GENETIC SURVEY OF *PINUS RADIATA*. 8: POPULATION DIFFERENCES IN MONOTERPENE COMPOSITION OF CORTICAL OLEORESIN

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ABSTRACT

Cortical oleoresin was taken from branches of young (c. 7-year-old) trees of *Pinus radiata* D.Don provenances for gas-liquid chromatograph analysis of monoterpenes. Two studies were undertaken: Study I involved 50 unrelated trees of each of the Californian mainland populations (Año Nuevo, Monterey, and Cambria), Guadalupe Island, and Kaingaroa and Nelson from New Zealand; Study II involved 50 trees from each of the Guadalupe and Cedros Island populations. Monoterpene levels were expressed in percentages of total monoterpenes.

Study I resolved seven frequent monoterpene peaks plus several other peaks that occurred infrequently, the levels of each peak varying widely both from tree to tree within populations and among population means. Several components showed indications of major gene effects. Multiple discriminant analysis, despite highly non-normal frequency distributions for most fractions, was a powerful tool for demonstrating patterns of population differences. Canonical variate distances were least between Cambria and Monterey, followed by those between Año Nuevo and Monterey and between Cambria and Guadalupe, suggesting some north-south trend. The analysis showed a striking intermediacy of the New Zealand populations between Año Nuevo and Monterey, corroborating other evidence that New Zealand stock had derived entirely from those two natural populations, and indicating around 66% and 52% of Año Nuevo genes in Kaingaroa and Nelson respectively.

Study II resolved 12 monoterpenes, the mean levels of 10 differing clearly between the two island populations. Monoterpene composition of Guadalupe differed substantially between the studies, pointing to a need to standardise sampling conditions closely, and preventing reliable cross-reference of Cedros with mainland and New Zealand populations. However, Cedros appeared not to fit a north-south trend. From Study II emerged the most definite correlation between levels of different monoterpenes, a strong positive association between sabinene and terpinolene.

Keywords: monoterpenes; provenance; variation; discriminant analysis; genetic distance; taxonomy; *Pinus radiata*.

INTRODUCTION

Pinus radiata occurs naturally in five discrete natural populations. Of these, three are at Año Nuevo, Monterey, and Cambria on the Californian mainland coast between latitudes

35°30'N and 37°N; the other two are Guadalupe and Cedros Islands off the coast of Baja California, Mexico, at latitudes 29°N and 28°N respectively. The morphological variability of the species, both between and within populations, has caused much confusion among taxonomists (Bannister 1959; Forde 1964; Axelrod 1980). Much of the confusion has centred around the island populations, particularly Cedros.

It is now widely accepted that the island populations belong within *P. radiata*, although as varieties *binata* and *cedrosensis*. This has been concluded from several lines of evidence—namely detailed morphology, phenological characteristics, crossability, and monoterpene composition of oleoresin from the bole. Even so, it is of interest to understand better the taxonomic relationships among the island populations, between them and the mainland ones, and even among the mainland ones. It is also of interest to determine the affinities between New Zealand "land-race" material and the various native populations. That should indicate the ancestry of local land-race stocks, which is of interest in interpreting the performance of the land-race stocks relative to native populations, and thus for decisions on long-term genetic management (Burdon 1988).

Considerable information is available on the population differences in growth rate, site tolerances, morphology, and disease resistance (*see* Burdon, Bannister & Low 1992; Burdon 1992), and wood properties (*see* Burdon & Low 1992), monoterpene composition in wood oleoresin (*see* Burdon, Gaskin, Low & Zabkiewicz 1992), allozyme (isozyme) frequencies (Moran & Bell 1987; Plessas & Strauss 1986; Moran *et al.* 1988), and immunoelectrophoretic properties (Murphy 1981). The population differences, while often subtle for individual traits, undoubtedly show a complex overall pattern. Moreover, while the New Zealand *P. radiata* appears to have derived entirely from the two northernmost of the native populations, Año Nuevo and Monterey, the evidence from different morphological traits and monoterpene composition of bole oleoresin has not consistently pointed to the same proportions of those populations in the ancestry (Burdon, Bannister & Low 1992).

The monoterpene fraction of cortical oleoresin of young shoots represents a much more complex mixture than the corresponding fraction of bole oleoresin (Zabkiewicz & Allan 1975) and the complex mixture is highly variable within the species. While the cortical oleoresin may pose greater problems of repeatability in its composition, in respect of both sampling and analysis, the complexity and variability of the mixture offer a greater content of information.

The Genetic Survey experiment (Burdon, Bannister, Madgwick & Low 1992) contained trees of the various native populations which were of the same age and in the same environment, along with two New Zealand control populations. Thus it was a logical trial to sample for oleoresin. However, since one of the five natural populations, Cedros, was missing from the block originally sampled, a complementary study was done in another planting to cross-reference Cedros with Guadalupe. This paper covers the results from the two studies.

MATERIALS AND METHODS

The populations and the experiment have been described by Burdon, Bannister, Madgwick & Low (1992). Briefly, 50 wind-pollinated progenies (families) were sampled from each of

the five natural populations and two New Zealand ones, Kaingaroa and Nelson. Each of the Californian mainland populations was sampled in five ecologically distinct localities (subpopulations), 10 families coming from seed parents scattered throughout each locality. On Cedros Island the families came from 25 almost uniformly spaced trees throughout each of the disjunct north and south subpopulations of Cedros Island. The Guadalupe families came from throughout the population, except for the high-altitude outlier trees.

Study I

Oleoresin sampling was done $6^1/_2$ years after planting in the Stage I block at Site A of the experiment in Kaingaroa Forest, which contained all families of all populations except Cedros. By taking one colour replicate of the interlocking block layout, one tree per family could be sampled, with a completely random layout. Of the intended 300 trees from the six populations 294 were successfully sampled, avoiding a few trees that were undersized, badly windbroken, or with severe dieback.

Resin sampling was during 2–9 February, when the trees averaged 9 m tall (range 6–12 m). A vigorous second-order lateral was cut from the free-growing crown of each tree at 5–6 m height (generally 5.5–6 m) with a pole pruner. Oleoresin was taken c. 30 cm from the shoot tip, often at the top of the zone of bare cataphylls which occurs at the base of a shoot cycle ("internode"). Almost all the actual shoots appeared to be well ripened (i.e., fully lignified) at the sampling point although they were current season's growth.

A small scalpel cut was made across the ridge of cortex subtending a cataphyll, to sever a prominent resin canal. Exuding resin was immediately collected in a fine glass capillary tube (2–20 µl) and sealed in a 1-cm^3 vial, and stored on return to the laboratory at –20°C pending gas-liquid chromatograph (GLC) analysis within 6 weeks.

For analysis c. 200 µl diethyl ether was added, and an aliquot (1–4 µl) injected into the gas chromatograph. Analytical conditions were: instrument—Pye 104; detector—FID; column—glass, 150 cm × 6.5 mm od, packed with Carbowax/Porasil C (80–100 mesh); oven temperature—125°C; carrier gas and flow rate—N_2, 40 ml/min. Amounts of the monoterpene fractions were initially measured as peak areas, using a Chromalog II integrator, and finally expressed as a percentage of the total monoterpene mixture. Samples were analysed in random order.

The following monoterpene fractions were detected, in order of elution: (1) α-pinene, (2) camphene, (3) β-pinene (+ sabinene), (4) carene (+ myrcene), (5) α-phellandrene + α-terpinene, (6) limonene, (7) β-phellandrene, (8) γ-terpinene, (9) terpinolene (+ p-cymene). Occasional very small amounts of other fractions appeared, but these were disregarded as likely contaminants or decomposition products.

Study II

Seedlings of the Guadalupe and Cedros populations, surplus to needs for the main field experiment, had been planted out as 1+1 stock in adjoining rows in the Forest Research Institute grounds. On 21 March, nearly 5 years after planting, 50 trees were sampled per population, representing one or sometimes two trees per progeny. Samples were collected from 2–3 m above the ground, 20–30 cm from shoot tips, making the scalpel cuts just below

needle fascicles, and were stored for 2 years pending analysis; otherwise, sampling procedures and storage were as for Study I.

GLC analysis was also as for Study I, except for the following features: column—metal capillary FFAP SCOT, 50' × 0.02" id; oven temperature—75°C; carrier gas—He, flow 6.7 ml/min; makeup gas—N_2, flow 40 ml/min. Compared with Study I, this resolved β-pinene and sabinene, carene and myrcene, and p-cymene and terpinolene into separate peaks, while the α-phellandrene/α-terpinene peak was not detected.

Statistical Analysis

Preliminary comparisons among populations were made by analysis of variance, one peak at a time, using both untransformed percentages and arcsin transformations. Arcsin transformation, which is widely advocated for data involving proportions of mixture mitigated the non-normality of most of the frequency distributions, and tended to give slightly sharper resolution of population differences, but it still tended to leave very non-normal distribution and exaggerated the determination errors that occur near the limits of detection. Tests for skewness and kurtosis were made routinely.

Data from Study I were used for multiple discriminant analysis adapted by Andrew (1972) from Blackith & Reyment (1971). Untransformed data were used, past experience having indicated that this analysis is highly robust with respect to both departures from normality and heterogeneity of within-class variances. Data for each monoterpene were coded into terms of standard deviations about the overall mean for the populations in question—this removed the interdependence between monoterpene levels which would have created singular matrices. Two peaks, α-phellandrene + α-terpinene and γ-terpinene, which occurred very infrequently, were not considered. The analysis was run for several combinations of populations: all six; the four native populations only; the three Californian mainland populations only; and Año Nuevo, Monterey, Kaingaroa, and Nelson only—all disregarding the subpopulations classification within the Californian mainland. Additional runs were made comparing the five subpopulations within each of the three Californian mainland populations, and a further run comparing all 15 mainland subpopulations together.

RESULTS

Study I

For all fractions both means (Table 1) and frequency distributions (Fig. 1) differed considerably among the populations. Almost all individual distributions showed significant ($p < 0.05$) skewness and/or kurtosis. Since arcsin transformation very often did not restore normality, some of the tests for significance of population differences must be viewed with caution.

Among the four native populations every one was distinctive in respect of the mean levels (Table 1), if not also the types of frequency distribution (Fig. 1), of at least two of the monoterpene fractions. Año Nuevo and Monterey differed significantly in levels of four fractions, α-pinene, limonene, β-phellandrene, and terpinolene (Table 2). For each of these Kaingaroa and Nelson had intermediate levels, with Kaingaroa being consistently closer to

TABLE 1–Mean percentages of monoterpene peaks, by populations, Study I

Population	Monoterpene peak								
	α-pinene	Camphene	β-pinene (+ sabinene)	Carene (+ myrcene)	α-pellandrene α-terpinene	Limonene	β-phellandrene	γ-terpinene	Terpinolene (+ p-cymene)
Año Nuevo	12.5 d	4.3 c	13.0 b	18.1 a	0.0 b	38.4 a	10.6 a	0.4 c	2.6 c
Monterey	26.5 b	4.5 c	12.4 b	23.7 a	0.2 b	18.7 c	2.0 c	0.8 ab	11.1 a
Cambria	43.5 a	6.6 b	12.6 b	13.5 b	0.2 b	6.5 d	3.5 b	1.5 a	12.1 a
Kaingaroa	17.9 c	5.5 bc	12.0 b	23.0 a	0.2 b	29.3 b	7.2 ab	0.5 b	4.3 bc
Nelson	19.3 c	5.2 c	13.2 b	23.5 a	0.3 b	28.7 bc	5.6 ab	0.5 b	5.7 bc
Guadalupe	28.9 b	10.8 a	19.6 a	6.4 c	1.0 a	9.5 d	11.8 a	1.7 ab	10.1 ab

Values in a column that are suffixed with a letter in common do not differ significantly ($\alpha = 0.05$, protected t-tests or χ^2-tests, using arcsin transformations).

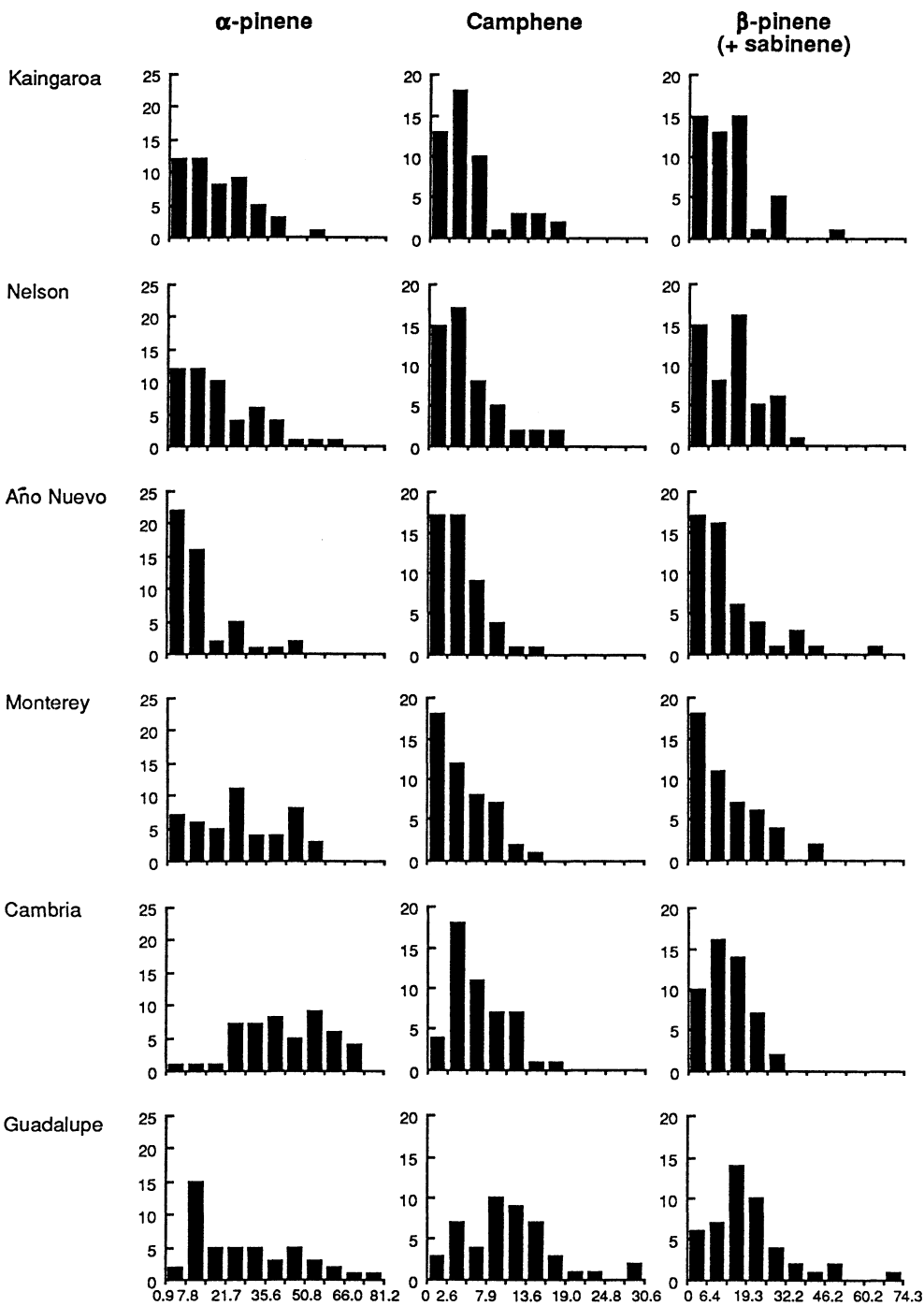
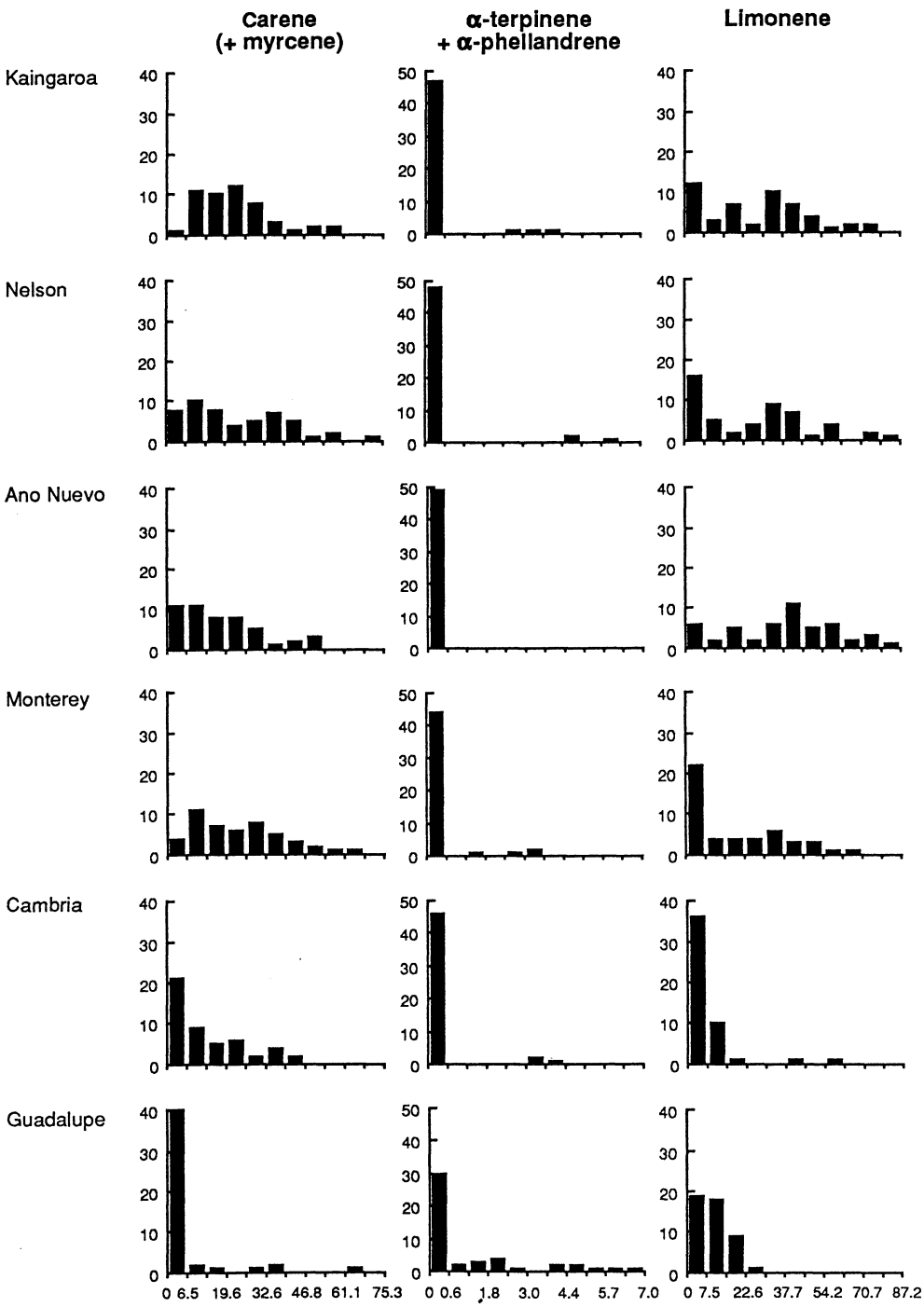


FIG. 1–Frequency distributions for percentage classes (equal percentage intervals between stated bounds for total sample) of individual monoterpene fractions, by populations.



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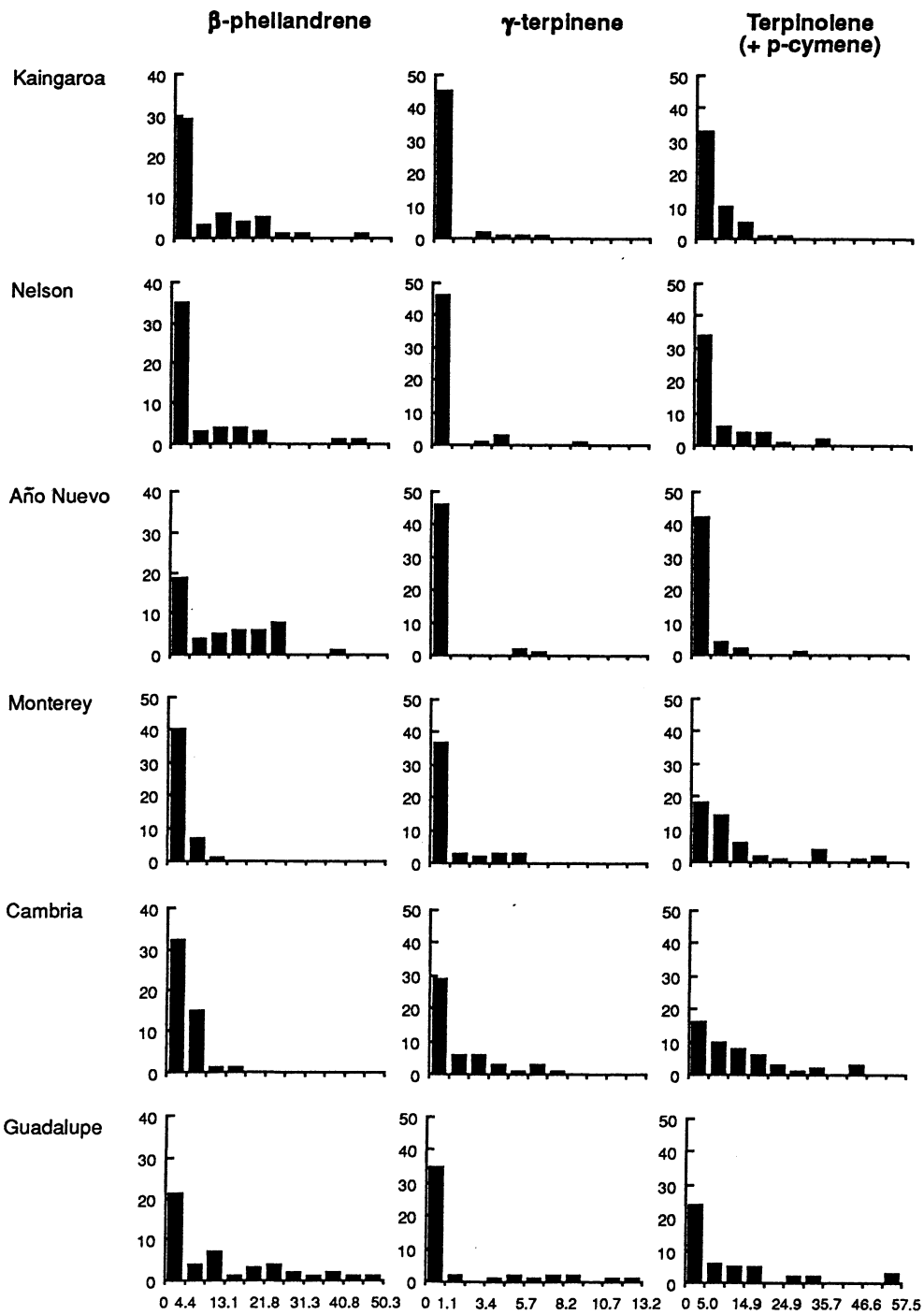

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TABLE 2–Estimated percentage resemblances to Año Nuevo *v.* Monterey of the New Zealand populations on the basis of levels of individual monoterpene fractions.

Fraction	Untransformed (%)		Arcsin transformed			
	Kaingaroa	Nelson	Kaingaroa	Nelson	$F_{3,194}$*	p*
α-pinene	61	51	56	46	8.67	<0.0001
carene (+ myrcene)	[12	3	0	9]	2.04	~0.09
limonene	54	49	58	40	7.61	<0.001
β-phellandrene	60	41	57	34	6.33	<0.001
terpinolene	80	69	69	60	11.4	<0.0001

Resemblance defined as (NZ – M)/(A – M) × 100, where A, M, and NZ are the averages for Año Nuevo, Monterey, and the New Zealand population respectively. Square brackets denote figures that mean little because of poor statistical separation of A and M.

* Test for differences among the four populations.

Año Nuevo than Nelson, although the resemblances of the New Zealand populations to Año Nuevo and Monterey respectively varied markedly among fractions.

The multiple discriminant analysis showed very clear differences (Wilk's Λ, $P \rightarrow 0$) among populations in all combinations studied, but showed no evidence of differences between subpopulations within populations ($p > 0.55$ in each case). When all the four native populations were considered, all three eigenvalues were highly significant ($p < 0.01$), demonstrating population differences in all available dimensions of "multidimensional space" and thence that all the populations differed significantly from each other. Even so, the third eigenvalue was only one-seventh as large as the second, so almost all the statistically significant differences are accounted for by the first two canonical variates. When the New Zealand populations were also considered no additional eigenvalues were significant, nor were the canonical vectors (not shown) greatly changed. Also, when just Año Nuevo, Monterey, and the New Zealand populations were considered only one eigenvalue was significant.

All tests (χ^2) for heterogeneity of covariance matrices were very highly significant ($p < 0.001$). Since this occurred even among subpopulations within single populations it presumably resulted mainly from non-normality of data, to which such tests are very sensitive. Thus the populations are liable to have different confidence limits about their means, and such confidence limits can be asymmetrical unless samples are large.

"Distances" between populations, on the basis of canonical variate scores, are shown in Table 3 for analyses involving various combinations of populations. The distances among natural populations increased according to geographic separation, allowing that distances from Guadalupe would be under-estimated through disregarding two fractions for which Guadalupe was distinctive. However, Monterey appeared to differ less than Cambria than from Año Nuevo while the (admittedly under-estimated) distance between Guadalupe and Cambria was not notably large. Considering subsets of the populations tended to increase the individual distances slightly, without greatly altering relativities, even though the canonical vectors were greatly altered when Guadalupe and Cambria were excluded from consideration. The general intermediacy of the New Zealand populations between Año Nuevo and Monterey, with Kaingaroa slightly closer than Nelson to Año Nuevo, was very evident.

TABLE 3–Estimated distances between population means, on basis of canonical variate scores, considering various combinations of populations.

	Año Nuevo	Kaingaroa	Nelson	Monterey	Cambria
All populations except Cedros					
Kaingaroa	0.79				
Nelson	0.97	0.34			
Monterey	1.785	1.19	0.90		
Cambria	2.75	2.23	2.09	1.51	
Guadalupe*	2.52+	2.19+	2.18+	2.16+	1.78+
Natural populations only					
Monterey	1.85				
Cambria	2.82	•	•	1.53	
Guadalupe*	2.75+	•	•	2.32+	1.71+
Californian mainland only					
Monterey	2.15				
Cambria	3.08	•	•	1.53	
Año Nuevo, Monterey, and New Zealand					
Kaingaroa	0.798				
Nelson	1.007	0.381			
Monterey	1.998	1.395	1.094		

* Pluses suffixing figures in Guadalupe arrays reflect omission from analysis of two fractions, α-phellandrene + α-terpinene and γ-terpinene (Table 1, Fig. 1), which were less scarce in this population than in the rest.

Plots of population means for the three statistically significant canonical variates (Fig. 2) show the status of the New Zealand populations even more clearly. For all pairs of variables they fell very close to the mid-points of the axes joining Año Nuevo and Monterey means, with negligible departures towards either Guadalupe or Cambria. Other features evident from similar plottings (not shown) were: the tight clustering of subpopulation means around their population means (shown in Fig. 2) when all 15 mainland subpopulations were plotted; and a much wider and somewhat asymmetric scatter of individual-tree plottings about their population means, with strong overlap among the populations.

Correlations between percentages of the various monoterpenes were calculated population by population. These are not reported in detail, but the following trends were evident:

- A tendency for major components in a population to be negatively correlated as a result of their being measured as proportions of a mixture (cf. Squillace 1976a);
- A tendency for correlations involving components of similar retention times to be positive, and vice versa with components of very different retention times;
- A tendency for minor components to be positively correlated with each other.

Otherwise, no strong positive correlations were observed consistently in the various populations.

Study II

Direct comparison between the two island populations showed major differences in levels of all but two of the monoterpenes (Table 4), Cedros having much higher average levels of carene and β-pinene and much lower levels of all other monoterpenes except γ-terpinene and

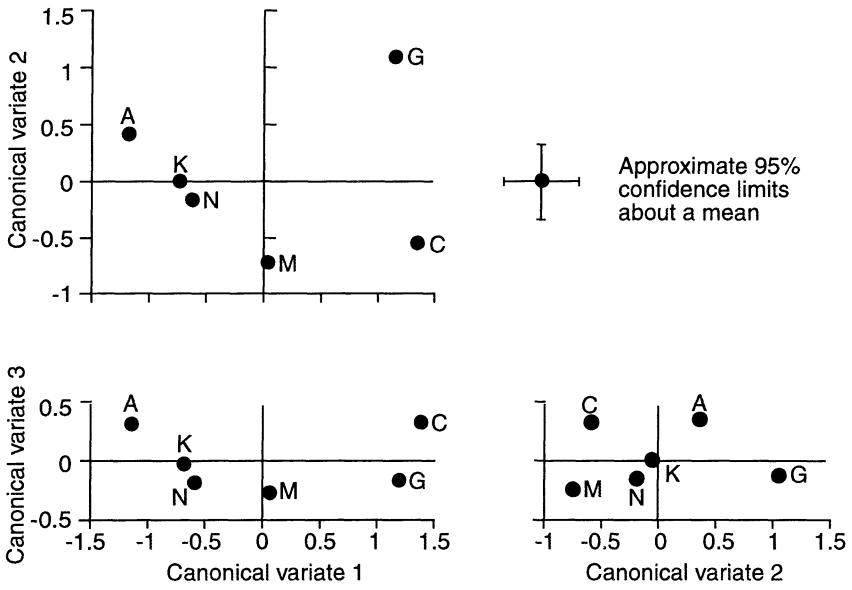

FIG. 2–Plots of population means for pairs of the three significant canonical variates, showing intermediacy of the New Zealand populations (K and N) between Año Nuevo (A) and Monterey (M), and distinctness from Cambria (C) and Guadalupe (G).

TABLE 4–Mean levels of individual monoterpenes (percentage of total monoterpenes) and tests (using arcsin transformation) for population differences.

Monoterpene	Study I Guadalupe	Study II			
		Guadalupe	Cedros	$F_{1,98}$*	p*
α-pinene	28.9	30.7	9.3	52	<0.0001
camphene	10.8	0.68	0.13	15	<0.001
β-pinene	19.6	39.9	70.2	59	<0.0001
sabinene		10.3	0.27	28	<0.0001
carene	6.4	2.3	15.9	40	<0.0001
myrcene		2.3	0.8	32	<0.0001
α-terpinene + α-phellandrene	1.1	–	–	–	–
limonene	9.5	1.1	0.75	30	<0.0001
β-phellandrene	11.8	5.7	1.0	15.4	<0.001
γ-terpinene	1.7	0.1	0.1	0.2	>0.5
p-cymene	10.1	0.06	0.02	0.9	>0.2
terpinolene		6.9	1.6	9.6	0.003

* Test for significance of difference between populations.

possibly p-cymene. As in Study I, the frequency distributions (Table 5) were generally very non-normal and varied markedly between monoterpenes. Not clear was whether and to what extent the distributions differed fundamentally between the two populations, given the obvious scalar effects combined with the need to express monoterpene levels in terms of percentages of total monoterpenes. These features of the frequency distributions would have caused some bias in tests for population differences, but that is unlikely to have been important in view of the large differences present.

Comparing the Cedros subpopulations, β-pinene averaged 74% in North and 64% in South ($F_{1, 48\ df} = 4.18$, $p \sim 0.05$, using arcsin transformation), while average carene levels were 24% and 34% respectively (corresponding $F = 9.08$, $p \sim 0.004$). However, these two differences are not independent, they are minor compared with the population differences, and the nature of the distributions could make the tests over-sensitive.

Considering the frequency distributions in more detail, they ranged from being essentially normal (at least after arcsin transformation) in α-pinene and β-pinene, to several that were both very skew-positive and showed signs of multinodality. This last category included carene, β-phellandrene, and terpinolene, although 50 trees were not enough to characterise a distribution very precisely for a population. The apparent binodality of sabinene (Study II)

TABLE 5–Frequency distributions for levels of individual monoterpenes (percentage of total monoterpenes) in Guadalupe (G) and Cedros (C) population samples, Study II.

Monoterpene	Bounds (lower/upper)	Pop.	Frequency class (equal intervals between bounds)									
			1	2	3	4	5	6	7	8	9	10
α-pinene	2.4/73.3	G	8	7	7	4	3	2	4	1	6	4
		C	34	14	0	2	0	0	0	0	0	0
camphene	0/6.3	G	34	8	4	2	0	1	0	0	0	1
		C	50	0	0	0	0	0	0	0	0	0
β-pinene	1.4/91.7	G	6	16	9	2	4	5	2	0	1	5
		C	1	0	2	1	4	4	6	10	8	14
sabinene	0/40.1	G	30	0	0	1	3	6	2	5	0	3
		C	49	0	0	1	0	0	0	0	0	0
carene	0/54.7	G	46	0	1	1	1	0	1	0	0	0
		C	20	4	6	3	5	1	5	3	2	1
myrcene	0/14.5	G	20	13	8	7	1	0	1	0	0	0
		C	42	3	3	1	0	0	0	0	0	1
limonene	0.4/2.5	G	6	7	9	9	7	6	1	2	2	1
		C	10	22	13	5	0	0	0	0	0	0
β-phellandrene	0/41.0	G	37	1	1	6	2	0	1	0	1	1
		C	50	0	0	0	0	0	0	0	0	0
γ-terpinene	0/1.5	G	41	2	3	0	2	0	0	0	1	1
		C	42	4	1	1	0	1	1	0	0	0
p-cymene	0/1.0	G	43	3	0	1	1	1	0	0	0	1
		C	46	2	1	0	1	0	0	0	0	0
terpinolene	0/42.5	G	32	5	1	3	3	2	2	0	0	2
		C	45	3	1	0	0	1	0	0	0	0

is viewed as being probably an artifact of the inability of the instrument to resolve low levels reliably from the β-pinene peak. Therefore, mean sabinene levels were probably biased downwards and β-pinene levels biased upwards.

One noteworthy association was evident between monoterpenes, a co-occurrence of sabinene (in Study II, when it was resolved from β-pinene) and terpinolene. In the Guadalupe population sample, the observed levels of the two compounds showed a correlation (R) of 0.66 ($p < 0.001$), all but one peak of terpinolene exceeding 2% being associated with a detected peak of sabinene although the ratios of the two compounds still differed quite widely. In the Cedros population sample much the highest level of terpinolene, 23.2%, was associated with 13.5% of sabinene but for the five other trees with significant terpinolene (3.0–9.4%) a sabinene peak was not detected.

Between Studies I and II the Guadalupe population samples differed greatly in the mean levels of several monoterpenes. In Study II β-pinene was present in substantially higher proportions compared with Study I, β-phellandrene and terpinolene in lower proportions, and camphene, limonene, and possibly γ-terpinene in much lower proportions.

DISCUSSION

The multiple discriminant analysis proved a very powerful heuristic tool for demonstrating the differences between four of the natural populations and the intermediacy of the New Zealand material between Año Nuevo and Monterey, despite the adverse data properties for individual monoterpene fractions. Thus, those data properties were evidently much less of a problem with such data analysis than was claimed by Birks & Kanowski (1988). Because prior evidence (Burdon & Bannister 1973) seemed to exclude Cambria as a progenitor of the New Zealand populations, Study I served in part to test the multiple discriminant analysis as a methodology for using the monoterpene data. Although alternative analyses (e.g., Aitchison 1982, 1983) may be even more powerful, the analysis used appeared to be extremely effective.

The failure in Study I to separate certain pairs of monoterpenes was not decisive, and may not have reduced the power of the study much. On the basis of Study II and other results (Burdon, Gaskin, Low & Zabkiewicz 1992; Burdon *et al.* in prep.) the separation of sabinene may have added little information to the terpinolene data, and myrcene levels appear not to differ sharply among populations, while p-cymene is suspected of being a contaminant and/or a degradation product.

The monoterpene composition within Study I may well have been influenced by factors such as differences between trees and even populations in shoot ripeness (cf. von Rudloff 1975) but, in that average shoot ripeness (reflecting average phase in the seasonal growth rhythm) would presumably be a feature of an individual population, the comparison in a common-garden experiment is no way invalidated. Indeed, this common-garden comparison of cortical monoterpenes appears to have been far more powerful for detecting the finer differences between populations than isozyme analysis (cf. Moran & Bell 1987). Comparisons using cortical monoterpenes in other situations, however, must clearly be made with great caution.

It is logical to use the comparisons between New Zealand populations and their Californian progenitors to estimate the percentage of genes contributed by each of the latter

populations. This initially entails characterising the distance between the populations concerned, which has been done piecemeal (Table 2), but the global estimates from the multiple discriminant analysis seem preferable. Such estimates could then be adjusted (Fig. 3) for departures of New Zealand populations from perfect intermediacy between Año Nuevo and Monterey. The relative distances were calculated as resemblances (cf. Table 2), to indicate 66% and 52% of Año Nuevo genes in the Kaingaroa and Nelson populations respectively, while the standard errors for these figures (Kendall & Stuart 1977, Ch. 10) appear to be around 12–14%. This inference of percentages of Año Nuevo genes assumes strictly additive inheritance, and although the genetic control of tree-to-tree variation in cortical monoterpenes is very strong (Burdon, Gaskin, Low & Zabkiewicz 1992) the degree to which it is additive has yet to be established.

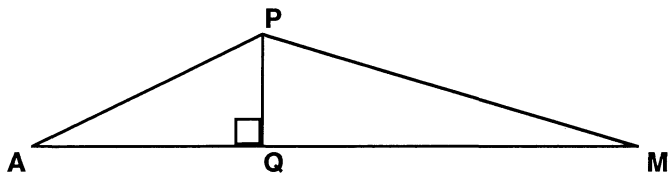

FIG. 3–Diagram illustrating calculation of adjusted differences between the New Zealand populations and their Californian progenitors. AM shows distance between Año Nuevo (A) and Monterey (M), and AP and PM show the unadjusted distances between a New Zealand population (P) and A and M respectively (Table 3), while AQ and QM denote the adjusted distances between P and A and M respectively (PQ perpendicular to AM).

The degree of additivity will depend on the scale used, which is inevitably somewhat arbitrary (cf. Birks & Kanowski 1988), and the use of arcsin transformation did suggest slightly greater resemblances to Monterey (Table 2). Certain genes could be inherently dominant in their effects; in other pine species, when major-gene effects have been observed, simple dominance is evidently common (Squillace 1976b). However, given the multiplicity of monoterpenes, and the overlapping frequency distributions which argue against fixation of major-gene differences between Año Nuevo and Monterey, it seems unlikely that departures from additivity were great. Another assumption was that monoterpene composition was not subject to significant pressures of directional selection in the various populations since the original importations of the New Zealand material from California. Earlier indications of an association between carene level and resistance to Diplodia-caused shoot dieback (Burdon & Bannister 1973), which may have exerted selection for higher carene levels, have not been borne out by more recent work (Burdon unpubl.). There is thus no strong evidence of genetic shifts resulting from natural and silvicultural selection.

Study II showed very strong differences between the two island populations. Evaluation of the affinities between Cedros and mainland populations, however, was severely complicated by the large differences in monoterpene composition between the Guadalupe samples in the two studies. Possible reasons are effects of differing ages (or maturation states) of trees, which could have been appreciable, given the different heights from which samples were collected, or differences in state of shoot ripeness. While almost all the shoots sampled in Study I appeared to be quite well ripened, the difference of nearly 2 months may have been important. Although the study of Burdon & Zabkiewicz (1973) did not address the effects of shoot ripeness directly, it did show relatively low levels of β-pinene in the spring when

shoot ripeness would have been least. Another study (Burdon, Gaskin, Low & Zabkiewicz 1992), involving cortical oleoresin samples taken in May from older New Zealand trees, albeit closer to shoot tips, showed much higher β-pinene levels and much lower levels of camphene, with slightly lower levels of limonene, β-phellandrene, and terpinolene than were observed in the New Zealand trees in Study I. Also, a more recent study (Burdon *et al.* in prep.), taking samples from well-ripened shoots at 2 m height in a $5^1/_2$-year-old trial of mainland Californian and New Zealand material, gave differences when compared with Study I that were comparable with differences obtained between Study I and Study II, which argues strongly against any important effect of the prolonged storage of the Study II samples. It is concluded, therefore, that the differences were presumably due to some combination of differences in shoot ripeness and maturation state, with a possible additional effect of distance from the shoot tip. The impacts of all these factors (which may not be consistent from genotype to genotype) would need thorough study before cortical monoterpene composition can be regarded as broadly cross-calibrated as a genetic fingerprint. If this were achieved, however, it might be possible to use changes in monoterpene composition as a good indicator of maturation state.

Any attempt to correct the Cedros data, on the basis of the differences in Guadalupe between the two studies, for cross-referencing Cedros with Californian mainland and New Zealand material, would be very tenuous. It would depend *inter alia* on the unproven assumption that certain factors change monoterpene composition in the same ways in all populations. However, it does appear that Cedros falls outside the slight north-south trend indicated by Study I. In the light of results from Study I and Burdon, Gaskin, Low & Zabkiewicz (1992), its high carene content suggests affinities between Cedros and both Año Nuevo and Monterey, while the low α-pinene and terpinolene levels of Cedros suggest affinities with Año Nuevo, and the low β-phellandrene affinities with Monterey, although the high β-pinene and low limonene might place Cedros at extremes beyond Guadalupe and Cambria respectively.

For several monoterpenes, notably carene, β-phellandrene and terpinolene, the frequency distributions suggested the existence of genes of large effect, or major genes (cf. Baradat *et al.* 1972, 1975; Squillace 1976a, b; Meier & Goggans 1978). Identifying such genes when monoterpenes are expressed as percentages of the volatile mixture is laborious and requires abundant data (cf. Squillace 1976a). It will be further complicated by any effects of variations in shoot ripeness or maturation state, which are likely to be problems with *P. radiata* with its comparative lack of discrete phenological states and its relatively gradual maturation process as trees become older and taller.

Correlations between the monoterpenes generally did not appear to be particularly meaningful. Many negative correlations appeared to be essentially artifacts of expressing monoterpenes as percentages of total monoterpenes. Positive correlations between monoterpenes of similar retention times or negative correlations between those of very different retention times could arise from correlated slope sensitivity errors. Positive correlations between levels of minor peaks may just reflect sample-to-sample variation in the general sensitivity of peak detection. While tree-to-tree variation in shoot ripeness might also generate correlations between certain monoterpenes, this was not clearly evident.

The one convincing case was the strong positive association between sabinene and terpinolene, which has also emerged strongly in other studies (Burdon, Gaskin, Low &

Zabkiewicz 1992; Burdon *et al.* in prep.). This and the co-occurrence of the two monoterpenes in wood oleoresin in *P. muricata* D.Don (Forde & Blight 1964; Mirov *et al.* 1966) and *P. radiata* (Burdon, Gaskin, Low & Zabkiewicz 1992) suggest a common biosynthetic pathway for the two monoterpenes in these pine species. There were some indications that, despite the co-occurrence of these two monoterpenes, the ratios of sabinene to terpinolene were lower in Cedros, although this could in some degree have been an artifact of the difficulties of detecting sabinene against a background of higher levels of β-pinene.

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