likiangensis* var. *purpurea* (Mast.) Dallim. & Jacks.

may shed new light on the evolutionary and geographic history of this systematically recalcitrant genus. The uniparental inheritance of the genome, its compact size, slow rate of evolution, and its ubiquitous distribution among plants are among the features furnishing its high resolving power for systematic comparisons (see Birky 1988; Palmer et al. 1988; Clegg 1989; Bremer 1991 for review). In the present study, we provide estimates of *cp*DNA diversity and advance hypotheses accounting for evolutionary patterns in *Picea*, inferred through the use of phenetic and cladistic analyses of *cp*DNA restriction fragment data. The aims of the present study are: (1) to infer genetic inter-relationships among *Picea* species; (2) to compare levels of *cp*DNA restriction fragment variation in *Picea* to that observed in *Pinus*; and (3) to examine the geographic partitioning of *cp*DNA variation in *Picea*.

Materials and methods

Plant material

Foliage for analysis was collected from single trees in arboreta (Tab. 1). We sampled 30 out of the 36 species recognized by Schmidt-Vogt (1977). In addition, we included *P. aurantiaca* in the analysis, listed by Schmidt-Vogt (1977) as a variety of *P. asperata*. Two species of *Pinus* (*P. cembra* and *P. sylvestris*) were included in all analyses. These represent one species from each of the two commonly recognized subgenera of *Pinus* (subgenus *Strobus* and subgenus *Pinus*, respectively; Mirov 1967; Price 1989). Each operational taxonomic unit (hereafter: OTU) was represented by the same individual in all analyses.

Chloroplast DNA isolation and restriction analysis

Isolation of *cp*DNA followed the procedure of White (1986), with modifications described by Szmidt et al. (1986). *Cp*DNA samples (1 μg) were digested separately with 10 units of the following eight six-cutter restriction endonucleases: *Bam*-HI, *Bcl*-I, *Bgl*-II, *Hind*-III, *Kpn*-I, *Sac*-I, *Sma*-I and *Xba*-I (Boehringer, Mannheim). Digestions were made according to the manufacturer's instructions.

The method chosen for scoring *cp*DNA restriction data in the present study (fragment "direct" analysis; FDA, sensu Bremer 1991) involves the inspection of purified *cp*DNA restriction fragment profiles for polymorphic fragments. This method is the least resource-demanding of the methods used for examining *cp*DNA restriction data for phylogenetic inference. It may, however, be least accurate, owing to risk of non-homology of characters (restriction fragments) scored. We chose to employ this method because of the large number of OTUs to be analyzed, and due to resource constraints. It involves treating restriction fragments of equal length from different OTUs as homologous, irrespective to their positions on the *cp*DNA molecule nor the mutational basis of restriction fragment differences. On the other hand it prevents the risk of scoring non-*cp*DNA polymorphisms (caused by cross-homology between different genomes; see Sederoff et al. 1986), which cause problems in experiments involving total DNA extracts and probe hybridizations.

Electrophoresis, scoring and size estimation of restriction fragments followed X.-R. Wang & Szmidt (1990). Fragments of double stoichiometric intensity were scored twice in the analysis, and coded with size numbers with one-base-pair differences. Repeated scoring of fragment sizes showed readings of fragments larger than 8 kb (kilobase-pairs) to be least accurate, and probably biased downward. Due to poor gel resolution and visibility of smaller-sized fragments of some *cp*DNA samples, we did not attempt to score fragments beyond a certain threshold fragment size. This threshold fragment size was dictated by the size (in kb) of the most poorly visualized fragment and DNA digest, and varied from 0.89 kb (for *Sma*-I) to 2.71 kb (for *Hind*-III).

Phenetic analysis

Estimates of the number of *cp*DNA nucleotide substitutions per site (weighted $\hat{d}$ values) were calculated from restriction fragment data for all pairwise OTU combinations following the method of Nei (1987; equations 5.53–5.55) and Nei & Miller (1990), using the RESTSITE program (version 1.1.) of the RESTSITE computer package (version 1.1.; developed by J. C. Miller, University of Wisconsin). Standard errors for $\hat{d}$ values were computed, using the "jack-knife" option of the RESTSITE program.

The unweighted pair-group method with arithmetic mean (UPGMA; Sneath & Sokal 1973), was used for inferring species inter-relationships phenetically. A phenogram was constructed on the basis of weighted $\hat{d}$ values, using the UPGMA program (v. 2.0), from the RESTSITE package.

Cladistic analysis

The restriction fragment data was cladistically analyzed through the use of Wagner parsimony, which assumes equal probability of character loss and character gain. In the analysis, we only included "phylogenetically informative" fragments (fragment presence or absence shared by two or more OTUs) and excluded all ambiguous (poorly resolvable) fragments from the analysis. These ambiguous fragments were mainly found in clusters of four or more fragments, where it was difficult to score apparent fragment length differences and occurrences with certainty. This approach produced identical data for *Picea asperata, P. aurantiaca* and *P. koyamai* (unresolved trichotomy). Hence, we excluded data for *P. aurantiaca* in all subsequent analyses. A presence/absence matrix of informative restriction fragments was then analyzed by Wagner parsimony. *Pinus sylvestris* was used as an outgroup to root cladograms, and *P. cembra* was included among the 30 species of *Picea* within the ingroup. Wagner parsimony dendrograms were constructed, using the MIX program with the global swapping option of the PHYLIP statistical package (Phylogeny Inference Package, version 3.1, developed by J. Felsenstein, University of Washington). From the equally parsimonious trees, a strict consensus tree was constructed with the CONSENSUS program of PHYLIP. In order to ascertain confidence intervals using the bootstrap method (Felsenstein 1985), we used the BOOT program of PHYLIP, with 100 replicate runs.

Results

Restriction fragment variation

A total of 4128 restriction fragments were scored for *Picea* species in the analysis (average of 133 restriction fragments per *Picea* species). When the two *Pinus* species were included the total number of restriction fragments scored rose to 4466. The analysis of data for *Picea* species alone resulted in a total of 212 discrete characters, of which 130 were variable, and 64 were phylogenetically informative. With the two *Pinus* species included the number of discrete characters rose to 264 and phylogenetically informative characters to 143. Fragment presence (shared by at least one *Pinus* and one

Tab. 2. Pairwise estimates of weighted number of nucleotide substitution per site between OTU's ($\hat{d} \times 100$; below diagonal); number of abbreviations, refer to Tab. 1.

ABI	**137**	130	129	124	125	116	126	119	117	134	126	132	131	127	123	126
ASP	0.27 (0.12)	**136**	135	121	122	112	124	117	115	130	125	132	135	126	120	124
AUR	0.29 (0.13)	0.02 (0.02)	**135**	120	122	112	123	116	114	130	125	131	134	125	120	123
BIC	0.54 (0.17)	0.66 (0.19)	0.68 (0.19)	**136**	132	111	130	114	112	123	124	124	121	125	120	130
BRA	0.51 (0.16)	0.63 (0.18)	0.61 (0.17)	0.19 (0.07)	**137**	112	130	116	114	125	126	124	122	125	121	131
BRE	0.87 (0.22)	1.05 (0.21)	1.03 (0.19)	1.10 (0.32)	1.07 (0.26)	**134**	112	108	106	116	112	114	113	116	111	111
CHI	0.47 (0.14)	0.54 (0.16)	0.56 (0.19)	0.27 (0.12)	0.29 (0.12)	1.07 (0.29)	**137**	113	111	125	126	124	124	124	120	129
ENG	0.81 (0.24)	0.89 (0.17)	0.92 (0.19)	1.04 (0.18)	0.96 (0.19)	1.30 (0.40)	1.11 (0.24)	**138**	132	118	115	120	118	118	115	116
GLA	0.87 (0.26)	0.94 (0.20)	0.97 (0.21)	1.10 (0.16)	1.02 (0.18)	1.37 (0.43)	1.17 (0.23)	0.21 (0.16)	**136**	116	113	118	116	116	113	114
GLE	0.10 (0.06)	0.25 (0.12)	0.23 (0.11)	0.56 (0.18)	0.49 (0.17)	0.85 (0.19)	0.49 (0.16)	0.84 (0.22)	0.90 (0.25)	**136**	126	132	130	126	123	125
JEZ	0.41 (0.15)	0.43 (0.15)	0.41 (0.13)	0.48 (0.12)	0.41 (0.10)	1.01 (0.30)	0.41 (0.08)	0.94 (0.24)	1.00 (0.26)	0.39 (0.13)	**134**	127	125	128	124	126
KOR	0.21 (0.13)	0.19 (0.10)	0.21 (0.11)	0.54 (0.18)	0.56 (0.19)	0.97 (0.26)	0.56 (0.18)	0.76 (0.18)	0.82 (0.22)	0.19 (0.12)	0.36 (0.12)	**137**	132	129	123	125
KOY	0.23 (0.12)	0.04 (0.04)	0.06 (0.04)	0.66 (0.19)	0.63 (0.18)	1.00 (0.20)	0.54 (0.16)	0.84 (0.18)	0.90 (0.21)	0.25 (0.12)	0.43 (0.15)	0.19 (0.10)	**136**	126	120	124
LIK	0.38 (0.14)	0.41 (0.12)	0.43 (0.13)	0.45 (0.18)	0.47 (0.17)	0.83 (0.25)	0.52 (0.16)	0.82 (0.17)	0.87 (0.18)	0.41 (0.17)	0.28 (0.01)	0.29 (0.10)	0.41 (0.12)	**135**	123	125
MAR	0.50 (0.16)	0.62 (0.19)	0.60 (0.18)	0.62 (0.17)	0.59 (0.15)	1.02 (0.31)	0.64 (0.21)	0.90 (0.24)	0.96 (0.26)	0.48 (0.14)	0.39 (0.15)	0.50 (0.15)	0.62 (0.19)	0.46 (0.13)	**132**	121
MAX	0.41 (0.14)	0.48 (0.16)	0.50 (0.17)	0.21 (0.08)	0.19 (0.09)	1.06 (0.28)	0.27 (0.10)	0.90 (0.18)	0.95 (0.18)	0.43 (0.16)	0.34 (0.09)	0.45 (0.18)	0.48 (0.16)	0.41 (0.18)	0.53 (0.16)	**134**
MEX	0.81 (0.24)	0.88 (0.17)	0.91 (0.18)	1.08 (0.17)	1.00 (0.18)	1.35 (0.40)	1.10 (0.25)	0.04 (0.02)	0.25 (0.18)	0.83 (0.22)	0.94 (0.25)	0.76 (0.18)	0.83 (0.17)	0.86 (0.17)	0.90 (0.25)	0.89 (0.19)
MEY	0.10 (0.08)	0.17 (0.11)	0.19 (0.10)	0.52 (0.16)	0.49 (0.15)	0.90 (0.22)	0.45 (0.13)	0.74 (0.16)	0.80 (0.20)	0.12 (0.07)	0.39 (0.15)	0.14 (0.07)	0.17 (0.11)	0.36 (0.14)	0.48 (0.16)	0.39 (0.13)
MOR	0.42 (0.13)	0.54 (0.14)	0.56 (0.15)	0.27 (0.08)	0.25 (0.07)	1.13 (0.28)	0.29 (0.11)	0.91 (0.14)	1.02 (0.16)	0.45 (0.14)	0.41 (0.09)	0.47 (0.15)	0.54 (0.14)	0.38 (0.15)	0.59 (0.14)	0.23 (0.05)
NEO	0.42 (0.14)	0.54 (0.16)	0.56 (0.16)	0.14 (0.06)	0.12 (0.06)	1.07 (0.29)	0.21 (0.12)	0.91 (0.16)	0.97 (0.15)	0.48 (0.15)	0.36 (0.09)	0.47 (0.16)	0.54 (0.16)	0.43 (0.16)	0.55 (0.15)	0.10 (0.05)
OMO	0.39 (0.11)	0.50 (0.14)	0.53 (0.15)	0.50 (0.16)	0.52 (0.14)	0.94 (0.32)	0.52 (0.15)	0.83 (0.19)	0.83 (0.23)	0.41 (0.12)	0.32 (0.12)	0.39 (0.12)	0.50 (0.14)	0.34 (0.14)	0.15 (0.07)	0.41 (0.14)
ORI	0.54 (0.16)	0.66 (0.17)	0.63 (0.17)	0.21 (0.05)	0.19 (0.07)	1.10 (0.33)	0.27 (0.13)	1.04 (0.21)	1.15 (0.21)	0.52 (0.18)	0.43 (0.11)	0.54 (0.17)	0.66 (0.17)	0.45 (0.20)	0.57 (0.20)	0.21 (0.05)
POL	0.42 (0.12)	0.54 (0.14)	0.56 (0.14)	0.14 (0.05)	0.12 (0.05)	1.07 (0.29)	0.21 (0.10)	0.91 (0.17)	1.02 (0.17)	0.45 (0.14)	0.36 (0.08)	0.47 (0.15)	0.54 (0.14)	0.38 (0.17)	0.55 (0.15)	0.10 (0.04(
PUN	0.36 (0.11)	0.38 (0.11)	0.41 (0.12)	0.52 (0.21)	0.49 (0.19)	0.85 (0.23)	0.54 (0.18)	0.89 (0.15)	0.95 (0.17)	0.38 (0.13)	0.25 (0.10)	0.32 (0.11)	0.38 (0.11)	0.19 (0.07)	0.48 (0.12)	0.39 (0.19)
PUR	0.47 (0.14)	0.54 (0.14)	0.56 (0.14)	0.19 (0.06)	0.16 (0.07)	1.12 (0.32)	0.25 (0.14)	0.91 (0.17)	1.02 (0.17)	0.49 (0.15)	0.41 (0.10)	0.47 (0.15)	0.54 (0.14)	0.43 (0.17)	0.55 (0.15)	0.15 (0.05)
RUB	0.48 (0.16)	0.59 (0.17)	0.57 (0.16)	0.64 (0.20)	0.57 (0.20)	0.99 (0.32)	0.66 (0.22)	0.87 (0.23)	0.93 (0.27)	0.45 (0.15)	0.32 (0.13)	0.52 (0.17)	0.59 (0.17)	0.39 (0.13)	0.15 (0.05)	0.50 (0.20)
SCH	0.34 (0.12)	0.41 (0.13)	0.43 (0.15)	0.36 (0.12)	0.34 (0.09)	0.93 (0.26)	0.43 (0.17)	0.82 (0.16)	0.83 (0.16)	0.36 (0.12)	0.32 (0.11)	0.34 (0.10)	0.41 (0.13)	0.25 (0.09)	0.46 (0.11)	0.32 (0.08)
SIT	0.62 (0.28)	0.70 (0.25)	0.68 (0.24)	0.89 (0.33)	0.81 (0.30)	1.04 (0.33)	0.91 (0.37)	0.98 (0.21)	1.04 (0.21)	0.60 (0.25)	0.70 (0.29)	0.58 (0.22)	0.65 (0.22)	0.72 (0.28)	0.76 (0.26)	0.75 (0.32)
SMI	0.51 (0.18)	0.54 (0.15)	0.56 (0.15)	0.63 (0.23)	0.60 (0.21)	1.07 (0.36)	0.70 (0.28)	0.76 (0.13)	0.92 (0.16)	0.54 (0.18)	0.54 (0.21)	0.47 (0.17)	0.54 (0.15)	0.47 (0.19)	0.41 (0.10)	0.50 (0.21)
SPI	0.39 (0.15)	0.36 (0.11)	0.39 (0.12)	0.45 (0.15)	0.43 (0.13)	0.98 (0.33)	0.52 (0.21)	0.72 (0.14)	0.83 (0.18)	0.36 (0.14)	0.36 (0.14)	0.29 (0.11)	0.36 (0.11)	0.30 (0.11)	0.46 (0.15)	0.32 (0.12)
WIL	0.48 (0.15)	0.60 (0.17)	0.62 (0.18)	0.21 (0.06)	0.14 (0.06)	1.14 (0.27)	0.27 (0.10)	1.02 (0.17)	1.08 (0.16)	0.51 (0.17)	0.42 (0.09)	0.53 (0.19)	0.60 (0.17)	0.49 (0.19)	0.61 (0.16)	0.16 (0.07)
PNC	5.18 (1.05)	5.15 (1.02)	5.25 (1.08)	5.15 (1.04)	5.30 (1.07)	5.22 (1.11)	5.18 (1.07)	4.97 (0.95)	5.15 (1.03)	5.27 (1.07)	5.34 (1.15)	5.18 (1.01)	5.15 (1.02)	5.13 (1.04)	4.82 (1.04)	5.10 (1.06)
PNS	5.66 (0.88)	5.51 (0.79)	5.49 (0.77)	5.75 (0.94)	5.66 (0.88)	5.96 (0.96)	5.54 (0.88)	5.68 (0.77)	5.63 (0.76)	5.75 (0.90)	5.58 (0.87)	5.54 (0.79)	5.51 (0.79)	5.73 (0.92)	5.78 (0.81)	5.58 (0.93)
	ABI	**ASP**	**AUR**	**BIC**	**BRA**	**BRE**	**CHI**	**ENG**	**GLA**	**GLE**	**JEZ**	**KOR**	**KOY**	**LIK**	**MAR**	**MAX**

restriction fragments shared by OTU's (above diagonal) and number of scored fragments per species (diagonal, blocked letters). For

120	134	127	127	126	124	127	128	126	124	128	123	125	128	127	53	52
118	132	124	124	123	121	124	127	124	121	126	121	124	127	124	53	53
117	131	123	123	122	121	123	126	123	121	125	121	123	126	123	52	53
114	124	130	133	123	131	133	124	132	120	127	117	122	125	133	53	51
116	125	131	134	123	132	134	125	133	122	128	119	123	126	135	52	52
108	115	111	112	113	111	112	116	111	112	114	113	112	113	112	52	49
114	126	130	132	123	130	132	124	131	120	126	117	121	124	132	53	53
138	120	117	117	117	114	117	117	117	116	118	116	120	120	116	55	52
132	118	114	115	116	111	114	115	114	114	117	114	116	117	114	53	52
119	133	126	126	125	124	126	127	125	124	127	123	124	127	126	52	51
116	126	126	127	126	125	127	129	126	126	127	120	123	126	127	51	52
121	133	126	126	126	124	126	129	126	123	128	124	126	129	126	53	53
119	132	124	124	123	121	124	127	124	121	126	122	124	127	124	53	53
118	127	127	126	126	125	127	131	126	125	129	120	125	128	126	53	51
116	123	121	122	129	121	122	123	122	129	123	118	125	123	122	55	50
117	126	130	133	124	130	133	126	132	122	127	119	124	127	133	53	52
140	121	118	118	118	115	118	118	118	117	119	117	121	121	117	55	52
0.74 (0.17)	**136**	127	127	126	124	127	128	127	124	128	124	127	130	127	54	53
0.90 (0.15)	0.40 (0.12)	**137**	134	124	131	134	126	133	122	128	121	124	127	133	53	52
0.90 (0.18)	0.40 (0.13)	0.12 (0.05)	**137**	125	133	136	127	135	123	130	121	125	128	136	53	52
0.82 (0.21)	0.36 (0.11)	0.48 (0.14)	0.43 (0.14)	**133**	123	125	126	125	130	126	120	126	126	125	54	50
1.03 (0.22)	0.52 (0.14)	0.23 (0.08)	0.14 (0.05)	0.50 (0.19)	**136**	134	124	133	121	127	120	123	126	134	54	53
0.90 (0.18)	0.40 (0.11)	0.12 (0.05)	0.04 (0.03)	0.43 (0.15)	0.10 (0.04)	**137**	127	136	123	130	120	126	129	136	54	52
0.88 (0.17)	0.34 (0.10)	0.45 (0.17)	0.40 (0.18)	0.36 (0.12)	0.52 (0.21)	0.40 (0.18)	**136**	126	126	129	121	127	128	127	52	51
0.90 (0.18)	0.40 (0.11)	0.16 (0.07)	0.08 (0.06)	0.43 (0.15)	0.14 (0.06)	0.04 (0.04)	0.45 (0.17)	**137**	123	129	121	127	129	135	54	52
0.87 (0.25)	0.45 (0.15)	0.57 (0.19)	0.52 (0.21)	0.13 (0.07)	0.59 (0.25)	0.52 (0.21)	0.36 (0.10)	0.52 (0.21)	**133**	124	119	126	124	123	53	49
0.81 (0.18)	0.32 (0.12)	0.34 (0.12)	0.25 (0.10)	0.34 (0.09)	0.36 (0.12)	0.25 (0.09)	0.27 (0.08)	0.29 (0.10)	0.43 (0.14)	**135**	122	126	129	130	53	51
0.97 (0.22)	0.56 (0.23)	0.72 (0.30)	0.72 (0.30)	0.68 (0.24)	0.74 (0.25)	0.76 (0.31)	0.70 (0.27)	0.72 (0.29)	0.73 (0.30)	0.63 (0.24)	**138**	121	123	122	54	52
0.76 (0.15)	0.40 (0.12)	0.56 (0.24)	0.51 (0.22)	0.39 (0.13)	0.58 (0.25)	0.47 (0.22)	0.40 (0.18)	0.42 (0.21)	0.39 (0.15)	0.43 (0.13)	0.72 (0.31)	**137**	129	125	55	50
0.72 (0.16)	0.23 (0.08)	0.38 (0.15)	0.34 (0.14)	0.34 (0.11)	0.41 (0.16)	0.29 (0.13)	0.32 (0.10)	0.29 (0.13)	0.43 (0.17)	0.25 (0.12)	0.58 (0.24)	0.29 (0.14)	**135**	128	55	53
1.01 (0.19)	0.46 (0.14)	0.23 (0.06)	0.10 (0.03)	0.49 (0.15)	0.16 (0.03)	0.10 (0.03)	0.46 (0.20)	0.14 (0.03)	0.58 (0.21)	0.31 (0.10)	0.73 (0.29)	0.57 (0.22)	0.40 (0.13)	**140**	53	52
5.02 (0.96)	5.03 (0.98)	5.18 (1.06)	5.18 (1.06)	4.96 (1.00)	5.03 (1.07)	5.06 (1.03)	5.27 (1.07)	5.06 (1.03)	5.08 (1.05)	5.13 (1.02)	5.08 (1.00)	4.94 (0.98)	4.90 (0.96)	5.25 (1.03)	**117**	61
5.73 (0.77)	5.51 (0.78)	5.66 (0.94)	5.66 (0.94)	5.81 (0.92)	5.51 (0.92)	5.66 (0.91)	5.75 (0.93)	5.66 (0.91)	5.94 (0.96)	5.173 (0.98)	5.68 (0.90)	5.90 (0.90)	5.49 (0.78)	5.73 (0.92)	4.18 (0.64)	**132**
MEX	**MEY**	**MOR**	**NEO**	**OMO**	**ORI**	**POL**	**PUN**	**PUR**	**RUB**	**SCH**	**SIT**	**SMI**	**SPI**	**WIL**	**PNC**	**PNS**

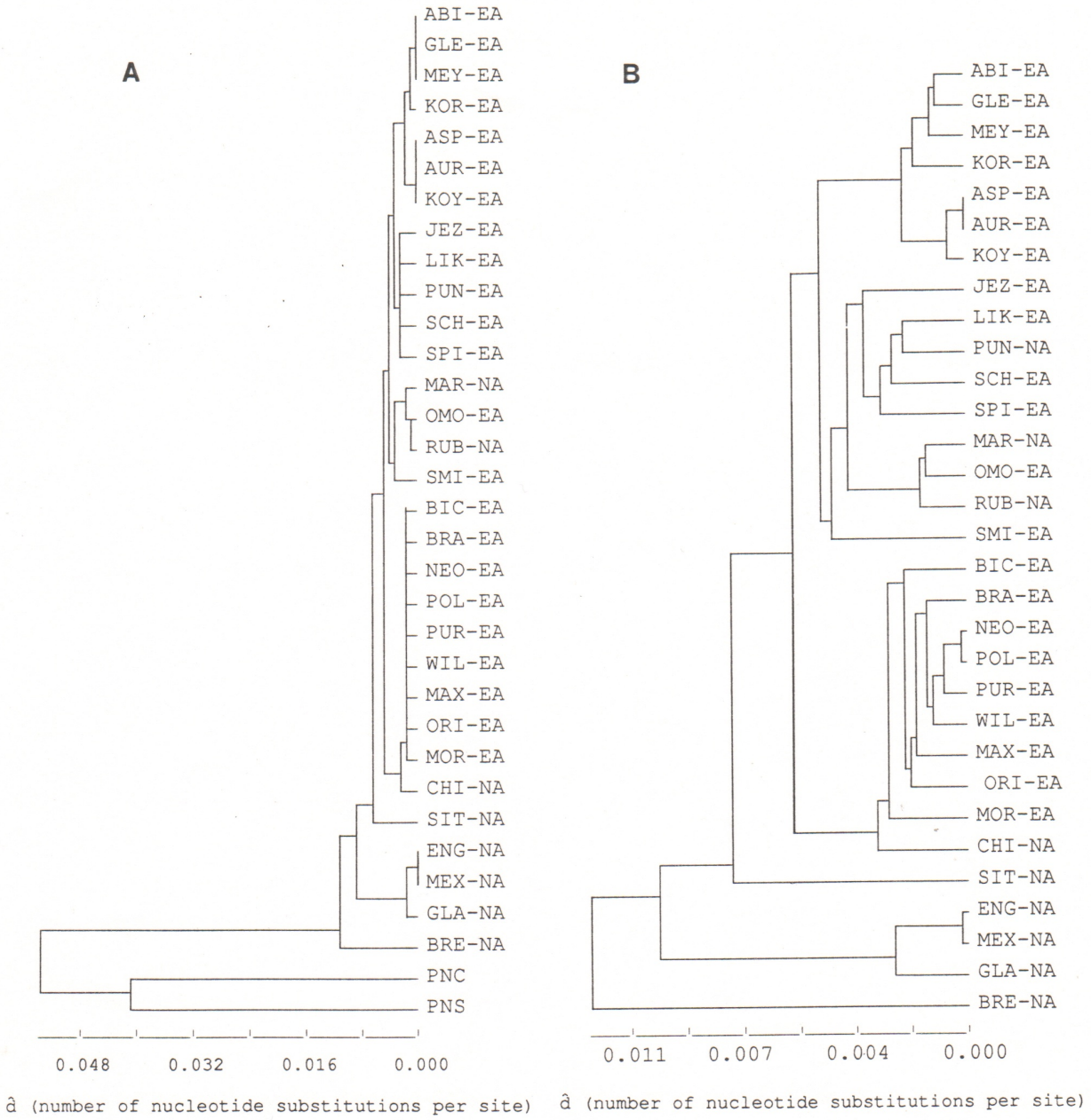

Fig. 1. UPGMA phylogenetic tree. – A: For *Picea* and *Pinus* species. – B: For *Picea* species only. For abbreviations of species names, refer to Tab. 1. Continental distributions are denoted by NA: North America and EA: Eurasia.

Picea species) was noted for 28 of these fragments, and shared, partial absence for 60 fragments. The documentation of informative and uninformative restriction fragments can be obtained from the senior author upon request.

Phenetic analysis

The number of nucleotide substitutions per site (weighted â values) between all pairs of species are summarized in Tab. 2. Within *Picea*, $\hat{a} \times 100$ values range from 0.02 to 1.37. The greatest differences noted for any pairwise comparison of *Picea* species were

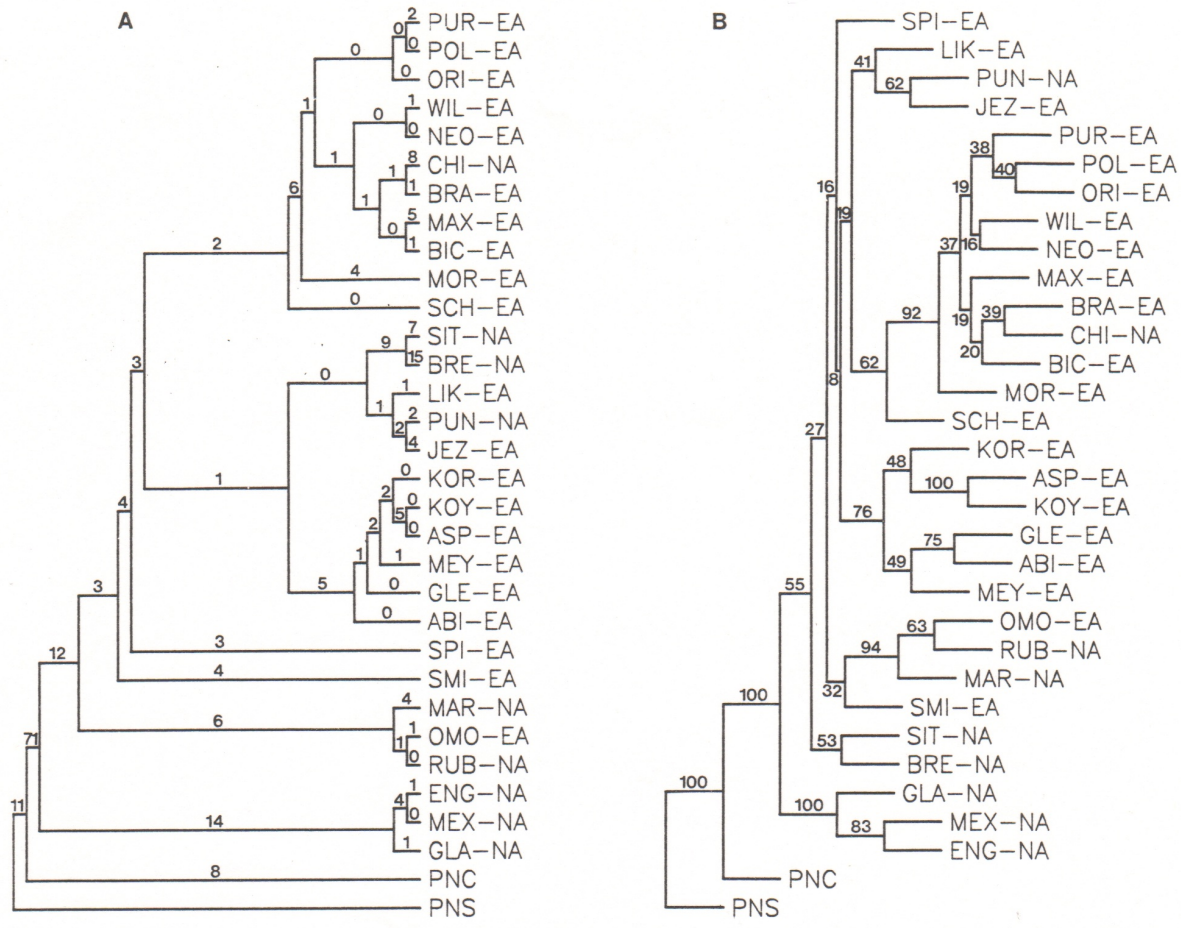

Fig. 2. A: Strict concensus tree for *Picea*, based on the 70 most parsimonious Wagner trees. Numbers at nodes denote the number of steps (reversions in character states). – B: Majority-rule consensus tree constructed with the use of bootstrap, with 100 replicate runs. Numbers at nodes refer to the percentage of bootstrap samples where this branching was observed.

those between *P. breweriana* and *P. glauca* (1.37); *P. breweriana* and *P. mexicana* (1.35); and *P. breweriana* and *P. engelmanni* (1.30). The smallest differences noted were between *P. asperata* and *P. aurantiaca* (= *P. asperata* var. *aurantiaca*) (0.02), *P. engelmanni* and *P. mexicana* (= *P. engelmanni* var. *mexicana*) (0.04) and *P. asperata* and *P. koyamai* (0.04). The $\hat{d} \times 100$ values were substantially greater between *Pinus cembra* and *P. sylvestris* than between the most divergent pair of *Picea* species (4.18 and 1.37, respectively). The $\hat{d} \times 100$ values for *Picea* species versus *Pinus cembra* and *P. sylvestris* ranged from 4.82 to 5.34 and 5.49 to 5.96, respectively. No significance can be placed on the apparent tendency for *P. cembra* to associate more closely with *Picea* spp. than did *P. sylvestris*, in light of the wide standard error (0.76–1.15) for pairwise estimates of $\hat{d} \times 100$ in these cases (Tab. 2).

We cannot dismiss the possibility that single length mutations have been scored twice in our analyses (when applying different restriction enzymes). As a result, $\hat{d}$ values may be biased somewhat upwards. Since some smaller fragments went undetected in our analyses, a further error in this estimate may occur. These values should be viewed as relative values for the group examined, and not as accurate estimates of nucleotide sequence divergence.

The UPGMA phenogram indicated several *cp*DNA groups within *Picea*, all clustering below a level from which the two *Pinus* species diverge (Fig. 1a). The phenetically most discrete *Picea* species was *P. breweriana*, followed by a group comprising *P. engelmanni*, *P. glauca* and *P. mexicana*, hereafter referred to as the "*P. glauca* alliance". *P. sitchensis* formed the third, phenetically discrete group on the UPGMA tree. The fourth group consisted of the remaining 26 species (see Fig. 1b).

Three main species clusters were nested within the fourth species group. Of these, one very cohesive sub-

group comprised *P. bicolor, P. brachytyla, P. chihuahuana, P. maximowiczii, P. morrisonicola, P. neoveitchii, P. orientalis, P. polita, P. purpurea* and *P. wilsonii* (hereafter: the "*P. brachytyla*" alliance). The second, more heterogeneous subgroup, was composed of *P. mariana, P. omorika, P. rubens* and *P. smithiana, P. jezoensis, P. likiangensis, P. pungens, P. schrenkiana* and *P. spinulosa*. The third, relatively weakly differentiated subgroup comprised *P. abies, P. asperata, P. aurantiaca, P. glehnii, P. koraiensis, P. koyamai* and *P. meyeri* (hereafter: the "*P. abies* alliance").

Cladistic analysis

The 70 most parsimonious Wagner trees each require 243 reversions in character states, which suggests 243 − 143 = 100 homoplasious steps. Out of these 243 reversions, 148 involved gains and 95 losses. Reversions occurring at the node connecting *Pinus* and *Picea* lineages were 71. The consistency index (Kluge & Farris 1969) was 59% (143/243). The "homoplasy excess ratio maximum" (HERM; Archie 1989) was 76% and the homoplasy excess ratio (HER) estimated from this value via equation 5 of Archie (1989) was 60%. Fig. 2a shows the strict consensus Wagner tree, and Fig. 2b the majority rule consensus tree resulting from the Wagner parsimony bootstrap. Lineages identified by Wagner parsimony generally corresponded to *cp*DNA groups identified by UPGMA. There were, however, instances where topologies differed between the phenogram and the cladograms.

One of these concerned *P. sitchensis* and *P. breweriana*. On the UPGMA tree, each of these species were discrete groups (Fig. 1b). On the strict consensus Wagner tree, however, these species represented branches within a lineage comprising *P. breweriana, P. jezoensis, P. likiangensis, P. pungens* and *P. sitchensis* (Fig. 2a). The assignment of these species to a *cp*DNA clade was not, however, supported by bootstrap (Fig. 2b).

Only four nodes on the Wagner tree were resolved at the 0.01 probability level (Fig. 2b). All 30 *Picea* species included in the analysis were resolved within a clade, separate from both *Pinus cembra* and *P. sylvestris*. *P. glauca, P. engelmanni* and *P. mexicana* formed a basal clade within *Picea*, supported by all 100 bootstrap samples. As noted above, *P. asperata, P. auriantiaca* and *P. koyamai* produced identical character state data of phylogenetically informative fragments, and hence *P. aurantiaca* was eliminated from subsequent analyses. *P. asperata* and *P. koyamai* (and, by inference *P. aurantiaca*) were resolved as a clade in all 100 bootstrap samples. Species groups which came close to being identified as clades at the 0.01 probability level included *P. mariana, P. omorika* and *P. rubens* (forming a clade in 94% of bootstrap samples), and the *P. brachytyla* alliance (in 92% of bootstrap samples).

Discussion

Intergeneric comparisons

Wright (1955) maintained that morphological variation within *Picea* is comparable to that found within a single series in *Pinus*. The lower level of *cp*DNA divergence observed phenetically among the 31 *Picea* species is in agreement with Wright's observation in suggesting greater phylogenetic cohesiveness of this genus relative to *Pinus*. Although we have only included two *Pinus* species in our analyses, these species should represent well the variation found within the genus *Pinus*. Abundant systematic evidence supports the "natural" division of *Pinus* into two separate subgenera, and the placement of *P. sylvestris* into subgenus *Pinus*, and of *P. cembra* into subgenus *Strobus*. This evidence includes phenetic (Mirov 1967; Frankis 1988; Price 1989) and cladistic (Hart 1987) analyses of morphological characters, immunological evidence (Prager et al. 1976; Price et al. 1987) and restriction fragment analyses (Szmidt et al. 1988b; Strauss & Doerksen 1990; X.-R. Wang et al. 1991). Furthermore, a *Pinus* species should provide a more suitable outgroup for rooting a *Picea* phylogeny than members of other Pinaceae genera. Affinities between the two have been suggested on the basis of morphology (Hart 1987; Frankis 1988). Furthermore, *Picea* and *Pinus* species produce hybridization patterns more similar to one another than to some other Pinaceae genera, when employing probes from conserved regions of *cp*DNA (c.f. Lidholm et al. 1988).

The present data are not sufficient to distinguish between two alternative scenarios explaining lower levels of morphological and *cp*DNA variation in *Picea*. The first scenario implicates retarded rates of evolution in *Picea*, relative to *Pinus*, a hypothesis first advanced by Wright (1955; in the form of "Perhaps there is something inherent in the spruce germ plasm that resists change"). The second scenario invokes a late radiation of *Picea* species from their common ancestor, occurring after *Pinus* had begun to diversify into the extant subgenera.

A proper distinction between these alternative scenarios must await the accumulation of comprehensive sequence data. Such data could shed light on the question whether rates of *cp*DNA evolution are different between the two genera. Nonetheless, the latter scenario would appear more likely, in light of the fossil record. By the Late Jurassic (ca. 140–135 Ma) *Pinus* had emerged from the ancestral plexus which gave rise to extant genera of the Pinaceae (Alvin 1960; Miller 1976; Axelrod 1986). By the Mid-Cretaceous (40–50 Ma), most extant subsections of *Pinus* are well represented in the fossil record (Axelrod 1986). In contrast, the fossil record of *Picea* is uncertain before the Cenozoic: the oldest confirmed fossil find of an ancient *Picea* species is *P. eichhornii* from the Oligocene (38–25 Ma), from the western United States (Miller 1989).

Phylogenetic implications

Earlier, rather discordant phylogenetic inferences for *Picea* have been derived independently from three main lines of evidence: morphology, patterns of crossability and chemical composition. A comprehensive, comparative synthesis from these lines of evidence is however not available (Page & Hollands 1987). *Picea* species are cytogenetically identical (Hizume et al. 1988) and this has prevented the use of karyotype analysis for phylogenetic inference in the genus (see Price 1989, and references therein). Assignments of *Picea* species to supraspecific groups on the basis of morphological criteria do not concur among authors. This lack of consensus is best explained by disagreement among taxonomists on what characters should be considered relevant for the classification of the genus (see Schmidt-Vogt 1977; Aldén 1987, for review). As a corollary, the most commonly proposed sections (*Eupicea, Omorika* and *Casicta*) are of uncertain interrelationship. Some authors, e.g. Wright (1955), recognize no sectional divisions within the genus. Apart from the work of Cheng et al. (1975), Wright (1955) and Schmidt (1989), most classifications for the genus have relied on few, easily scorable characters from gross morphology for circumscribing supraspecific taxa. The remarks of Wright (1955) suggest that the abundance of parallelism and convergence in these characters may compromise the use of these schemes as general-purpose classifications, reflecting phylogeny. Indeed the systematic validity of some classications may be questioned in light of evidence that the character most commonly used for delimiting sections; the morphology of the cone scale, is polymorphic in alternative character states within *P. abies* (Schmidt 1989).

We note agreement between the *cp*DNA results and the classification schemes of Cheng et al. (1975) and Wright (1955). These schemes were based on many characters (27 and 32, respectively), and Wright (1955) additionally included a consideration of distributional and crossability data. Several species groups delimited by these schemes are mirrored in the conclusions of the present study, including the *P. glauca* alliance, the *P. abies* alliance and the *P. brachytyla* alliance.

Wright (1955) considered *P. purpurea* to be morphologically closer to certain species of the *P. brachytyla* alliance than to *P. likiangensis*, despite its commonly-accepted varietal status as *P. likiangensis* var. *purpurea*. *P. purpurea* also shows nearer *cp*DNA affinities to the *P. brachytyla* alliance than to *P. likiangensis* (see Figs 1a,b, 2a,b). These lines of evidence suggest that *P. likiangensis*, as currently circumscribed, is paraphyletic.

Species of the *P. abies* alliance have earlier been reported to share several morphological features (Wright 1955; Rushforth 1987; Schmidt 1989) and to exhibit high interfertility in artificial crossing experiments (Mikkola 1969). The very close similarity between *P. asperata* and *P. aurantiaca* at the *cp*DNA level agrees with the placement of *P. aurantiaca* as a variety or ecotype of *P. asperata* (Schmidt-Vogt 1977).

Species of the *P. glauca cp*DNA alliance are all native to North-America. These species are commonly assigned to separate sections, on the basis of their distinguishing cone scale and branch morphology (Lacassagne 1934; Gaussen 1966; Bobrow 1970; Aldén 1987; Schmidt 1989). Fowler's (1983) subsection *Glaucoides* (designated on the basis of high cross-compatibility) encompasses these species, however. Their evolutionary affinities, as reflected here in their placement within a common *cp*DNA clade, concur with the conclusions of T. M. C. Taylor (1959), where *P. engelmanni* has been given subspecific rank to *P. glauca* (*P. glauca* spp. *engelmanni* Taylor). Furthermore, R. J. Taylor & Patterson (1980), give *P. mexicana* varietal rank to *P. engelmanni*, as *P. engelmanni* Parry var. *mexicana* (Martinez) Silba.

Our results indicate near evolutionary affinities between *P. omorika, P. mariana* and *P. rubens*. Morphologically, *P. omorika* differs from *P. mariana* and *P. rubens* in having flattened cross-sectional needle form and arrangement of stomata on the lower surface of needles. On this basis *P. omorika* is assigned to section *Omorika* while *P. mariana* and *P. rubens* are generally assigned to other sections (*Picea* or *Eupicea*) (e.g. Schmidt-Vogt 1977). The close relationship between *P. mariana* and *P. rubens* is widely accepted (Gordon 1976). *P. omorika* and *P. rubens* are among the few *Picea* species that can be crossed with *P. mariana* (Mikkola 1969; Gordon 1976, 1990). In addition *P. omorika* and *P. mariana* share several features rare within the genus, such as cone serotiny (Wright 1955) and tolerance of swampy soils (Rushforth 1987).

P. chihuahuana is morphologically distinct and reproductively isolated from other North-American species (R. J. Taylor & Patterson 1980). A morphological affinity to the *P. brachytyla* alliance of East Asia, especially *P. polita*, has however been noted (Gordon 1968; R. J. Taylor & Patterson 1980). *P. maximowiczii* (from Japan) and *P. orientalis* (from Caucasus) have been found to cross with ease, although both are reported to cross poorly with species from outside this *cp*DNA alliance (Gordon 1986). This evidence for evolutionary affinities in this species group is corroborated by evidence from the present study.

Our results indicate *cp*DNA affinities between *P. breweriana, P. jezoensis, P. likiangensis, P. sitchensis* and *P. pungens* (Fig. 2a). Although this group is not supported as a clade by bootstrap, it is in accord with the common assignment of the first four species to section *Omorika* (Schmidt-Vogt 1977), and similarities among the five species in needle micromorphology (unpublished work cited in Page & Hollands 1987), and crossability data (Mikkola 1969).

Although the clustering of species within groups was to a large degree consistent between the cladogram and the phenogram, topologies sometimes differed. Two

causes may explain this discordance. Firstly, rates of *cp*DNA evolution may be different among lineages. UPGMA assumes that rates are constant in separate lineages, but Wagner parsimony is less susceptible to distortion due to rate heterogeneity (Nei 1987). Secondly, the data used differed between the two analyses. All scored fragments were analyzed phenetically, while only informative fragments were analyzed cladistically.

Geographic partitions of cpDNA variation

Wright (1955) postulated that *Picea* had its origin in East-Asia, from where it later colonized its present range throughout Europe, Asia and North America. His postulate was based on the large number of endemic species in East-Asia, and the presence of *P. koyamai*, which he considered synonymous with *P. koraiensis*. He argued that *P. koyamai* represented a likely choice for the most "primitive" species in the genus, due to its "generalized" taxonomic characters and interfertility with five other species. Wright's postulate of an Asian origin for *Picea* emerged as an oft-cited and widely accepted working hypothesis for the evolutionary origin of *Picea* (e.g. Moir & Fox 1977; O'Driscoll 1977; Page & Hollands 1987). Although the premises for his postulate have been questioned (Mikkola 1969; Schmidt-Vogt 1977; Aldén 1987), they have not been tested to date.

The continental partitioning of *cp*DNA diversity, reported here, allows an interpretation which contradicts Wright's conclusions (1955) and suggests the origin and primary diversification of *Picea* in North America. We find North-American species branching closest to the hypothesized root of the genus on the cladogram. Furthermore, *cp*DNAs of North-American species are phenetically more divergent inter se than those of Asian species. Neither of these results would implicate Asia as the center of diversity for *Picea*. We find no support from *cp*DNA for the designation of *P. koyamai* (= *P. koraiensis*) as a "primitive" species. The *cp*DNA evidence suggests close evolutionary affinity to several widespread Eurasian species, including *P. asperata* and *P. aurantiaca*, with which it forms a significant clade. It should be noted, furthermore, that the five species with which *P. koyamai* could be crossed in Wright's investigation, are all members of the *P. abies cp*DNA alliance.

Schmidt-Vogt (1977) suggested that all 22 *Picea* species described from China could be combined within one of three species: *P. asperata*, *P. likiangensis* and *P. brachytyla*. Aldén (1987), Dallimore & Jackson (1966) and Wright (1955) have also pointed out the unsatisfactory taxonomy of many Asian *Picea* species, and suggested that many of these would be reduced to synonymity if subjected to a more rigorous taxonomic treatment. Near *cp*DNA affinities among Asian species, especially those within the *P. brachytyla* alliance, together with the profusion of taxonomic species in Asia, may reflect taxonomic artifact. Many of these species grow in inaccessible regions and are therefore represented by meager collections (Wright 1955; Aldén 1987). Most taxonomic descriptions of these species are derived from cursory surveys carried out during a time when conceptual distinctions were not made between species and ecotypes (Schmidt-Vogt 1977). C. W. Wang (1961; not seen, cited in Schmidt-Vogt 1977) tenders the explanation that the current fragmented distribution of East-Asian *Picea* species is a result of the uplift of the Tibetan plateau, concomitant with the submergence of mountain ranges throughout insular East-Asia during the Pliocene (2–5 Ma). According to C. W. Wang (1961) these changes resulted in the contraction of the ranges of few, formerly widespread species to smaller, disjunct populations.

A close agreement between *cp*DNA affinities and their occurrence in adjacent geographic ranges is observed among certain species groups, e.g. the *P. abies* alliance and the *P. glauca* alliance. Nonetheless, the division of the genus on the basis of *cp*DNA affinities does not follow closely boundaries between Eurasia and North America. The geographic distribution of the *cp*DNA lineages rather suggests that intercontinental migrations have occurred after the original spread of *Picea* between continents. Species native to separate continents are observed tightly nested within common *cp*DNA clades. An example of this involves *P. omorika*, an endemic confined to a small range in Southern Europe, and the widespread and partially sympatric *P. mariana* and *P. rubens*, native to North America. As an explanation for the present, disjunct distribution of this clade, vicariance would appear more likely than long-range dispersal. According to fossil evidence, species closely resembling *P. omorika* and *P. rubens* in morphology were widespread in Eurasia prior to the onset of Quaternary glaciations (Weber 1898; Müller-Stoll 1938; Szafer 1954). Another case of trans-continental *cp*DNA-lineage distributions concerns the Mexican *P. chihuahuana* and the remainder of the *P. brachytyla* alliance, distributed throughout Asia. *P. chihuahuana* is considered a relict species with a very restricted range in Mexico.

Cronquist (1988) states that a particular species should be expected to show ecogeographic coherence, reflecting genetically-determined limits in ecological amplitude. As a corollary to this line of thought, separate species belonging to a monophyletic group should be expected to show similarities in habitat preference, reflecting suites of adaptations derived through synapomorphy (Wanntorp 1983; Dobson 1985; Olmstead 1989). Association between *cp*DNA affinities and similarities in ecogeographic preference is suggested by the present results. Climates within the ranges of all species within the *P. brachytyla* alliance, including the Mexican *P. chihuahuana*, are characterized by "quasioceanity" with prevailing cool temperatures associated with small seasonal amplitude (c.f. Schmidt 1989; Ohsawa 1990). In contrast, the *P. abies* alliance occupies the more

strongly seasonal, boreal forest zones of Eurasia, north of the range of the *P. brachytyla* alliance. Kuan (1981) tenders the explanation that the southward distribution of species in China assigned here to the *P. abies* alliance is limited by their high light demand during the summer.

Reliability of the data

Deriving *cp*DNA phylogenies through our approach to scoring restriction data (FDA) may bring about several sources of potential error. The first factor which may bring about bias in our results are possible homologous recombination events during the evolution of *Picea* *cp*DNA. Among most groups of higher plants the cp genome is highly conserved in structure and organization (Palmer 1985; Palmer & Stein 1986). Conifer *cp*DNA lacks the inverted repeat structure, present among most plant groups (Lidholm et al. 1988; Strauss et al. 1988; White 1990; White et al., in press). Conifer *cp*DNA has been reported to exhibit unique duplications of transcribed gene sequences (Lidholm et al. 1991) and to possess accumulations of dispersed repetitive sequences (Tsai & Strauss 1989; White 1990). These characteristics are considered likely to promote or to suggest the past occurrence of *cp*DNA rearrangement (Palmer & Thompson 1982; Palmer 1985). Such rearrangements could undermine the robustness of phylogenetic hypotheses based on unmapped restriction fragment data, such as those presented here. The occurrence of a single recombination event may affect many fragments scored and bias the derived phylogeny. Rearrangements of this kind would probably necessitate the construction of restriction maps for individual species prior to phylogenetic analysis (c.f. Sytsma et al. 1990). Based on the positions of mapped restriction sites among four of the species included in the present study (*P. abies*, *P. glauca*, *P. mariana* and *P. sitchensis*) no rearrangements are apparent (White et al., in press). As these species appear to represent well separated clades on the *cp*DNA phylogeny, this evidence would lessen the likelihood that restriction fragment differences observed between congeneric species in the present study could be accounted for by rearrangements.

Secondly, by inspection of restriction fragment profiles without probe hybridizations, it is not possible to ascertain the homology of two fragments of equal length. There is therefore a risk of erroneously scoring fragments coming from different parts of the genome as homologous, thereby inflating the level of homoplasy (Bremer 1991). The present study cannot clarify to what extent such fragments have been scored as homologous. Based, however, on hybridization data for six gene-specific probes, four endonucleases and all species included in the present study, we have not observed instances where non-homologous fragments had been identified as homologous (A. Sigurgeirsson, unpubl. data). In our mind, this source of error may represent a more serious problem in intergeneric comparisons, e.g. between *Picea* and *Pinus*.

Thirdly, Palmer et al. (1988) maintain that phylogenetic analyses of endonuclease-digested *cp*DNA should be based solely upon point mutations and should not include length mutations as these are prone to generate homoplasies. As pointed out by Bremer (1991), however, a single, small *cp*DNA deletion may affect several restriction sites. Both restriction sites and fragments may therefore be dependent characters and both can therefore generate homoplasies and cause problems in phylogenetic interpretation. The values obtained for CI, HER, and HERM in the present study are relatively high when compared to values earlier reported (Tab. 1 in Archie 1989; Tab. 1 in Sanderson & Donoghue 1989) and do not indicate rampant homoplasy, despite our use of restriction fragment data. The value obtained for CI is perhaps high in light of the large number of OTUs examined, as CI has been shown to decrease as the number of taxa included in the analysis increases (Archie 1989; Sanderson & Donoghue 1989).

As discussed by Bremer (1991), the choice of method for scoring restriction data involves a trade-off between accuracy and time (resources). Compared to other methods of scoring *cp*DNA RFLPs, the FDA approach used here allows the greatest savings in resources but may potentially yield the lowest accuracy. By comparisons between different methods applied to data from the family Rubiaceae, Bremer's (1991) findings indicate no appreciable bias in phylogenetic trees based on this approach. Moreover, separate studies conducted upon the same species groups have yielded concordant topologies of *cp*DNA-based phylogenetic trees regardless of whether the method employed was restriction profile inspection of purified *cp*DNA or restriction site analysis based on probe hybridizations (c.f. Hosaka et al. 1984 and Spooner et al. 1991, respectively, for *Solanum*).

A final factor which could compromise the use of *cp*DNA for deriving phylogenetic inference in *Picea*, regardless of RFLP scoring method or plant group, is introgression (Anderson 1949) and its evolutionary consequences, reticulation and phylogenetic sorting of *cp*DNA lineages. Introgression is recognized as a potentially confounding factor in phylogenetic inference (see e.g. Raven 1976; Grant 1981). The tendency of the *cp* genome to cross species boundaries in certain groups of angiosperms has suggested the need for caution in promoting the use of *cp*DNA variation for phylogenetic reconstruction (Smith & Sytsma 1990; Rieseberg & Soltis 1991; Rieseberg et al. 1991). That the same may apply to *Picea* or other conifers is not strongly supported by the available evidence (Wagner et al. 1987; Rieseberg and Soltis 1991; A. Sigurgeirsson, unpubl.). Nevertheless, there is reason for prudence in interpreting organismal phylogeny from *cp*DNA variation in *Picea*. The weak barriers to interspecific gene exchange and several hypothesized instances of introgression have lead to the suggestion that introgression has played

an important role in the evolution of *Picea* (Wright 1955; Bobrow 1972). Hence discordance between a *cp*DNA-derived lineage which only follows the evolution of a uniparentally inherited genome and the species ("true") phylogeny of *Picea* should be anticipated. In *Picea* and other conifer genera mitochondrial (*mt*) DNA is maternally inherited (Sutton et al. 1991) while *cp*DNA is paternally inherited (Szmidt et al. 1988a; Stine et al. 1989; Stine & Keathley 1990; Sutton et al. 1991). Owing to the separate modes of inheritance for these genomes in conifers, the validity of phylogenetic hypotheses derived from DNA data is testable. A comparison can be made between phylogenies derived separately for the same species group via two independent pathways of evolution; a matriarchal, *mt*DNA-derived one; and a patriarchal, *cp*DNA-derived one.

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