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GEOGRAPHICAL VARIABILITY OF MONOTERPENES FROM *ABIES BALSAMEA* AND *A. FRASERI*

EUGENE ZAVARIN and KAREL SNAJBERK
University of California, Forest Products Laboratory, Richmond, Calif. 94804, U.S.A.

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Abstract—Nearly 300 trees of *Abies balsamea* and *A. fraseri* from 29 locations covering the entire ranges of these species were examined for composition of their cortical monoterpenes. Eastern *A. balsamea* was intermediate in terms of most of its monoterpenes, with *A. fraseri* and western *A. balsamea* representing two opposite chemical extremes. Both *A. fraseri* and the westernmost populations of *A. balsamea* were found to possess poorer genetic pools than the eastern populations of *A. balsamea*. These findings were interpreted on the basis of available paleobotanical evidence as suggestive of long-time separation of the western and eastern *A. balsamea* in Rocky Mountain and Appalachian glacial refugia, respectively, with *A. fraseri* evolving from the eastern *A. balsamea* by gene-loss during the xerothermic period.

INTRODUCTION

Abies balsamea (L.) Mill. and *A. fraseri* (Pursh) Poir. are two North American firs which together with the western *A. lasiocarpa* (Hook.) Nutt. form a group of three closely related species. In Canada, *A. balsamea* ranges from the central Alberta in the west to Labrador and Newfoundland in the east south of the 60th parallel (Fig. 1). In the United States it occurs in Minnesota, Wisconsin, Michigan, Iowa, northern Pennsylvania, New York, and the New England states. *Abies balsamea* occupies generally lower elevations than *A. lasiocarpa*, ranging from sea level to about 5000 ft; it is a small- to middle-sized tree with a slender, spire-like crown and smooth, blistery bark which becomes rough in its older age.[1] The morphologically similar *A. fraseri* is a higher-elevation species, occurring in the Appalachian Mountains of southwestern Virginia, western North Carolina, and eastern Tennessee between 4000 and 6000 ft.[2] Several isolated fir populations of a status intermediate between the two above species occur in northern Virginia and West Virginia.

At the present time the taxonomic status of the three taxa mentioned can be regarded as settled in the main features only, with most taxonomists accepting them on species level, although in the past *A. fraseri* was classified as *A. balsamea* var. *fraseri* Nutt.,[3] and even in recent times a proposal has been made to consolidate *A. lasiocarpa* and *A. balsamea* into one *A. balsamea*, species.[4] The morpholigical distinction of the three is not too clear, however. Separation of *A. lasiocarpa* and *A. balsamea* is based mainly on the distribution of leaf stomata and color and shape of leaves,[5] while *A. fraseri* and *A. balsamea* are best distinguished by the number of hypodermal cells and the bract-to-scale ratio of cones; in

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[1] *Silvics of Forest Trees of the United States*, compiled and revised by H. A. Fowells, Agr. Handbook No. 271, U.S. Dept. of Agr., Forest Service, 1965, Washington, D.C., p. 10.

[2] E. L. LITTLE, JR., *Check List of Native and Naturalized Trees of the United States*, Agr. Handbook No. 41, U.S. Dept. of Agr., Forest Service, 1953, Washington, D.C., (a) p. 27; (b) p. 29.

[3] W. H. LAMB, *Proc. Soc. Am. For.* **9**, 528 (1914).

[4] B. BOIVIN, *Nat. Can.* **86**, 219 (1959).

[5] R. C. HOSIE, *Native Trees of Canada*, pp. 88, Dept. of Fisheries and Forestry, Canad. Forestry Service, Ottawa (1969).

[6] J. F. ROBINSON and E. THOR, *Forest Sci.* **15**, 238 (1969).

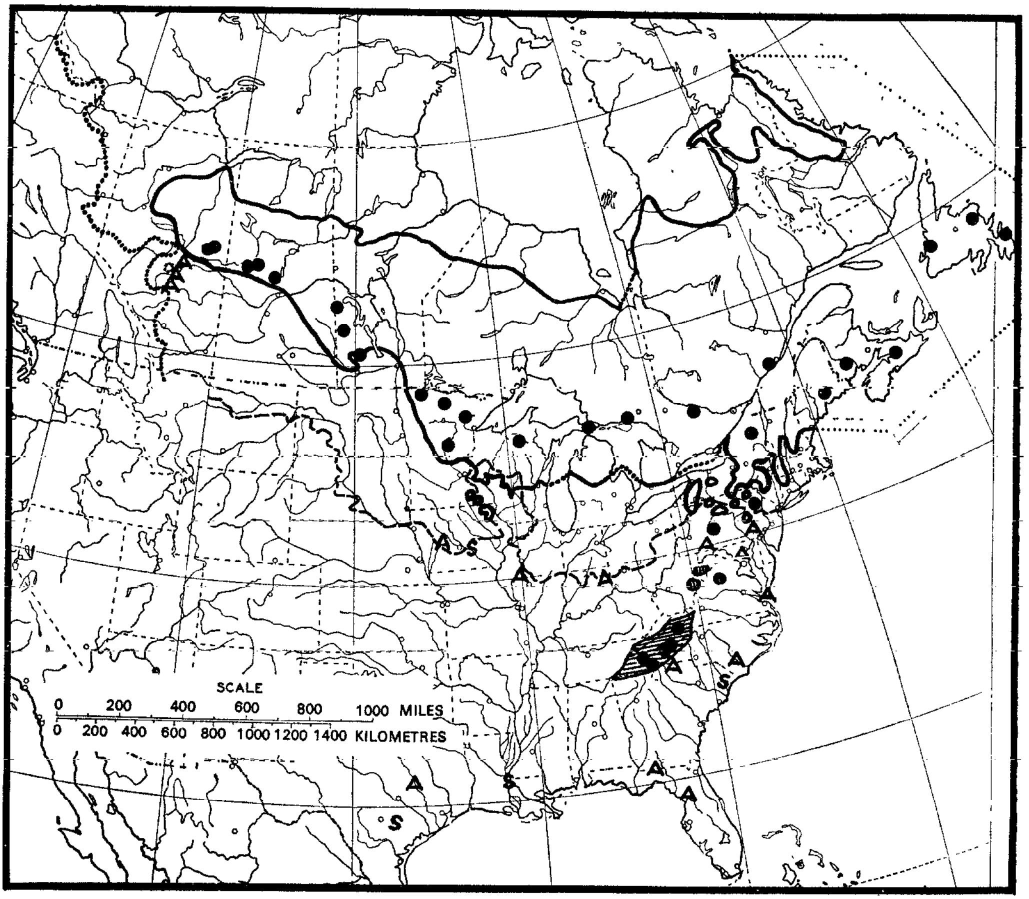

Fig. 1. Ranges of *A. balsamea* (heavy lines), *A. fraseri* (horizontal lines) and intermediate populations (vertical lines) drawn according to refs. 1, 5, and 59. The populations sampled are indicated in black. The dotted lines indicates easternmost extension of *A. lasiocarpa*. Broken line delineates maximal extension of the Wisconsin glaciated areas,[24] with letters denoting areas where *Abies* (A) or *Picea* (S) pollen or other fossils of Wisconsin age were identified.

A. fraseri the bract is much longer than the cone scale and protrudes outside, while in *A. balsamea* the bract is much shorter and is hidden beneath the cone scale.[6] The taxonomic situation is further complicated by the existence of *A. balsamea* var. *phanerolepis Fern.* with bract-to-scale ratio intermediate between those of *A. fraseri* and *A. balsamea* var. *balsamea.*[2,7] The trees belonging to var. *phanerolepis* are found in the eastern part of the *A. balsamea* range. In Canada they occur in the Province of Ontario and eastward, and are particularly common in Newfoundland.[5] In the United States they grow in Maine, New Hampshire, Vermont, and New York. In their study of geographic and altitudinal variations in phenotypic expressions of *A. balsamea*, Myers and Bormann reported the existence of an altitudinal gradient on Mount Washington, New Hampshire, with *var. phanerolepis* predominating at higher altitudes; they interpreted their results on geographic

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[7] O. Myers, Jr. and F. H. Bormann, *Ecology* **44**, 429 (1963).

variability as being due to the existence of an east-west cline, possibly maintained by the precipitation patterns.[7] In a later study Lester[8] re-examined the variation in cone morphology of the 49 populations sampled from Saskatchewan to Nova Scotia, and reported moderate but statistically significant correlations with annual precipitation and temperature variations.

Two intermediacy problems are connected with the *A. lasiocarpa*/*A. balsamea*/*A. fraseri* species. As mentioned, trees from northern Virginia and West Virginia are similar to those of var. *phanerolepis* from the northern states and Canada; some authors classify them as such.[2,9] Other believe them to be intermediates of *A. balsamea* and *A. fraseri*;[2,10,11] still others regard them as relics of a recently broken-up north-to-south cline,[6] and in the past some regarded them even as a separate species—*A. intermedia.*[12] The second intermediacy problem exists in the west where *A. lasiocarpa* meets *A. balsamea* (essentially in Alberta) and the two intergrade.

The present report represents a continuation of our investigations of the North American fir species using chemical and quantitative taxonomic methods, and explores the problems of the geographic variability of *A. balsamea* and *A. fraseri*, as well as the relationship of the two species, on the basis of cortical monoterpene variability. The investigation of the *A. lasiocarpa*/*A. balsamea* intergrading populations, as well as that of the taxonomic status of the intermediate *A. balsamea*/*A. fraseri* populations from northern Virginia and West Virginia, will become the subject of our forthcoming communications.

The volatile fraction of *A. balsamea* cortical blister oleoresin was the subject of numerous chemical investigations, as the oleoresin 'Canada Balsam' represents an important commercial product.[13] The bulk of the constituents form monoterpene hydrocarbons, composed of α-pinene (17·0%), camphene (tr), β-pinene (31·0%), 3-carene (4·0%), myrcene (0·5%), limonene (28·5%), β-phellandrene (19·0%) and terpinolene (tr). However, a markedly different composition has been reported for *A. fraseri* monoterpenes with α-pinene (59·0%), camphene (0·5%), β-pinene (21·5%), 3-carene (12·5%), sabinene (tr), myrcene (0·5%), limonene (4·0%), β-phellandrene (1·0%), and terpinolene (1·0%).[14] Oxygenated monoterpenes and sesquiterpenes apparently represent a minor portion of either oleoresin, and exhibit no striking differences.[15]

RESULTS

This report is based on chemical analyses of cortical monoterpene hydrocarbons from 242 *A. balsamea* trees sampled in 24 geographically different locations and from 56 *A. fraseri* trees sampled in 5 locations (Table 1). The analyses cover the entire range of either species, aside from areas of morphological intermediacy and more northern and inaccessible latitudes. No monoterpene components different from those reported previously were found, although an impressive quantitative variability in monoterpene composition was encountered. Results for the populations from geographically extreme areas of *A. balsamea*

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[8] D. T. Lester, *Rhodora* **70**, 80 (1968).
[9] F. R. Fosberg, *Virginia J. Sci.* **2**, 106 (1941).
[10] E. L. Core, *Torreya* **34**, 92 (1934).
[11] G. S. Ramseur, *Assoc. Southeast. Biol.* **8**, 31 (1961).
[12] E. H. Fulling, *So. Appalachian Bot. Club* **1**, 91 (1936).
[13] *Die Aetherischen Oele*, E. Gildemeister and Fr. Hoffman, Rev. by W. Treibs and K. Bournot, Akademie Verlag, Berlin (1956).
[14] E. Zavarin and K. Snajberk, *Phytochem.* **4**, 141 (1965).
[15] L. A. Smedman, K. Snajberk, E. Zavarin and T. R. Mon, *Phytochem.* **8**, 1471 (1969).

TABLE 1. *Abies* POPULATIONS SAMPLED

Population No.	No. of samples	Empirical designation	Latitude		Longitude		Elevation	Collector
			Abies balsamea					
62	10	Green Lake, Sask.	54	18	107	49	2000	Snajberk
89	8	Sled Lake, Sask.	54	24·4	107	19·8	1666	Critchfield
52	10	Candle Lake, Sask.	53	50	105	18	2000	Snajberk
51	14	Armit River, Sask.	52	50	101	51	2000	Snajberk and Parker
48	14	Duck Mnt., Mann.	51	35	101	00	2400	Snajberk and Parker
49	14	Riding Mnt., Mann.	50	37	99	37	2300	Snajberk and Parker
73	10	Warroad H'way, Minn.	48	53	95	28	1400	Snajberk
72	10	Rainy River, Minn.	48	30	93	48	1200	Snajberk
70	10	Aitkin Lake, Minn.	46	30	93	43	1800	Snajberk
71	10	Laurentia Divide, Minn.	47	39	92	32	2000	Snajberk
69	10	Land O'Lakes, Wisc.	46	10	89	11	2000	Snajberk
68	10	Kinross, Mich.	46	07	84	31	1500	Snajberk
67	10	Espanola, Ont.	46	15	81	46	1200	Snajberk
66	10	Petawawa Forest, Ont.	46	00	77	27	1500	Snajberk
46	7	Saranac Lake, N.Y.	44	20	74	10	2000	Duffield
93	10	Quebec, Qu.	46	57	71	30	690	Wolff
47	7	Orono, Maine	44	57	68	38	150	Duffield
91	11	Fredericton, New Brunsw.	45	55	66	40	350	Fowler
92	10	Truro, Nova Sc.	45 45	14– 36	63 63	01– 32	100–1000	Cumming
94	11	Corner Brook, Newfoundl.	48	55	57	54	450	Nicholson
95	11	Gander, Newfoundl.	48	56	54	31	450	Nicholson
96	12	St. John, Newfoundl.	47	37	52	47	450	Sharpe and Earle
117	8	Promised Land Lake, Pa.	41	19·5	75	13·3	1720	Critchfield
80	5	Bear Meadows, Pa.	40	44	77	45·4	1820	Critchfield
			Abies fraseri					
78	8	Mount Rogers, Va.	36	39·6	81	32·7	5720	Critchfield
63	8	Roan Mnt., N.C.	36	05	82	05	6200	Duffield
53	10	Mount Mitchell, N.C.	35	45	82	15	6600	Duffield
64	22	Richland, N.C.	35	20	83	00	6200	Duffield
115	8	Newfound Gap, N.C.	35	36·4	83	26·9	5300	Critchfield

range-western from Candle Lake, Saskatchewan, and eastern from near St. John, Newfoundland, as well as for a population of *A. fraseri* from Roan Mountain, North Carolina, are given in Tables 2–4 on an individual tree basis. Tables 5 and 6 describe on a population basis the information obtained, using median and quartile statistics; use of the latter was necessitated by the nonparametric nature of much of the data obtained.

TABLE 2. COMPOSITION OF TERPENES FROM WESTERN *Abies balsamea*

Sample No.*	Candle Lake, Saskatchewan					Total terpenes
	α-Pinene	β-Pinene	Myrcene	Limonene	β-Phellandrene	
888	9·0	32·5	2·5	52·0	4·0	26·6
889	10·5	46·0	2·0	37·0	4·5	28·5
890	7·0	37·0	2·0	50·5	3·5	30·9
891	10·0	45·0	3·0	37·0	5·0	24·6
892	8·5	44·5	1·0	41·0	5·0	28·3
893	10·0	36·0	3·0	47·5	3·5	25·9
894	10·0	38·5	3·5	44·5	4·0	26·0
895	11·0	47·5	1·5	34·5	5·5	32·2
896	7·5	28·0	3·0	58·0	3·5	29·6
897	7·5	43·0	3·0	41·5	5·0	27·7
Mean	9·1	39·8	2·5	44·4	4·4	28·0

* No camphene, sabinene, α-phellandrene, 3-carene or terpinolene.

In terms of most monoterpenes, western *A. balsamea* populations and populations of *A. fraseri* apparently represent two opposite chemical extremes, with eastern *A. balsamea* somewhere in the middle. Thus, while limonene and β-pinene account for more than 80% of monoterpenes in the west, with α-pinene percentages small and 3-carene absent, the last two terpenes attain their maximal values in *A. fraseri*, with β-pinene content reduced by roughly half and with limonene either small or present in traces only. β-Phellandrene represents the only exception to this pattern—it occurs in largest quantities in the eastern

TABLE 3. COMPOSITION OF TERPENES FROM EASTERN *Abies balsamea*

Sample No.*	St. John, Newfoundland						Total terpenes
	α-Pinene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	
1642	10·2	27·2	0·0	2·0	23·4	37·1	32·3
1643	19·4	48·6	2·2	0·5	12·3	16·8	30·7
1644	47·1	18·2	2·1	1·0	1·9	29·3	30·3
1645	42·8	31·1	0·8	0·5	0·8	23·3	30·6
1646	56·2	35·7	5·5	0·2	0·4	1·4	25·5
1647	22·3	19·6	0·0	1·0	39·0	17·8	35·6
1648	15·5	29·5	0·9	1·1	20·5	32·2	33·0
1649	35·5	36·8	0·0	1·1	23·7	2·6	29·2
1650	47·8	22·4	20·6	1·2	0·6	5·9	30·6
1651	30·5	29·8	1·9	1·4	1·5	34·5	34·4
1652	16·9	54·1	2·7	1·0	20·4	4·7	34·1
1653	17·7	38·8	1·5	0·9	20·6	20·2	30·9
Mean	30·2	32·7	3·2	1·0	13·8	18·8	31·4

* Camphene was present in samples 1645 (0·3%), 1646 (0·3%), 1650 (0·2%), and terpinolene in 1650 (0·8%); α-thujene, sabinene and α-phellandrene were absent.

A. balsamea and reaches minimal levels in the west as well as in *A. fraseri.*[16] Myrcene is significantly less in *A. fraseri* than in either *A. balsamea* variant although the generally low percentages in which it occurs prohibit attaching much meaning to that fact[15] due to potential analytical errors. The total monoterpene content of the Saskatchewan oleoresins was significantly lower than in the east or in *A. fraseri*, although differences were again rather small.[16]

Changes in *A. balsamea* monoterpene composition with longitude were statistically examined by determining the regression statistics separately for each terpene, with medians for each fir population as a dependent variable and with longitude as an independent variable. Variations in longitude explained from 30 to 80% of the variability in most terpenes, with both correlation coefficients and nonparametric rank correlation coefficients significant on more than a 1% level (Tables 7 and 8). Myrcene and total monoterpene content of oleoresins did not correlate however.

TABLE 4. COMPOSITION OF TERPENES FROM *Abies fraser*

Sample No.*	α-Pinene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	Total terpenes
		Roan Mountain, North Carolina					
948	77·5	14·0	5·0	tr	1·0	1·5	40·0
949	51·5	21·5	17·0	tr	7·5	2·0	33·1
950	77·0	21·0	0·0	tr	0·5	1·5	39·4
951	68·5	26·5	1·0	tr	0·5	2·5	38·6
952	65·0	23·0	7·5	0·0	tr	4·0	35·9
953	68·0	25·5	3·5	0·5	0·5	1·5	36·6
954	45·5	16·5	35·0	0·5	0·5	1·5	29·9
955	65·5	28·5	1·5	0·0	2·0	2·5	35·9
Mean	64·8	22·1	8·8	tr	1·6	2·1	36·2

* Camphene was present in samples 948, 951 (1·0%) and samples 949, 952, 953 and 954 (0·5%). α-Thujene, sabinene, α-phellandrene and terpinolene were absent.

Within *A. balsamea* populations the western extremes of α-pinene, limonene, and β-phellandrene percentages were associated with the westernmost Saskatchewan data, but geographically were less well-defined in case of 3-carene and β-pinene (Table 5). In the east, α-pinene (Fig. 2) and β-phellandrene were highest in easternmost Newfoundland longitudes (50–60°), while 3-carene exhibited a weak maximum around 70°, geographically closer to *A. fraseri*, with limonene and β-pinene less definite again. The 3-carene curve (Fig. 3) suggested a break somewhere between 85 and 75°; towards the west oleoresin samples were essentially devoid of this terpene (only 8% of the samples contained in it measureable amounts, mostly below 2%), while in the east 3-carene was common (present in 77% of the samples, in amounts mostly above 2%). With other terpenes west-to-east changes were gradual, with no noticeable breaks. In α-pinene, the slope was continuously increasing towards the east; thus the difference in regression constants-*b*-(slope) determined separately

[16] The significance of these differences was tested using population medians from the geographic areas compared (Table 4), with < 0·01 levels attained in all cases, with exception for mycrene with levels >0·05 (*t*-test).

for the regions east and west of 85°, proved significant on the 5% level (*t*-test). Possibly this also applied for β-pinene and β-phellandrene, although differences in slopes were not statistically significant.

In the absence of any genetic information about terpenes of *A. balsamea* it is impossible to give any exact interpretation of the increase in β-phellandrene in the allegedly chemically intermediate eastern *A. balsamea* populations. The existence of genes enabling β-phellandrene to be produced in large amounts cannot be disproved, and in this case the eastern fir would not represent a chemical intermediate. A possibility exists, however, that intermediate genotypes by themselves produce monoterpenes richer in β-phellandrene than either genotypic extreme, as analogous cases of this have been reported before.[17]

Table 5. Median values for populations sampled*

Population No.	α-Pinene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phellandrene	Total monoterpenes
62	9·0	43·5	0	1·0	41·5	4·5	29·7
89	9·2	40·0	0	2·4	43·9	4·1	29·4
52	9·5	40·8	0	2·8	43·0	4·3	28·4
51	8·3	37·3	0	2·0	45·0	6·3	29·6
48	11·0	43·5	tr	1·3	32·0	12·0	30·2
49	13·5	50·0	0	1·0	28·3	5·0	28·1
73	9·5	41·3	0	4·3	31·8	9·3	31·3
72	13·3	47·8	0	3·5	26·8	7·0	36·4
70	9·5	38·8	0	1·3	45·0	4·5	35·2
71	10·8	41·0	0	1·3	33·5	8·3	33·9
69	11·3	38·0	0	4·8	38·3	4·8	30·6
68	12·3	38·5	0	1·3	23·0	18·0	34·6
67	15·0	52·0	1·5	tr	25·5	4·8	34·8
66	11·3	47·3	1·0	1·0	19·3	12·8	31·6
46	17·5	25·5	3·5	1·5	27·5	11·5	28·9
93	23·5	37·2	2·8	1·4	4·8	20·5	35·1
47	21·0	29·0	3·5	1·0	18·5	13·0	30·1
91	20·0	31·8	4·0	1·3	10·1	23·8	32·1
92	19·6	40·4	1·7	2·1	14·7	18·5	32·6
94	21·6	36·0	0·9	0·9	10·9	22·4	31·4
95	33·4	31·5	1·6	0·4	8·3	26·5	34·0
96	26·4	30·5	1·7	1·0	16·4	19·0	30·8
117†	13·4	35·4	1·6	1·9	24·1	25·7	33·8
80†	37·5	31·5	1·5	1·5	8·5	3·0	28·0
			Abies fraseri				
78†	69·5	14·3	2·8	0·8	1·0	5·5	34·1
63	66·8	22·3	4·2	tr	0·5	1·8	36·3
53	59·0	17·0	15·5	0·5	1·8	2·5	32·7
115	49·7	16·5	16·4	0·6	6·3	1·3	33·6
64	62·5	18·5	13·5	tr	0·5	1·5	33·7

* Populations from No. 62 (Saskatchewan) to No. 96 (Newfoundland) arranged according to decreasing longitude.

† Pennsylvania *A. balsamea* and Virginia *A. fraseri* populations tested for intermediacy.

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[17] W. B. Critchfield, *Silvae Genetica* **16**, 89 (1967).

TABLE 6. VARIABILITY STATISTICS

Populations and longitude	Statistic	α-Pinene	β-Pinene	3-Carene	Myrcene	Limonene	β-Phell-andrene	SII*	Total monoter-penes
A. balsamea	Quartiles	7·8–10·0	37·4–45·5	0·0–0·0	1·0–3·0	37·1–48·6	3·5–5·5	25	27·7–30·5
110°–102° 30′	Range	6·8–13·0	16·0–52·1	0·0–5·5	0·0–3·7	29·6–44·3	1·6–9·6		20·2–32·9
A. balsamea	Quartiles	8·3–14·0	37·3–52·5	0·0–0·0	0·9–2·8	26·8–44·3	4·5–12·0	49	27·7–31·0
102° 30′–97° 30′	Range	6·5–21·5	21·5–65·0	0·0–6·5	0·0–5·5	9·5–64·5	1·5–27·5		25·8–35·6
A. balsamea	Quartiles	8·9–11·6	35·4–48·6	0·0–0·0	1·5–4·6	26·9–43·3	4·5–13·8	45	30·8–35·2
97° 30′–87° 30′	Range	6·5–21·5	24·0–60·0	0·0–1·5	0·0–8·5	3·0–64·0	2·5–45·0		27·8–42·0
A. balsamea	Quartiles	11·0–16·4	36·4–56·0	0·0–1·8	0·0–1·4	13·5–41·8	3·6–19·3	72	32·0–38·6
87° 30′–80°	Range	8·5–22·5	23·0–64·0	0·0–9·0	0·0–3·0	1·0–48·0	2·0–41·0		30·4–41·5
A. balsamea	Quartiles	10·5–24·0	25·5–47·5	0·5–4·0	0·5–2·0	3·2–35·0	6·0–24·7	91	29·6–34·5
80°–70°	Range	9·0–31·6	14·0–59·0	0·0–18·0	0·0–7·0	0·5–57·5	1·7–42·6		24·4–43·9
A. balsamea	Quartiles	17·3–30·2	27·7–42·1	1·4–8·5	0·9–3·2	5·3–21·1	7·7–26·0	71	29·7–33·8
70°–60°	Range	10·9–47·8	11·0–69·6	0·0–12·3	0·0–6·6	0·6–52·5	0·0–40·5		26·0–47·5
A. balsamea	Quartiles	20·1–44·1	26·5–38·6	0·0–27·0	0·3–1·2	0·9–20·4	8·9–32·0	82	30·3–34·4
60°–50°	Range	10·2–56·2	18·2–54·1	0·0–20·6	0·0–2·6	0·0–40·5	0·7–38·8		25·5–49·8
A. fraseri	Quartiles	47·5–68·0	13·5–26·5	8·0–20·0	0·0–0·6	0·5–4·5	1·0–3·0	42	30·9–35·9
	Range	29·5–78·5	4·5–44·5	0·0–53·0	0·0–1·5	0·0–17·6	0·0–26·0		22·6–40·0

* Sum of interquartile intervals.

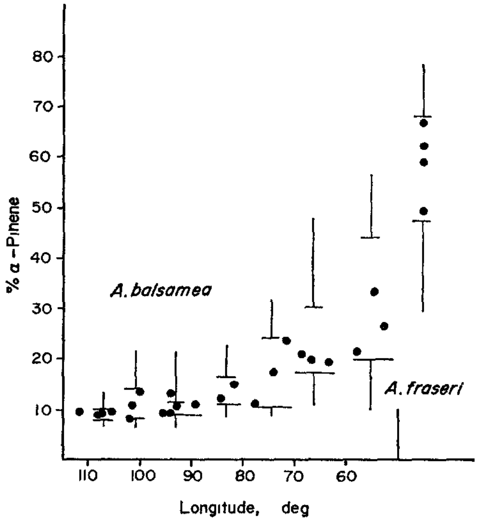

FIG. 2.

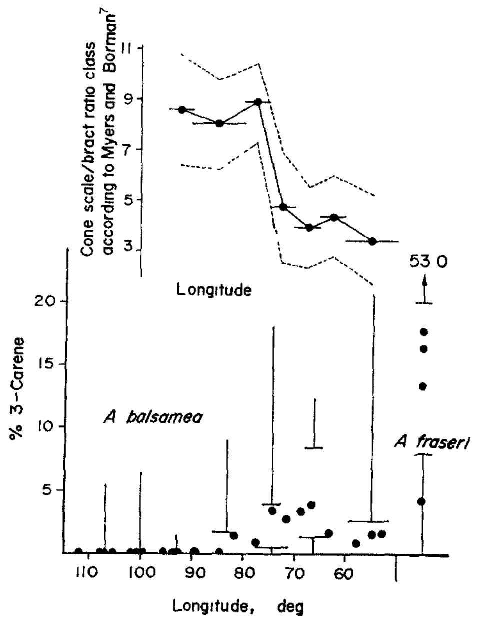

FIG. 3.

FIG. 2. PLOT OF α-PINENE MEDIANS AGAINST LONGITUDE FOR *A. balsamea*, WITH MAXIMAL AND MINIMAL VALUES AND QUARTILES INDICATED FOR SEVEN LONGITUDINAL RANGES AS GIVEN IN TABLE 6.

FIG. 3. PLOT OF 3-CARENE AGAINST LONGITUDE FOR *A. balsamea*, WITH MAXIMAL AND MINIMAL VALUES AND QUARTILES INDICATED FOR SEVEN LONGITUDINAL RANGES AS GIVEN IN TABLE 6. THE UPPER PART OF THE DIAGRAM REPRESENTS THE PLOT OF CONE SCALE/BRACT RATIO CLASS MEANS (SOLID LINE) AND STANDARD DEVIATIONS (BROKEN LINES) FOR SEVERAL LONGITUDINAL RANGES (HORIZONTAL LINES) OF THE SAME SPECIES, CALCULATED FROM THE DATA OF MYERS AND BORMANN.[7]

Geographic changes in variability of monoterpene composition were explored by separating the total *A. balsamea* data into seven longitudinal groups, with *A. fraseri* populations from North Carolina forming the eighth, and by determining the first and third quartiles separately for each group and terpene. The results are presented in Table 6, together with the sums of interquartile intervals for all terpenes (SII); the latter statistics thus represent a measure of total variability of monoterpene composition within a group. The SII values reached their maximum in the populations east of 87° 30′, with no statistically significant differences encountered among the three easternmost SII values. Minimal variabilities were exhibited by the *A. fraseri*, and particularly by the Saskatchewan populations, with the differences in SII values between these and eastern *A. balsamea* data statistically significant on the <0·005 level. The data between 102° 30′ and 87° 30′ were transitional, with the SII values intermediate between those of Saskatchewan and the east and with respective differences significant on the <0·005 level again.

TABLE 7. TEST FOR GAUSSIAN FIT USING χ^2 METHOD

Terpene	*A. balsamea* west (110°–102° 30′) χ^2	*df*	*A. balsamea* east (50°–60°) χ^2	*df*	*A. fraseri* χ^2	*df*
α-Pinene	9·2	4	11·6	6	13·3	9
β-Pinene	5·5	10	11·0	14	3·1	5
3-Carene	—	—	78·2†	8	12·6*	6
Limonene	8·5	9	28·5†	5	157·2†	5
β-Phellandrene	7·0	4	23·1*	13	64·8†	5
Total terpenes	3·3	7	7·8	7	3·5	5

* Deviation from Gaussian on 0·95 level.

† Deviation from Gaussian on 0·99 level.

Terpene per cent on total monoterpene basis, total terpenes on oleoresin per cent basis. Expressing individual terpene percentages on oleoresin basis did not influence the goodness of fit for all practical purposes. *Abies fraseri* included populations 53, 63, 64 and 115 only. *A. balsamea* west included additional 10 samples collected near Touchwood, Alberta, (54° 52′; 111° 26′), indistinguishable from Saskatchewan material.

Further comparison between westernmost (102° 30′ to 110°) and easternmost (50 to 60°) *A. balsamea* and *A. fraseri* was made by computation of the goodness of fit of data into the normal distribution and examination of the distribution diagrams. In the west, only myrcene exhibited significant deviation from the Gaussian curve (Table 7). As this terpene represents only a quantitatively minor consituent, however, it is entirely possible that some systematic analytical error is responsible for that. In the east as well as in *A. fraseri* limonene, 3-carene and β-phellandrene exhibited significant deviations from normality which restricted our use of parametric methods in statistical calculations. The distribution diagrams (Fig. 4) were mostly skewed, and in some cases suggested bi- or even trimodality. These abberrations are in good accord with what has been previously reported[18] and suggest control of terpene levels by a few genes only. The differences in general shapes of the distribution patterns again suggested, in 3-carene and limonene, the chemically intermediate position of the eastern *A. balsamea.*

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[18] A. E. Squillace and G. S. Fischer, U.S. Forest Serv. Res. Paper NC-6, p. 53 (1966).

Thus, chemical data indicate the existence within the *A. balsamea/A. fraseri* complex of three geographically more or less distinct gene pools. Two pools are rather poor (as evidenced by simpler turpentine composition and lower variability) and comprise populations of *A. fraseri* and populations of *A. balsamea* between 110° and 102° 30′; the third pool is richer, is characterized by more complicated turpentine composition and higher variability, and comprises eastern *A. balsamea* populations roughly east of 72° 30′. As shown in Tables 5 and 6 and Figs. 2 and 3, the populations between 102° 30′ and 87° 30′ seem predominantly western and identical to the Saskatchewan material in respect to the absence of 3-carene, although showing some minor affinity with east in terms of other terpenes. The area between 87° 30′ and 72° 30′ is apparently the area where west-to-east transition largely takes place.

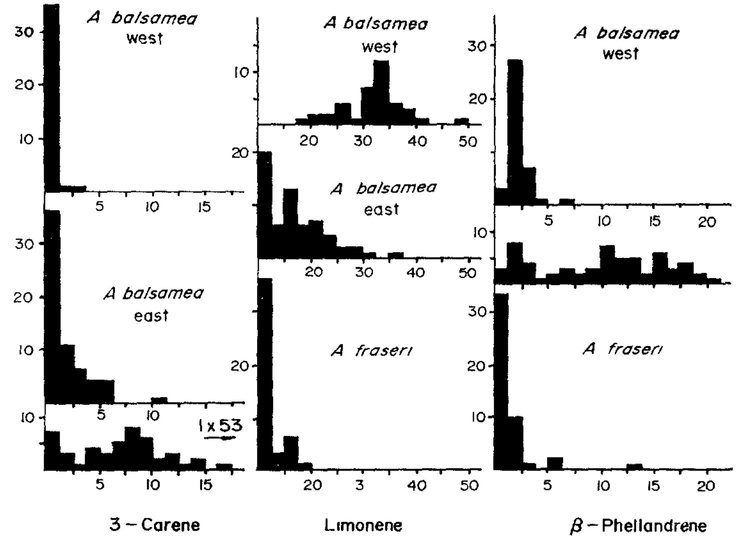

FIG. 4. DISTRIBUTION DIAGRAMS OF 3-CARENE, LIMONENE AND β-PHELLANDRENE CONSTRUCTED FOR *A. fraseri* (POP. 53, 63, 64, 115), EASTERN *A. balsamea* (POP. 47, 91–96) AND WESTERN *A. balsamea* (POP. 25, 52, 63, 89).

In populations of *A. fraseri* from North Carolina the median values for 3-carene in Roan Mountain, and for limonene in Newfound Gap materials, appeared aberrant; however, the differences did not prove significant on the basis of total data from these and other *A. fraseri* locations sampled (Wilcoxon's *T*-test). Nor were definite north-to-south gradients noticed with *A. balsamea*; the most northern populations of this fir were not included in this study, however, and the data do not allow us to make a final judgment.

Due to their proximity to the intermediate populations of West Virginia, the two Pennsylvania *A. balsamea* populations and *A. fraseri* material from Mount Rogers, Virginia, were not included in calculations discussed so far, although the respective stands are generally regarded as 'pure' species. The more northern Pennsylvania population from Promised Land Lake did not show any significant trends towards *A. fraseri*. However, material from Bear Meadows suggested some affinity with this fir in terms of median values for α-pinene, limonene, and β-phellandrene—the latter were significantly closer to *A. fraseri* (*t*-test, level of significance 0·01) than to *A. balsamea* populations of comparable longitude (60° to 85°). These differences were also significant in case of α-pinene ($> 0{\cdot}01$ level) and limonene (0·05 level) when tested using entire data rather than medians (Wilcoxon's T-test). The northernmost *A. fraseri* population from Mt. Rogers suggested some affinity with *A. balsamea*, with its median values significantly different from medians of other *A. fraseri* populations in the case of 3-carene (0·05 level) and β-phellandrene (0·01 level) (t-test); the differences did not maintain their significance, however, when entire data were used (T-test).

In our previous paper[5] a suggestion was made, based on data obtained with eastern *A. balsamea*, that chemical differences separating *A. lasiocarpa* from *A. balsamea* were small and not too promising for study of intergradation of the two species. Our present investigations indicated, however, that the chemical differences between the two become more pronounced in the west (i.e. towards the intergradation area), and afford a good method for study of intermediate populations. The differences include higher β-pinene content in *A. balsamea*, greater variability in β-phellandrene and limonene, and presence of 3-carene in *A. lasiocarpa*, as well as the 10:1 β-pinene to β-phellandrene ratio in *A. balsamea* as contrasted with 1:2·2 ratio between the same compounds in *A. lasiocarpa*. This suggests that not only quantities of certain compounds but also their percentage ratios should be considered in chemotaxonomy.

TABLE 8. REGRESSION OF POPULATION MEDIANS WITH LONGITUDE*

Terpene	Intercept a	Slope b	Correlation coefficient r†	Standard deviation from regression	% variance explained	Rank correlation coefficient r_s
α-Pinene	26·7	−0·339	−0·895	3·07	80	−0·902
β-Pinene	32·1	+0·211	+0·551	5·82	30	+0·588
3-Carene	2·8	−0·053	−0·702	0·99	49	−0·812
Myrcene	0·8	+0·026	+0·384	1·13	15	+0·414
Limonene	5·6	+0·627	+0·872	6·39	76	+0·860
β-Phellandrene	23·7	−0·350	−0·850	3·95	72	−0·836
Total monoterpenes	33·4	−0·500	−0·356	2·37	13	—

* Longitude as distance in degrees from 50°.
† Significance: $r_{5\%} = 0{\cdot}432$; $r_{1\%} = 0{\cdot}537$.

The correlations between percentages of individual terpenoids can be used for speculation on biosynthesis of these compounds, provided the results are obtained from an area for which randomization of genes within gene pool can be assumed.[19] Present data were examined for existence of such correlations, using separately the westernmost (110° to 102° 30′), easternmost (50 to 60°), and North Carolina *A. fraseri* populations. Results are given in Table 9. A relatively large number of significant negative correlations was obtained but only a few good positive correlations were encountered. Thus, β-pinene and β-phellandrene are produced in western *A. balsamea* and *A. fraseri* in the same ratio, with slope $b = 0{\cdot}07$ and the intercept -a- small in both cases. The rather low value for the slope

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[19] E. ZAVARIN, *Phytochem.* **9**, 1049 (1970).

appears unique and specific for the *A. balsamea*/*A. fraseri* complex; in other firs (such as *A. lasiocarpa*, *A. magnifica*, *A. procera*, and several Japanese firs) it was found to be always about 2–3, and in still other cases these compounds did not correlate. Other positive correlations found were those between 3-carene and terpinolene, and α-pinene and β-pinene. All appear to be rather common in Pinaceae; the biosynthetic meaning of these has been previously discussed.[19]

TABLE 9. REGRESSION STATISTICS FOR PERTINENT TERPENE PAIRS

		Data expressed on total monoterpene % basis				Data expressed on oleoresin % basis	
Independent variable	Dependent variable	Intercept *a*	Slope *b*	Corr. coeff. *r*	Stand. error of estimate	Slope *b*	Corr. coeff. *r*
A. balsamea	112° 30′–102° 30′		$r_{0\cdot 99} = 0\cdot 413$				
Limonene	α-Pinene	13·8	−0·106	−0·612	1·43	−0·0904	−0·498
β-Pinene	α-Pinene	4·97	0·104	0·519	1·55	0·116	0·669
β-Pinene	β-Phellandrene	1·50	0·068	0·464	1·18	0·073	0·594
β-Pinene	Limonene	89·2	−1·12	−0·971	2·48	−0·591	−0·619
Limonene	β-Phellandrene	7·35	−0·071	−0·555	0·555	−0·052	−0·400
A. balsamea	50°–60°		$r_{0\cdot 99} = 0\cdot 437$				
α-Pinene	Limonene*	25·7	−0·462	−0·557	9·55	−0·465	−0·507
A. fraseri			$r_{0\cdot 99} = 0\cdot 385$				
α-Pinene	Limonene*	17·5	−0·236	−0·592	3·90	−0·198	−0·606
α-Pinene	β-Pinene	45·02	−0·423	−0·552	7·74	−0·167	−0·237
β-Pinene	β-Phellandrene*	0·714	0·0673	0·418	1·36	0·0691	0·494
β-Pinene	3-Carene	25·46	−0·529	−0·461	9·44	−0·456	−0·459
3-Carene	Terpinolene	−0·944	0·0189	0·558	0·299	0·0208	0·570

* Correlation coefficients tested by calculation of nonparametric rank correlation coefficients, with similar values of −0·620, −0·400 and +0·532, respectively, obtained in each case.

As before, in neither case did the monoterpene content of oleoresins correlate with percentages of terpenes—i.e. the data suggested again that biosynthesis of monoterpenes as a whole is not coupled to the formation of individual terpenoids. In most cases it made no large difference whether the regression and correlation statistics were based on data expressed on total terpene or on oleoresin per cent basis.

DISCUSSION

In order to rationalize the chemical and morphological relationships present in the *A. balsamea*/*A. fraseri* complex it appears desirable to observe results obtained from the viewpoint of paleobotany. The *Abies* fossils are known from Upper Jurassic and Lower

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[20] P. I. DOROFEEV, V. V. ZAUER, I. N. SVESHNIKOVA, I. A. SHILKINA and A. A. YAZENKO-CHMELEVSKII, [Semeistvo Pinaceae Lindley, 1836 in *Osnovy Paleontologii* (edited by A. L. TACHTADZIAN, V. A. VACHRAMEEV and G. P. RADCHENKO), p. 266, Gos, Nauch. Tech. Izd. po Geol. i Okhr. Nedr. Moscow (1963).

[21] H. GAUSSEN, *Les Gymnospermes actuelles et fossiles* (edited by A. JULES-GUESDE), Fasc. VII, p. 456, Faculté des Sciences, Toulouse (1964).

[22] *Ibid.* p. 462.

Cretaceous[20] and are not uncommon in the Tertiary deposits[21] from the Eocene on. The *Abies* species began to assume their present form at least by the Miocene[22] and at the end of the Pliocene were probably similar to the species of today. During the Pleistocene the glaciation of the north, with intervening, warmer, interglacial periods, resulted in north–south–north shifts of vegetation and exerted strong selective pressures on the species involved by forcing them to adapt themselves to the changing climatic conditions. Towards the end of the last interglacial or Sangamon, which ended about 70 000 yr before present (B.P.), the climatic conditions throughout the United States and Canada were probably not much unlike from what they are today[23a,24a,25] and the coniferous forests were not too dissimilar from what they are now in terms of their composition and geographic extent.[26–28] *Abies* pollen considered to be Sangamonian was reported from Illinois,[29] Indiana,[30] New York,[31] South Carolina,[32] Iowa[33] and Ontario.[25]

The ensuing Wisconsin glaciation drastically modified the species distribution[24b] (Fig. 1). The ice destroyed nearly all vegetation in the Canadian and northern U.S. part of today's general range of *A. balsamea*, but created to the south of the ice sheet more moderate, cooler, climatic conditions better suitable for boreal forests. In the general region of the western plains, the glaciers came in contact with grasslands and split the boreal forests into a larger eastern and a smaller western part.[28,34–36]

The western refugium proposed earlier by Raup,[36] Hulten[37] and Halliday and Brown,[35] comprised small area or areas along the drainage channels of the glaciers at the foot of the Rocky Mountains and possibly extending between the Cordilleran glaciers in the west and the Laurentide ice sheet in the east.[28,38] *Abies* pollen, estimated to be of late Wisconsin age, has been reported by Hansen[39] from several peat deposits near Edmonton, Alberta and is regarded as evidence for the existence of a corridor-like refugium between the two ice sheets.

The boreal forests including *Abies* occupied a more extensive area in the east, growing at much lower altitudes than now and probably ranging from at least South and North Carolina to close to the southern margin of the ice sheet in the north, and from Pennsylvania in the east to Illinois and Iowa in the west. *Abies* pollen of Wisconsin age is

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[23] D. R. Whitehead, Palynology and Pleistocene phytogeography of unglaciated Eastern North America, in *The Quaternary of the United States* (edited by H. E. Wright and D. G. Frey), (a) p. 426; (b) p. 418, Princeton University Press, New Jersey (1965).

[24] R. F. Flint, *Glacial and Pleistocene Geology*, (a) p. 340; (b) p. 321; (c) p. 324; (d) p. 350. Wiley, New York (1957).

[25] C. Emiliani, *Science* **171,** 571 (1971).

[26] J. Terasmae, *Can. Dept. Mines, Tech. Surv., Geol. Surv.*, Canada Bull. **56,** 23 (1960).

[27] C. W. Yeatman, *Can. J. Bot.* **45,** 2201 (1967).

[28] D. Loeve, *Can. J. Bot.* **37,** 547 (1959).

[29] J. Voss, *Bot. Gazz.* **94,** 808 (1933).

[30] R. O. Kapp and A. M. Gooding, *J. Geol.* **72,** 307 (1964).

[31] J. J. Donner, *Am. J. Sci.* **262,** 355 (1964).

[32] E. B. Leopold, *U.S. Geol. Surv. Bull.* 1079, 49 (1959).

[33] G. H. Lane, *Iowa Geol. Surv. Ann. Rep.* **37,** 233 (1941).

[34] E. Hulteń, *Kungl. Vetensk.-Akad. Handl.* Ser. 4, Vol. 7, No. 1, Stockholm (1958).

[35] W. E. D. Halliday and A. W. A. Brown, *Ecology* **24,** 353 (1943). While advocating the existence of a Cordilleran boreal refugium they accepted only eastern refugium for *A. balsamea.*

[36] H. M. Raup, *Bot. Rev.* **7,** 147 (1941).

[37] E. Hulteń, *Outline of the history of Arctic and Boreal Biota during the Quaternary Period*; Stockholm 1937, Bokforlags Aktiebolaget Thule.

[38] J. S. Rowe, 1956, as quoted in. Ref. 27.

[39] H. P. Hansen, *Am. J. Bot.* **36,** 54 (1959); *Bull. Torrey Bot. Club* **76,** 278 (1949).

known from Virginia,[40] Carolinas,[23b,41–44] Pennsylvania,[45] Illinois,[29,46] Iowa,[47] and Indiana.[30]

The existence of the two *Abies* refugia is in good agreement with the chemical differences encountered in our work. The exceptionally low variability of the western variant (poor gene pool) is suggestive of long-time isolation and confinement within a rather limited area. Higher variability of the eastern variant is in good agreement with a much more extensive eastern distribution range, including larger variety of habitats.

The northern expansion of the boreal forests began with retreat of the glaciers which started roughly 13 000 yr B.P. most likely in the west;[24c] the boreal flora spread eastwards from its western refugium through Saskatchewan and reached Manitoba during the Two Creek interstadial (11 750 B.P.).[28] This idea is supported by several pollen diagrams from Manitoba,[48,49] and is in good agreement with the predominantly western chemistry of Manitoban *A. balsamea*. In the east the boreal forests moved north into the liberated territories of New York, New England, and coastal Canada, as well as into the area of the post-glacial equivalents of the Great Lakes as evidenced by extensive palynological records.[50–53] It is possible that some contact between western and eastern floras was established at this time.[28]

With further warming up and the advent of the xerothermic or hypsithermic period (8000–4000 B.P.) a general expansion of boreal forests in northern latitudes and at higher elevations took place, while the same forests were eliminated or reduced at the lower elevations and in more southern latitudes. In the southern Appalachians, *A. balsamea* disappeared from all but the highest elevations and was broken up into several isolated populations.[54,55] During that time according to Mark, *A. balsamea* suffered a reduction in biotypes yielding *A. fraseri* as we know it today[7,8,56] which is in agreement with the much lower variability of our chemical data for this fir.

In the north the western *A. balsamea* variant spread in all directions from Manitoba (as was proposed for spruce[28]) and occupied the newly-liberated territories south of Hudson Bay, where it came in contact with the eastern variant of the same fir moving northwest; at the same time the boreal flora receded from the more southerly locations,[52] including habitats south of the Great Lakes.

With the advent of the cooler present-day climate (4000 B.P.) the boreal forests shifted again, with *Abies* occupying its present range. Today, the *Abies* west of Lakes Michigan

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[40] W. HARRISON, R. J. MALLEY, G. A. RUSNAK and J. TERASMAE, *J. Geol.* **73,** 20 (1965).
[41] D. R. WHITEHEAD and E. S. BARGHOORN, *Ecol. Monogr.* **32,** 347 (1962).
[42] S. A. CAIN, *Torrey Bot. Club Bull.* **71,** 11 (1962).
[43] D. R. WHITEHEAD, *Ecology* **44,** 403 (1963).
[44] D. R. WHITEHEAD, *Ecology* **45,** 767 (1964).
[45] P. S. MARTIN, *Am. J. Sci.* **256,** 470 (1958).
[46] J. VOSS, *Ecology* **20,** 517 (1939).
[47] R. V. RUHE, M. RUBIN and W. H. SCHOLTES, *Am. J. Sci.* **255,** 671 (1957).
[48] J. A. ELSON, *Science* **126,** 999 (1957).
[49] J. C. RITCHIE and S. LICHTI-FEDEROVICH, *Can. J. Earth Sci.* **5,** 873 (1968).
[50] S. JELGERSMA, *Am. J. Sci.* **260,** 522 (1962).
[51] M. B. DAVIS, Phytogeography and palynology of Northeastern United States and E. J. CUSHING, Problems in quaternary phytogeography of the Great Lakes Region, in *The Quarternary of the United States* (edited by H. E. WRIGHT and D. G. FREY), Princeton University Press, New Jersey (1965).
[52] J. G. OGDEN, III, *Bot. Rev.* **31,** 481 (1965).
[53] J. H. MCANDREWS, *Mem. Torrey Bot. Club* **22** (1966).
[54] H. Y. OOSTING and W. E. BILLINGS, *Ecology* **32,** 84 (1951).
[55] W. E. BILLINGS and A. F. MARK, *Ecology* **38,** 140 (1957).
[56] A. G. MARK, *Ecol. Monograph* **28,** 293 (1958).

and Superior represent, according to the chemical data, a predominantly western variant, so the reinvading genotypes must have been largely western. The western invasion could also account for the fact that the geographically more distant Newfoundland populations exhibit more chemical affinity to *A. fraseri* than does material from the east/central Great Lakes area. This is in agreement with the cone scale/bract ratio data of Myers and Bormann,[7] with a definite break between western and eastern *A. balsamea* suggesting itself between 70 and 80° longitude (Fig. 3). The slight eastern-chemical traits of Manitoba, Minnesota, and Wisconsin *Abies* populations are probably due to the limited gene exchange from the time the two variant met in the north, although the idea of some gene exchange during the early postglacial cannot be excluded either.

EXPERIMENTAL

Oleoresin samples were collected in localities listed in Table 1, spread as randomly as possible through the ranges of the two species investigated. To reduce the possibility of close parentage, the trees standing within 50 ft or so from each other were not sampled. Concurrently with oleoresin collection a small branchelet was secured from each or marjority of trees sampled and preserved dry between newspapers for later reference. Collection, preservation and analysis of the oleoresin samples were described earlier.[57]

The normality of data distribution was tested by χ^2. With reasonably normal data the differences in means were tested by *t*-test, while rank *T*-test was applied with data strongly deviating from normality. The significance of differences in sums of quartile intervals (SII), was tested using theoretical number of individuals falling into and outside of the quartiles interval ($Q_1{}^a - Q_3{}^a$) and the number computed on the basis of the $M^a - (M^b - Q_1{}^b)$, $M^a + (M^b - Q_3{}^b)$ interval. The numbers obtained for all monoterpenes were pooled and X^2 test applied ($df = 1$).[58] If $M^a - (M^b - Q_1{}^b)$ or $M^a + (M^b - Q_3{}^b) < 0$, the data were divided into those falling above and below the other quartile only. M^a, M^b = medians from data sets a and b. $Q_1{}^a$ $Q_3{}^a$, $Q_1{}^b$, $Q_3{}^b$ — first and third quartiles from the same two data sets.

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[57] E. Zavarin, K. Snajberk, Th. Reichert and E. Tsien, *Phytochem.* **9,** 377 (1970).

[58] G. W. Snedecor, *Statistical Methods*, pp. 212, Iowa University Press, Ames, Iowa (1961).

[59] E. L. Little, Jr., *Atlas of U.S. Trees*, U.S. Dept, of Agr. Forest Service Misc. Publ. No. 1146 (1971).

Key Word Index—Abies balsamea; *Abies fraseri*; Pinaceae; monoterpenes; geographical variation.