Chemotaxonomic Relationships within the Central American Closed-cone Pines

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(Received 19th June 1987)

Summary

Samples of stem-xylem oleoresin from 617 trees of *Pinus patula* Schiede and Deppe ssp. *tecunumanii* (Eguiluz and Perry) Styles from 21 provenances in Central America were collected together with samples of the associated species *P. patula* Schiede and Deppe and *P. oocarpa* Schiede. The taxonomic status of *P. patula* ssp. *tecunumanii* was investigated by analysis of monoterpenes and sesquiterpenes by gas-liquid chromatography on OVI and CW20M capillary columns. Because of constraints in the statistical analysis of compositional data, evaluation of analytical results was limited to the production of a classification of resin types based on four monoterpenes (α-pinene, $\triangle$-3-carene, β-phellandrene, limonene). The rationale used in the formulation of type classes was supported by the use of a graphical technique for the display of multi-variate data (Andrews curves). Little quantitative or qualitative intra-specific variation in terpene composition was expressed in the associated species, however, variation in terpene composition was indicated in *P. patula* ssp. *tecunumanii*, both within and between provenances. Clinal trends in some of the terpene patterns were apparent with altitude. It was shown that the chemical profile of the sub-species does not resemble that of *P. patula*. No close similarities in terpene phenotypes with *P. oocarpa* were suggested and excessive intra-specific variation in the sub-species discourages designation of specific rank. The chemotaxonomic status of *P. patula* ssp. *tecunumanii* remains obscure.

Key words: *P. patula* ssp. *tecunumanii*, *P. patula*, *P. oocarpa*, terpene variation, gas-liquid chromatography.

Introduction

As a result of the recommendations of the Eighth British Commonwealth Forestry Conference in 1962, the Central American Pine Provenances Research Project was initiated at Oxford (see Kemp, 1973). The project initially focussed on investigations of *Pinus caribaea* Morelet and later expanded to include the first comprehensive seed collection of *P. oocarpa* Schiede across its natural range. As the study progressed, taxonomic problems arose both within and between species (Barnes and Styles, 1983). During investigations of *P. oocarpa* a number of provenances in the International Provenance Trial showed atypical morphological features and superior growth and form over all sites. In particular, what were thought to have originated from higher-altitude southern populations of *P. oocarpa*, in Nicaragua, differed from typical *P. oocarpa* chemically, because they were rich in $\triangle$-3-carene, and morphologically. Styles (1985) declared these anomalies as a sub-species of *P. patula* Schiede and Deppe: *P. patula* Schiede and Deppe ssp. *tecunumanii* (Eguiluz and Perry) Styles, on botanical grounds, thus extending the range of *P. patula* as the second most southerly Central American pine species, after *P. caribaea* var. *hondurensis* Barr. and Golf. A full account of the taxonomic background and detailed botanical descriptions can be found in Styles (1985).

It was therefore imperative to examine more fully the relationships within and between provenances of *P. oocarpa* and *P. patula* ssp. *tecunumanii*. An intensive programme of exploration, seed, resin and botanical specimen collection of *P. patula* ssp. *tecunumanii* and associated species *P. patula* and *P. oocarpa* from their natural range was launched in 1982. Traditionally, qualitative descriptions of morphological characters have often been adequate for discriminating taxa. Within the sub-section Oocarpae of the genus *Pinus* a number of variable botanical characteristics exist and overlap in the occurrence of these characteristics in the natural range and the possibility of hybridisation makes discrimination between the species difficult. Numerical techniques have recently been applied to *P. patula* ssp. *tecunumanii* morphological data (McCarter and Birks, 1985) in an attempt to resolve the taxonomic problem using a small number of variable characters. Seven variables were found to contribute to a discriminant function which identified trees as belonging to one of the two species, *P. oocarpa* or *P. patula* ssp. *tecunumanii*. Comparisons between *P. patula* ssp. *tecunumanii*, *P. patula* and its variety *longipedunculata* suggest that the citation as a subspecies of *P. patula* on botanical grounds is probably correct. Eguiluz Piedra and Perry (1983), however, maintain that this tree is a distinct species and that it is more closely related to *P. oocarpa*.

Chemical methods have been increasingly used since the 1950s as an aid in taxonomic identification. Mirov (1965) reported his life-long study of the genus *Pinus* and suggested that " . . . as taxonomic markers, pine terpenoids could be invaluable for separating many species . . . ". Although not all species possess a characteristic pattern, Mirov believed " . . . the knowledge [of terpenoid patterns] added to the understanding of the geography and evolutionary history of the pines". The benefits derived from gas chromatographic analysis of pines for taxonomic studies have been reported by, among others, Zavarin (1968) and Turner and Flake (1974). Terpene patterns of *P. oocarpa* in particular were thoroughly examined during earlier stages of the Central American Pine Provenances Research Project (see Green, Keeble and Burley, 1974; Burley and Green, 1979). More recently Eguiluz Piedra (1986) evaluated measurements of 24 morphological traits in 108 trees of *"P. tecunumanii"* from five regions of Guatemala in support of his designation of specific rank and included gas chromatographic analysis of 57 oleoresin samples from three regions of Guatemala. Comparative chemical analysis included only 15 trees of *P. patula* var. *longipedunculata* and 15 trees of *P. oocarpa* var. *ochoterenae*[1]). Presentation of the terpene analysis results was

[1]) Styles reported evidence to show that *P. oocarpa* var. *ochoterenae* Martinez had been wrongly classified and really belonged to *P. patula* and allied to var. *longipedunculata*. It is now believed to be *P. patula* ssp. *tecunumanii* and is cited as a synonym in Styles and McCarter (1988).

confusing. Mean values for each species were based only on those trees in which a given constituent was present rather than the total number of trees analysed. Para-cymene, for instance, was present in only three out of 57 trees but at levels up to 50.4% with a resultant 'mean' value of 29.6%. In the author's experience, and that of others (J. COPPEN, pers. comm.)[2]) para-cymene is rarely present in pine resin in anything more than trace amounts (< 0.5%). It is, however, a degradation product readily detected when resin has been exposed to oxidative conditions. A sample containing 50.4% para-cymene is therefore highly suspicious. EGUILUZ PIEDRA also reported values for trans-caryophyllene of 1.1% to 53.9% (present in all 57 trees, mean = 16.5%) and longifolene 0% to 3.0% (present in 21 trees, mean = trace). It is the opinion of both the author and COPPEN that these terpenes may have

2) J. J. W. COPPEN, Overseas Development and Natural Resources Institute, 56—72 Gray's Inn Road, London WC1X 8LU

Table 1a. — Details of provenances — *P. patula* ssp. *tecunumanii*.

Site Number	Provenance	State/Department Country	Latitude Longitude	Altitudinal Range (m)	Number of Samples
1	Yucul	Matagalpa Nicaragua	12° 55' N 85° 47' W	900-1100	26
2	San Rafael	Matagalpa Nicaragua	13° 14' N 86° 08' W	1000-1200	24
3	Zambrano	Comayagua Honduras	14° 16' N 87° 25' W	1500-1600	6
4	Siguatepeque	Comayagua Honduras	14° 32' N 87° 50' W	1280-1650	23
5	La Paz	La Paz Honduras	14° 19' N 87° 45' W	1750-2000	41
6	Villa Santa	El Pariaso Honduras	14° 11' N 86° 20' W	850-950	47
7	Culmi	Olancho Honduras	15° 06' N 85° 21' W	550-650	51
8	Guajiquiro	La Paz Honduras	14° 11' N 87° 50' W	1835-2250	47
9	Montaña Sumpul	Ocotepeque Honduras	14° 24' N 89° 08' W	1950-2050	24
10	Cusuco	Cortes Honduras	15° 30' N 88° 11' W	950-1600	38
11	Montaña Celaque	Lempira Honduras	14° 34' N 88° 39' W	1600-1800	9
12	San Esteban	Olancho Honduras	15° 22' N 86° 19' W	800-900	27
13	San Francisco de la Paz	Olancho Honduras	15° 05' N 86° 20' W	870-1100	29
14	Jocon	Yoro Honduras	15° 16' N 86° 55' W	1000	37
15	San Pastor Pine Ridge	Cayo Belize	16° 41' N 88° 58' W	650-750	30
16	Mountain Pine Ridge	Cayo Belize	17° 00' N 88° 55' W	700-800	37
17	Juquila	Oaxaca Mexico	16° 15' N 97° 17' W	2000-2250	19
18	Las Piedrecitas	Chiapas Mexico	16° 44' N 92° 38' W	2300-2600	27
19	La Soledad	Jalapa Guatemala	15° 03' N 90° 14' W	2300-2500	22
20	San Jeronimo	Baja Verapaz Guatemala	14° 33' N 90° 19' W	1700-2000	28
21	Pachoc	Totonicapan Guatemala	14° 56' N 91° 16' W	2500-2700	25

Table 1b. — Details of provenances — *P. patula* and *P. oocarpa.*

Site Number	Species Provenances	State/Department Country	Latitude Longitude	Altitudinal Range (m)	Number of Samples
1	P. patula Xoxocotla	Veracruz Mexico	18° 40' N 97° 06' W	2500-2600	40
2	P. patula var. longipedunculata	Oaxaca Mexico	17° 27' N 96° 29' W	2600-3000	29
3	P. oocarpa Guimaca	Francisco Morazan Honduras	14° 33' N 86° 46' W	900	27
4	Siguatepeque	Comayagua Honduras	14° 37' N 87° 54' W	1200	29
5	Conacaste	Zacapa Guatemala	15° 13' N 89° 21' W	550	29
6	Malacatancito	Huehuetenango Guatemala	15° 13' N 91° 32' W	1700	15
7	Abosolo	Chiapas Mexico	17° 20' N 92° 07' W	1300	24

been misidentified and that it is longifolene which is present in the greater quantity. The maximum value recorded for caryophyllene in 617 samples of *P. patula* ssp. *tecunumanii* analysed in the author's laboratory was 2.35% and longifolene values were in the range 0% to 24.67%. Caryophyllene was detectable in 100 out of 617 trees and longifolene in all 617 trees.

This paper presents the results of oleoresin monoterpene and sesquiterpene analysis of 617 trees of *P. patula* ssp. *tecunumanii* from its natural range in Mexico and Central America and compares them with the terpene composition of the associated species *P. patula* and *P. oocarpa.*

Materials and Methods

1. *Resin collection*

Oleoresin was collected from living *P. patula* ssp. *tecunumanii, P. patula, P. patula* var. *longipedunculata* and *P. oocarpa* trees during the seed collecting seasons of 1982 to 1983. *Tables 1a* and *b* show site details. Wolf and badly mis-shapen trees were excluded. Trees were selected after botanical identification of gross morphological characters/features of species. Glass screw neck vials were inserted into holes-drilled through the bark of selected trees at breast height and left until filled with oleoresin or, in low yielding trees, until oleoresin flow ceased. Following collection, the vials were closed with screw caps. Distilled water was added to vials from low-yielding trees to exclude air. On arrival at the laboratory, samples were refrigerated at 4° C until analysed.

2. *Sample preparation*

Aliquots of 0.25 ml oleoresin were dissolved in 1.75 ml ANALAR cyclohexane. Samples prepared in this way were analysed for monoterpenes and sesquiterpenes by capillary gas-liquid chromatography using a Carlo Erba Fractovap with LT Programmer and Varian series 8000 autosampler.

3. *Conditions of analysis*

The following conditions were established:
Column: SGE BPl fused silica capillary, 25 m × 0.32 mm ID, film thickness 0.5 μm.
FID: air = 500 ml/min
H_2 = 10 ml/min
H_e = 3 ml/min (carrier gas)
Injector temperature = 200° C
Detector temperature = 250° C
Temperature programme: Initial 50° C for 2 mins
Final 170° C
Rate 4° C/min
Sample size = 1 μl
Split ratio = 10:1

As the BPl column was unable to resolve limonene and β-phellandrene, all samples were additionally analysed on SGE BP 20 capillary column (equivalent to Carbowax 20M). Separated chemical constituents were recorded as percentage of total terpenes and identified by comparison with known purified chemical standards.

Results and Discussion

Throughout the analyses, up to 20 terpenes were identified in oleoresin samples. In some cases additional unidentified chemicals were resolved, but only in trace quantities. A subset of seven terpenes were chosen as those chemicals which could provide a discriminatory approach (see Burley and Green, 1977); α-pinene, β-pinene, $\triangle$-3-carene, limonene, β-phellandrene, estragole (methyl chavicol) and longifolene. Provenance mean values of terpene composition are presented in *Figure 1a* and *b*. Because of contraints in the statistical analysis of compositional data (Aitchison, 1982; Birks and Kanowski, 1988), no multivariate techniques that produced statistically significant definitions were attempted. A subjective classification of resin types based on preset levels of four monoterpenes was adopted and the use of a multi-variate graphical display technique described by Andrews (1972) investigated.

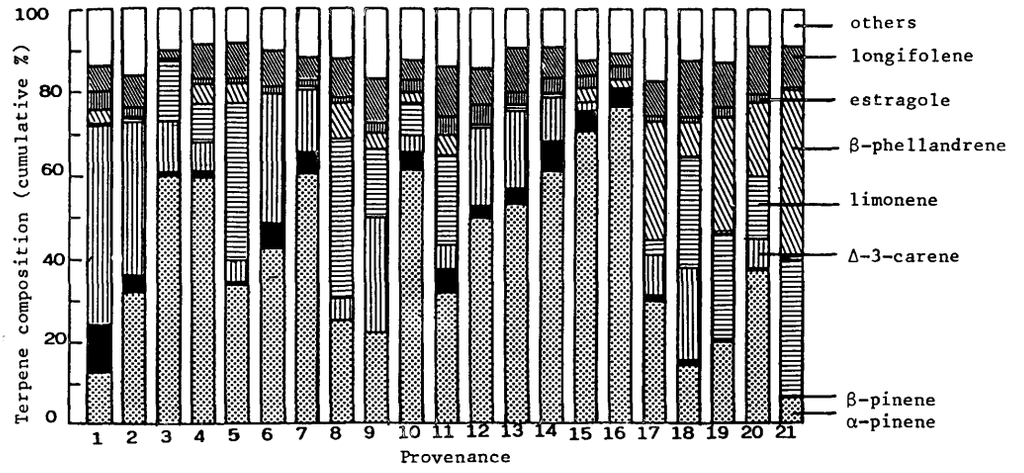


Figure 1a. — Terpene composition of *P. patula* ssp. *tecunumanii* — provenance mean.

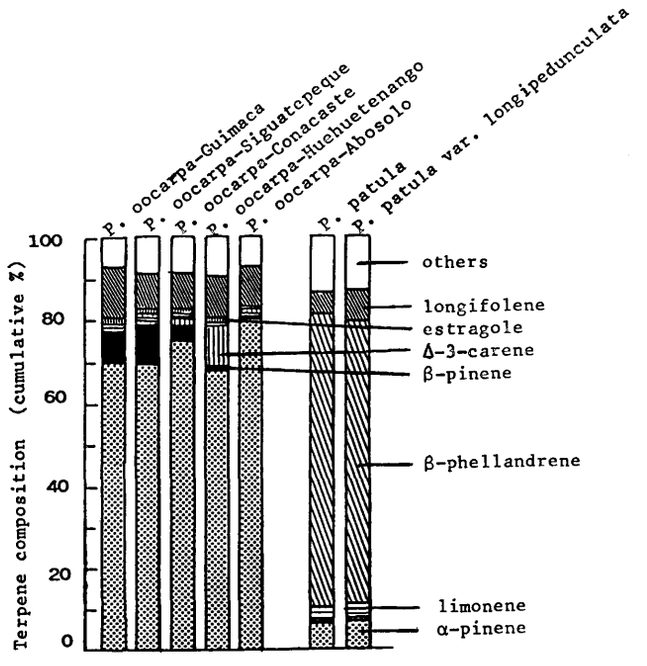


Figure 1b. — Terpene composition of associated species — provenance mean.

Of the subset of seven main components, four (α-pinene, $\triangle$-3-carene, β-phellandrene, limonene) were used to define five type classs. *Tables 2a* and *b* specifiy the components of these classes and the allocation to type class by country.

P. occarpa provenances contained high percentages of trees in type 1 (α-pinene greater than 60% of total terpenes). Provenances of *P. patula* and its variety *longipedunculata* were virtually exclusively type 4 (greater than 30% β-phellandrene). Major differences between countries were found in the type class allocation of *P. patula* ssp. *tecunumanii* provenances. Marked clines existed in both resin type and in the proportional presence of some terpenes, particularly with altitude.

1. Resin type and altitudinal variation

Figure 2 demonstrates the relationship of dominant terpenes with altitude. Weaker clines existed for latitude and longitude in *P. patula* ssp. *tecunumanii* but it is possible to predict geographic distribution based on chemical composition.

Resin type 1 — high α-pinene

In Honduras, with the exception of Villa Santa, provenances were separable into two groups based on altitude and resin type. Group 1 corresponded to provenances at low altitudes, 500 m to 1600 m, with resin type 1 profiles:

Zambrano	50% of trees with > 60% α-pinene
Siguatepeque	68% of trees with > 60% α-pinene
Cusuco	60% of trees with > 60% α-pinene
San Francisco de la Paz	66% of trees with > 60% α-pinene
San Esteban	63% of trees with > 60% α-pinene
Culmi	67% of trees with > 60% α-pinene
Jocon	70% of trees with > 60% α-pinene

Group 2 provenances at over 1600 m could not be allocated to type 1 terpene composition:

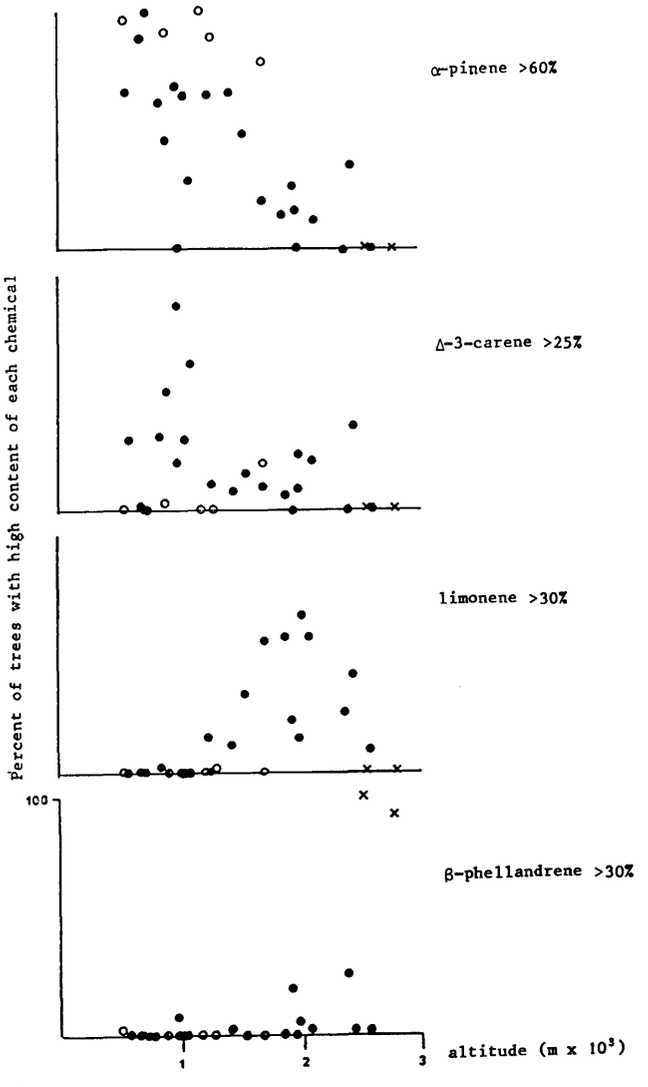
Table 2a. — Percentage of trees in 5 monoterpene classes for 21 provenances of *P. patula* ssp. *tecunumanii.*

COUNTRY	BELIZE	GUATEMALA	HONDURAS	MEXICO	NICARAGUA
No. of provenances	2	3	12	2	2
No. of trees	67	75	379	46	50
Type Main components					
1 α-pinene > 60%	92.9	3.3	46.7	6.4	16.0
2 $\triangle$-3-carene > 25%	1.4	4.0	25.1	29.8	76.0
3 limonene > 30%	-	13.3	18.5	21.3	-
4 β-phellandrene > 30%	1.4	9.3	0.1	19.1	2.0
5 limonene > 10% + β-phellandrene > 10%	-	52.0	0.1	12.8	-
Others	2.9	8.1	9.7	10.6	6.0
Total	100.0	100.0	100.0	100.0	100.0

Table 2b. -- Percentage of trees in 5 monoterpene classes for provenances of *P. oocarpa* and *P. patula*.

Species	*P. oocarpa*	*P. oocarpa*	*P. oocarpa*	*P. patula*	*P. patula* var. *longipedunculata*
COUNTRY	HONDURAS	MEXICO	GUATEMALA	MEXICO	MEXICO
No. of provenances	2	1	2	1	1
No. of trees	56	24	44	40	29
Type Main components					
1 α-pinene > 60%	87.5	95.8	90.9	-	6.9
2 △-3-carene > 25%	1.8	4.2	6.8	-	-
3 limonene > 30%	-	-	-	-	-
4 β-phellandrene > 30%	1.8	-	2.3	100.0	93.1
5 limonene > 10% + β-phellandrene > 10%	-	-	-	-	-
Others	8.9	-	-	-	-
Total	100.0	100.0	100.0	100.0	100.0

Montaña Celaque	22% of trees with > 60% α-pinene
Montaña Sumpul	17% of trees with > 60% α-pinene
La Paz	22% of trees with > 60% α-pinene
Guajiquiro	0% of trees with > 60% α-pinene



● = *P. patula* ssp. *tecunumanii*
○ = *P. oocarpa*
× = *P. patula*

Figure 2. — Relationship of dominant terpenes with altitude.

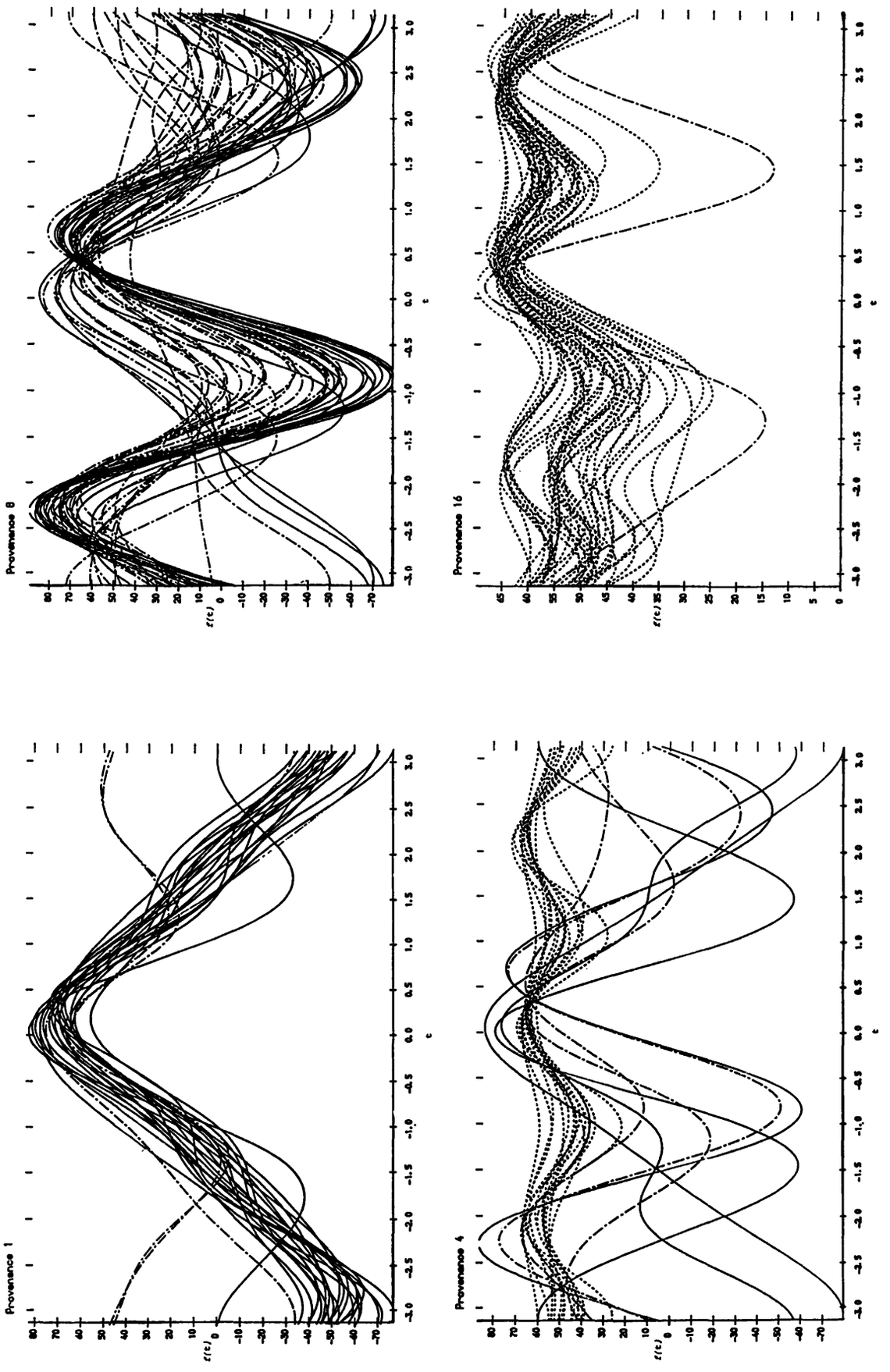
Levels of α-pinene clearly decreased with increased altitude in *P. patula* ssp. *tecunumanii*. *P. oocarpa* populations clustered quite strongly for α-pinene (*Figure 2*), but suggested distinction from the clinal patterns of *P. patula* ssp. *tecunumanii*. Provenances of *P. patula* at the highest altitudes, with little or no α-pinene were also dissimilar to most *P. patula* ssp. *tecunumanii*. Geographically, increased frequencies of high α-pinene types were found to occur in easterly regions of the natural range of the subspecies in Belize and north-east Honduras.

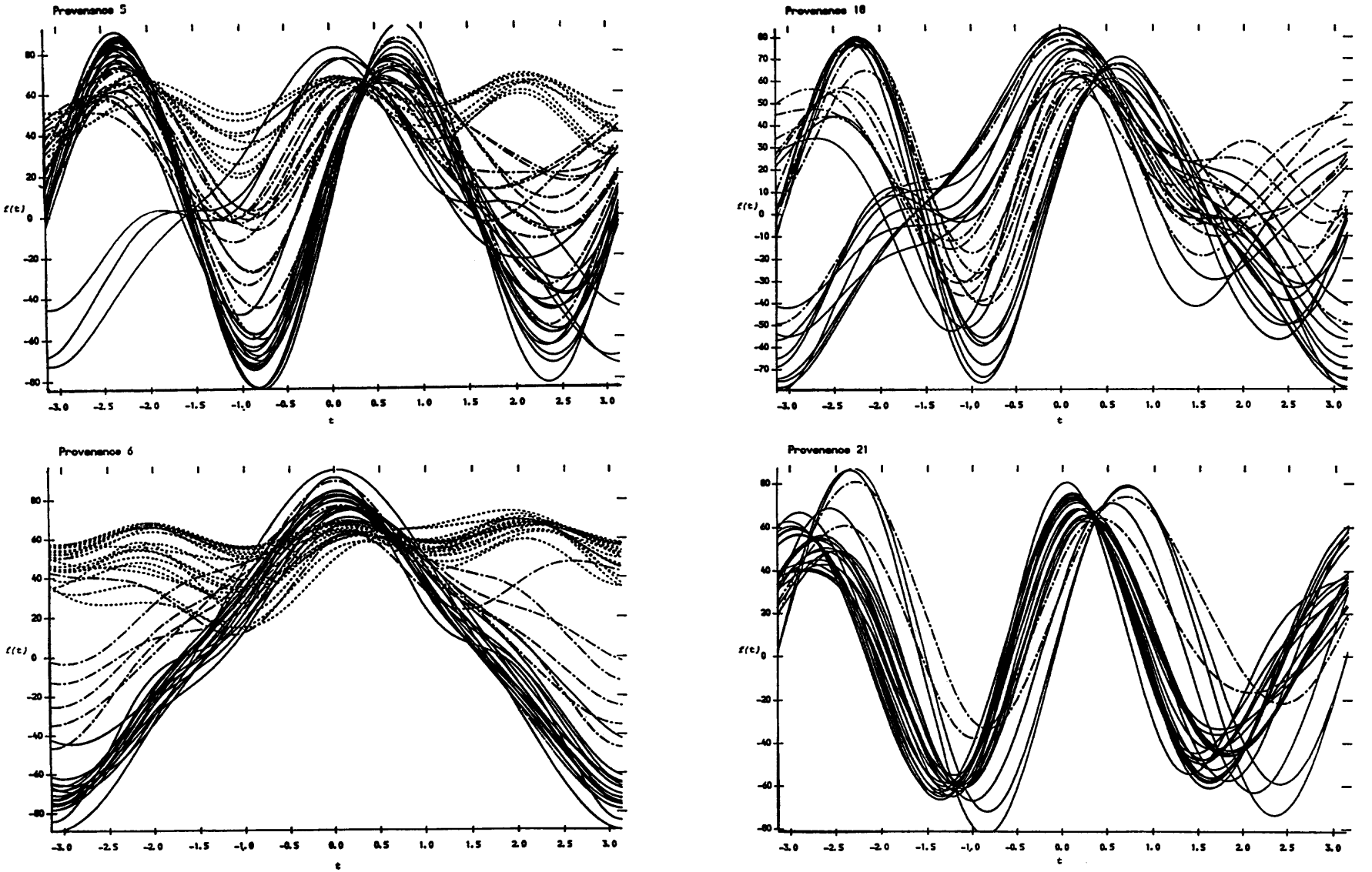
Resin type 2 — high △-3-carene

Patterns for △-3-carene were less obvious but demonstrated how the majority of *P. patula* ssp. *tecunumanii* trees differed from *P. oocarpa*. In the latter species trees contained little or no △-3-carene regardless of altitude. Seven populations of *P. patula* ssp. *tecunumanii* had medium to high frequencies of trees with greater than 25% △-3-carene at low elevations (below 1200 m.). Resin type 2 trees predominated in eastern Honduras and Nicaragua, particularly in those trees which lacked α-pinene.

Resin types 3, 4, 5 — high limonene/β-phellandrene

Limonene and β-phellandrene type classes were restricted to Mexico and Guatemala. The frequency of *P. patula* ssp. *tecunumanii* trees of resin type 3 (greater than 30% limonene) increased sharply above 1500 m and demonstrated a clear elevational trend (see *Figure 2*). It was a noteable and discriminatory feature that the population of *P. oocarpa* at 1700 m contained no trees with high levels of limonene. *P. patula* ssp. *tecunumanii* populations at this and at lower altitudes did contain some high limonene trees. Except at the higher altitudes, *eg* at Juquila, populations of *P. patula* ssp. *tecunumanii* did not contain high frequencies of trees with resin type 4 (greater than 30% β-phellandrene). *P. patula* and its variety *longipedunculata*, however, showed respectively, 100% and 93% type class 4 trees and β-phellandrene values were in the range 47% to 80% of total terpenes. The range of values at Juquila, for comparison, was 0.92% to 71.59%. All Mexican and Guatemalan provenances of the sub-species ranged equally widely with reduced upper and lower limits. Type 5 terpene profiles (limonene and β-phellandrene both greater than 10%) were predominant in Guatemala, noticeably so at Pachoc where 80% of trees were allocated to this type class. Limonene and β-phellandrene values ranged from 8% to 80% and 0% to 63% of total terpenes, respectively.

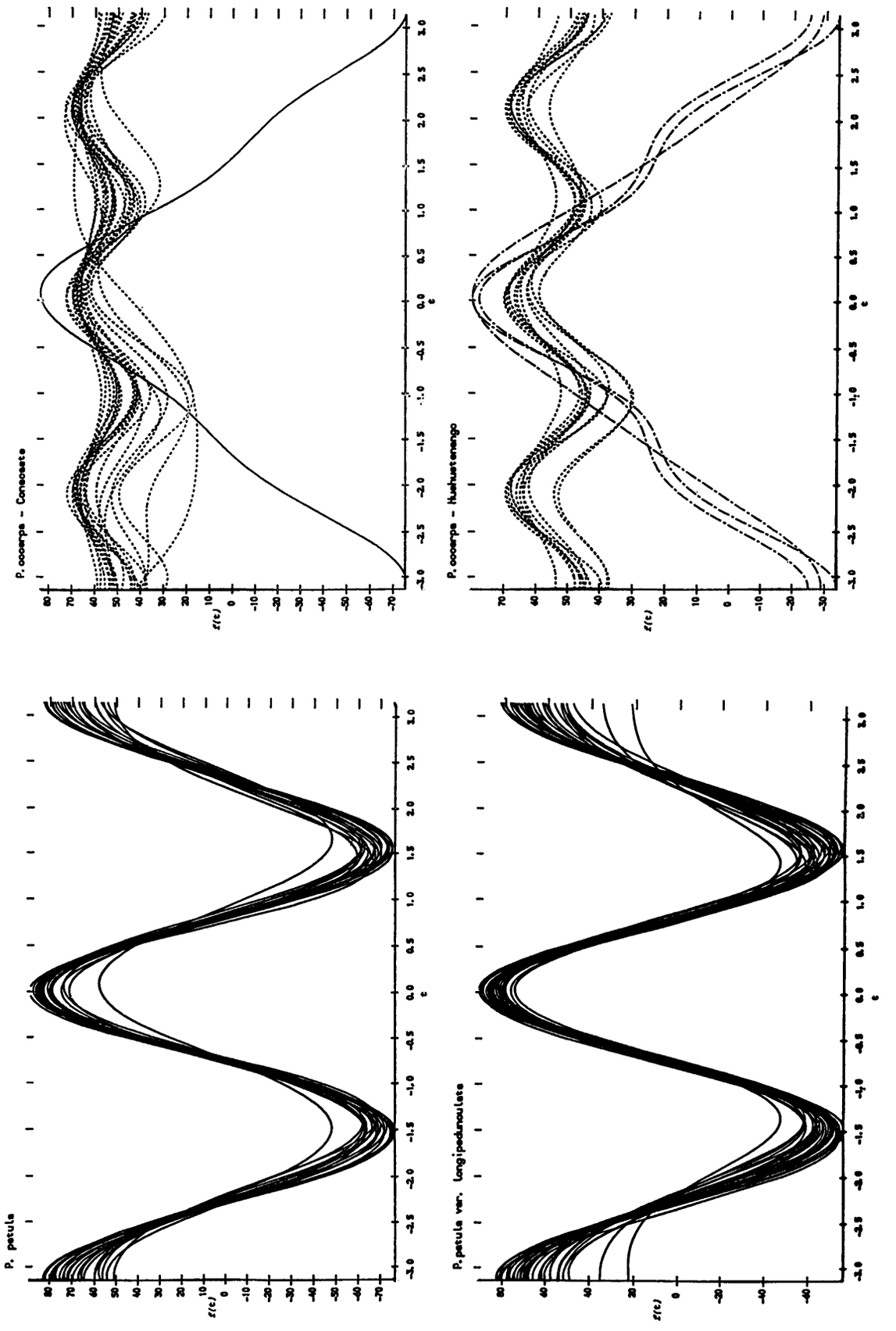

Provenance 8
Provenance 16
Provenance 1
Provenance 4



..... α-pinene greater than 60% of total terpenes
. — . — . — α-pinene 20% to 60% of total terpenes
——————— α-pinene less than 20% of total terpenes

Figure 3a. — Andrews curves of selected provenances of *P. patula* ssp. *tecunumanii*.

In addition to the four main components, β-pinene and longifolene were frequently found in substantial quantities and may be useful to further disciminate between some provenances. *Table 3* lists these provenances and the frequency of trees with greater than 10% longifolene. The dissimilarity between *P. patula* ssp. *tecunumanii* and *P.*



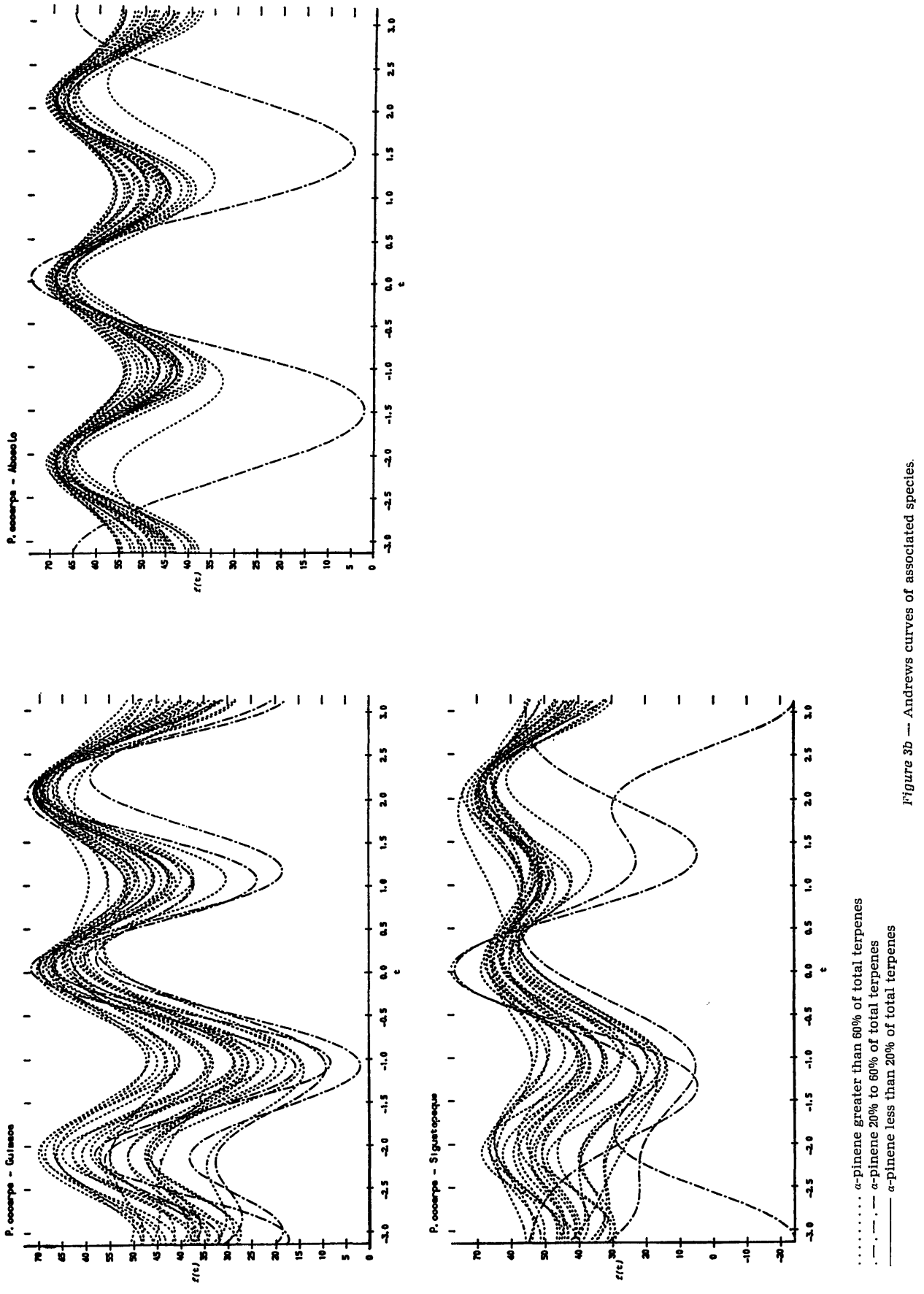


Figure 3b — Andrews curves of associated species.

patula is thus further highlighted. Longifolene results initially suggested a greater similarity with *P. oocarpa*. This similarity, however was only found where *P. patula* ssp. *tecunumanii* grows in association with *P. oocarpa*, particularly in the mid- to higher elevations in Honduras and Guatemala. High frequencies of trees with longifolene

Table 3. — Frequencies of trees with > 10% longifolene.

P. patula ssp. *tecunumanii*	
Yucul	15%
San Rafael	17%
Villa Santa	8%
Culmi	6%
Cusuco	8%
Siguatepeque	30%
San Esteban	26%
Jocon	27%
San Francisco de la Paz	45%
La Paz	39%
Guajaquiro	38%
Montaña Sumpul	58%
Montaña Celaque	67%
La Soledad	50%
San Jeronimo	50%
Pachoc	36%
Juquila	47%
Las Piedrecitas	85%

P. patula	22%
P. patula var. *longipedunculata*	11%
P. oocarpa	
Siguatepequ	76%
Guimaca	33%
Abosolo	37%
Huehuetenango	60%
Conacaste	59%

at Las Piedrecitas were confusing as *P. oocarpa* is not thought to grow in association with *P. patula* ssp. *tecunumanii* at this location. *P. patula* ssp. *tecunumanii* in Belize was virtually bereft of longifolene. *P. oocarpa* does not grow in this area. Similarly in the north-eastern provenances of Honduras, *P. oocarpa* is rarely found in association with *P. patula* ssp. *tecunumanii* and only low frequencies of trees with longifolene were detected. Morphological mis-identification of the sub-species for *P. oocarpa* was common in these areas and high α-pinene levels in both species further confused identity. Exchange of the genetic material responsible for the production of longifolene is suggested at mid- to high altitudes whilst lack of such interaction and little or no longifolene confirms the identity of 'pure' *P. patula* ssp. *tecunumanii*. Of particular importance in the discrimination between populations within country were the high levels of β-pinene in samples from Yucul, Nicaragua. Nearly 70% of trees sampled contained β-pinene in the range 10% to 27% of total terpenes. Only 16% of trees in the Nicaraguan provenance of San Rafael had β-pinene at levels above 10%. High $\triangle$-3-carene in association with β-pinene further characterised this provenance.

2. *Andrews curves*

This technique for the display of multi-variate data, proposed by Andrews (1972), maps each multi-response observation (*ie* the seven terpene variables) as a function f (t) of a single variable t (in these data t is α-pinene). The function f (t) produces wave patterns depending on observed values of p variables plotted over the range $-\pi$ to $+\pi$. The multi-response observations are displayed with:

y axis = function value f (t)

x axis = ranging values of t between $-\pi$ and $+\pi$

(Note that for comparison of provenances, the scale of the y axis may differ between provenances).

Each line represents the function of the multi-response observations for a single tree. Examples of Andrews curves are presented in *Figures 3a* and *3b*. Explanatory notes accompany in *Table 4*. The shape of the curve enables judgement of the predominant resin phenotype and allows comparisons between populations and species. In addition to the qualitative function of these curves indications of within provenance variation were gained from the cohesiveness or spread of curves within a population. Clusters of chemically similar trees were identifiable, as were occasional outliers.

Nicaragua

Samples from Nicaraguan provenances were predominantly terpene phenotype B. The dominant component of which is $\triangle$-3-carene. Cohesiveness of curves was good although a number of outliers in San Rafael provenance were identified as phenotype A. The value of β-pinene to distinguish between the Nicaraguan provenances was not evident using Andrews curves. Had the function been based upon β-pinene the distinction may have emerged.

Honduras

Substantial variation was evident between Honduran provenances as indicated by the large number of outliers and wide spread of curves along the y axis. With the exception of La Paz, Guajiquiro and Montaña Sumpul, provenances were a mixture of phenotype A, α-pinene dominant, and phenotype B, $\triangle$-3-carene dominant. La Paz, Guajiquiro and Montaña Sumpul reflected phenotype C (limonene and β-phellandrene) and support the altitudinal groupings defined in the above section.

Belize

Little variation within or between provenances was found. Resin samples were predominantly phenotype A.

Mexico

Analysis of *P. patula* and *P. patula* var. *longipedunculata* data produced archetypal diagramatic representation of phenotype D (see *Figure 3b*). Less clear patterns emerged for Mexican provenances of *P. patula* ssp. *tecunumanii*. Spread of curves was substantially greater than in *P. patula* and its variety. Curves tended to phenotype C, an indication of limonene with β-phellandrene. A number of resin samples form Juquila provenance were classical phenotype D.

Guatemala

Phenotype C predominated but the spread of curves and some gradation to phenotype D pointed to within-provenance variation. Cohesiveness was greater in samples from Pachoc. This provenance was irrefutably phenotype C. Andrews curves for Pachoc closely resembled those of

Table 4. — Explanatory notes for Andrews curves.

Terpene phenotype	Wave shape	Major troughs/peaks	Dominant chemical	eg of species or resin type
A		Peaks at: pi = -2.1, +0.1, +2.1	∝-pinene	P. oocarpa Resin type 1
B		Peak at: pi = 0.0	△-3-carene	Resin type 2
C		Troughs at: pi = 1-1.2, +2.0	Limonene with β-phellandrene	Resin type 3 or 5
D		Troughs at: pi = -1.5, +1.5	β-phellandrene	P. patula P. patula var. longipedunculata Resin type 4

P. greggii, also a member of the Oocarpae, but with a different flowering season to those species discussed here and therefore reported elsewhere (LOCKHART, 1985).

Provenances of *P. oocarpa* from three countries were consistently of phenotype A with little spread of curves, except in Guimaca, Honduras.

No numerical test of similarity between curves was attemped due to the nature of the data. Work continues at the Oxford Forestry Institute on further techniques for the presentation of compositional data. Andrews curves supported the categories subjectively formed by resin type classification and offered immediate visual appreciation of the importance and contribution of the major variables to the overall resin phenotype. They did not, however, provide the sensitivity of resin type class and frequency data to further identity and distinguish using minor compounds.

Conclusions

In only limited examples do the analytical results indicate similarities in terpene features between *P. patula* and *P. patula* ssp. *tecunumanii* eg Juquila, Mexico.

Some provenances more closely resemble *P. oocarpa*, particulary in Belize and eastern Honduras, however, the lack of β-pinene and longifolene in noteable quantities confirms the identity of *P. patula* ssp. *tecunumanii vis-a-vis P. oocarpa* in Belize. Dissimilarities in △-3-carene levels and the clinal variation of limonene concentration in the subspecies corroborate this.

Because of its greater altitudinal range, *P. patula* ssp. *tecunumanii* can be found in association with both *P. oocarpa* and *P. patula*. Zones of morphological transition exist and these species are known to have similar flowering seasons. Over a range of elevations it is possible that interchange of genetic material between *P. oocarpa* and *P. patula* ssp. *tecunumanii* has occurred and still does. At upper elevations the same may be true between *P. patula* and its subspecies. Originally *P. patula* ssp. *tecunumanii* may have been very high in limonene and relatively low in other constituents. Secondary contact rather than incomplete differentiation may have occurred (W. B. CRITCHFIELD, pers. comm.) [3]). The origins of the introduction of △-3-carene are obscure.

The chemical analyses do not therefore appear to support the designation (originally on botanical grounds) of the test pine as a sub-species of *P. patula*. Nor do they suggest any close similarities with *P. oocarpa*. Moreover excessive intra-specific variation dissuades the designation of specific rank. The taxonomy, is therefore, still uncertain but if the taxon is to continue as *P. patula* ssp. *tecunumanii* on morphological grounds then the existence of 'chemical races' or 'varieties' must be recognised.

Further sampling of *P. patula* ssp. *tecunumanii* is desirable, particularly at the margins of its natural range and resin type boundaries, for confirmation of the continuous nature of the clinal trends. Chemical analysis of additional provenances of the associated species is also recommended to assess intra-specific variation levels with greater confidence.

Acknowledgements

I am grateful to numerous colleagues at the Oxford Forestry Institute for help and advice throughout the project. In particular, thanks are due to Mr. P. MCCARTER for resin collections, Mrs. J. POWER for statistical help and Miss C. BUDDEN who typed the manuscript.

The Central American Pine Provenance research was entirely funded by the UK Overseas Development Administration.

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Inheritance of Allozymes in Larix decidua Mill.

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(Received 4th July 1988)

Summary

The inheritance of eleven enzyme systems at 19 loci was investigated using both macrogametophyte and embryo tissues of open pollinated seeds of *Larix decidua*. Eleven polymorphic loci were tested for deviations from the Mendelian segregation. Four loci (Fdh, Mdh4, Mnr2, Sod1) were monomorphic and another four loci (Gdh, Idh, Sod2, Srdh) possessed rare alleles observed only in bulked seed collections from 11 populations throughout the natural range of *L. decidua*.

Key words: Larix decidua, inheritance, allozymes.

Zusammenfassung

Die Vererbung von 11 Enzymsystemen wurde an 19 Enzymloci von *Larix decidua* in Macrogametophyten und Embryogeweben anhand von Einzelbaumsaatgut analysiert. Die Abweichungen von der Mendelspaltung wurden an 11 polymorphen Loci geprüft. Vier Loci (Fdh, Mdh4, Mnr2, Sod1) waren monomorph, an vier anderen (Gdh, Idh, Sod2, Srdh) wurden seltene Allele nachgewiesen, die nur in der Mischprobe von 11 Populationen aus dem natürlichen Verbreitungsgebiet von *L. decidua* auftraten.

Introduction

The number of enzyme gene markers known in *Larix decidua* is still small compared with other conifers. So far only few enzymes have been analysed in macrogametophyte tissue of this species (MEJNARTOWICZ and BERGMANN, 1975; KOSINSKI and SZMIDT, 1984; BERGMANN and RUETZ, 1987). The aim of our report was to present electrophoretical patterns for 11 enzyme systems in *L. decidua* and to determine the inheritance of allozymes controlled by 11 polymorphic loci. These investigations are a first part of a study which also includes the genetic structure and mating system of this species.

Material and Methods

Cones were collected from 70 trees growing in four natural populations of *L. decidua* in Poland and 25 clones growing in a clonal seed orchard near Kórnik.

Open pollinated seeds were extracted from cones for each tree separatly.

For the isozyme study initialy 6 seeds were analysed from each tree. Afterwards, 18 trees with polymorphic loci were chosen to complete the study.

Additionaly, bulked seed collections, from 11 populations throughout the natural range of *L. decidua*, were used to identify possible other allozymes of the investigated enzymes.

Macrogametophyte tissue and embryo were isolated separately trom the seeds and homogenized in 35 μl and 15 μl of Tris-HCl buffer (pH 7.2) respectively. A 0.15% 2-mercaptoetanol was added to the homogenate buffer as an antioxidant. Homogenates were subjected to horizontal (12%) starch gel electropharesis.

Two different buffer systems were used. System I according to RIDGEWAY *et al.* (1970): electrode buffer — 0.06 M lithium hydroxide and 0.3 M boric acid, pH 8.1 Gel buffer: 0.03 M Tris, 0.005 M citric acid and 1 % electrode buffer, pH 8.5. System II after SICILIANO and SHAW (1976): electrode buffer — 0.13 M Tris and 0.043 M citric acid, pH 7.0. Gel buffer: 1:10 dilution of electrode buffer or 1:6 dilution of electrode buffer designated as system IIa.

Gel silices were stained for the activity of 11 different