NEEDLE FLAVONOIDS AND POPULATION DIFFERENTIATION IN THE *ABIES* SECTION *NOBILIS* ENGELM.

by

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ABSTRACT

The *Abies* section *Nobilis* Engelm. consists of two high-elevation tree species that grow in the mountains of Washington, Oregon and California. The two varieties of *A.magnifica* A.Murray grow south of *A.procera* Rehd. One of these varieties, *A.magnifica* var.*shastensis* Lemm., is intermediate in some characteristics and locations between the two "typical" species. The goal of this thesis was to clarify species and population differentiation in the section *Nobilis*. To this end, nine populations of mature and twelve populations of immature trees were collected along a north-south transect that cut through most of the range of the section. The flavonoids were extracted from dried needle samples selected from each of the sample trees, and a flavonoid profile was determined for each tree. These individual tree profiles were used to prepare a composite flavonoid profile for the whole section.

Sixty-four probable flavonoid compounds occurred in the sample trees, of which forty-seven were at least partially identified. Twenty-four variable compounds were chosen for analysis. Variation was assessed using product-moment correlation co-efficients, frequency histograms, principal components analysis, weighted-pair-group cluster analysis, discriminant analysis and cladistic analysis. These different statistical techniques allowed comparison between the different interpretations of population variation.

Four factors appear to underly the pattern of population variation in the *Abies* section *Nobilis* : (1) evolution of the section under the diverse and changing conditions that have prevailed in the Pacific Northwest and California since the Oligocene epoch; (2) hybridization between morphologically, ecologically and/or chemically differentiated populations of trees within the section; (3) the presence of a genetically variable gene pool in the section as a consequence of these two factors;

and (4) the expression of a portion of that variability as adaptation to different stages of forest succession.

A.procera is an early-successional species with a gene pool that is more uniform than *A.magnifica*, which is essentially a late-succession or climax species. Flavonoid differentiation between these two species is not as distinct as has been observed in the *Abies* section *Balsamea* Engelm. *A.procera* tends to exhibit acetylated monoglycosides consistently whereas *A.magnifica* does not, and *A.magnifica* tends to accumulate glycosides of dihydrokaempferol and taxifolin where *A.procera* does not. These differences apparently have been obscured somewhat by variation between the flavonoid complement of mature and immature trees, by migration and fragmentation of the range of the section *Nobilis* since the Oligocene epoch, and by hybridization within the section.

The needle flavonoid results reported by this thesis, supported by published ecological, morphological and terpene data, confirm the current recognition of three taxa in the section. However, the variety *shastensis* exhibits enough differentiation to warrant further investigation into separation of that taxon into three or four separate taxa: (1) *A. procera* x *A.magnifica;* (2) relictual populations of *A.magnifica* var.*shastensis* in Tulare County, California and the Klamath Mountains of California and Oregon; and (3) *A.magnifica* var.*shastensis* from the North Coast Ranges of California.

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INTRODUCTION

Taxonomic definition within the genus *Abies* Mill. (the true firs) is difficult because of morphological variation within individual trees, within populations and between populations of the same and of contiguous species. Large and overlapping ranges, ready species interbreeding and phenotypic plasticity contribute to the taxonomic confusion (Klaehn and Winieski,1962; Liu,1971). Repeated migration and consequent possible speciation processes during climate changes have contributed both to this variability in species characteristics and to irregularities in temperate species' occurrence (Whittaker,1961; Parker,1963; von Rudloff,1975).

Recent population studies that examine both morphological and chemical variability in North American *Abies* species delimit several gene complexes, including one that is the section *Nobilis* Engelm. (eg. Jacobs*et al.*,1984; Parker*et al.*,1981; Hunt and von Rudloff,1974; Thor and Barnett,1974; Zavarin and Snajberk,1972; Robinson and Thor,1969; Smedman*et al.*,1969). Each gene complex contains a "species-pair" that is composed of two easily recognized extreme forms or species that are separated by an intergrading series of intermediates. Depending upon the degree and pattern of variability within and between populations throughout each complex, and considering reliable fossil evidence, several possibly complementary hypotheses can be suggested to explain the variation in each complex :

1. The intermediate populations, if they have the same amount of variation compared to the "typical" species populations, could be an intermediate taxon or taxa derived from the same ancestor and deserving specific status. This pattern of variation implies the existence of at least three species or subspecies, and is likely particularly if each taxon is discrete from all the others.

2. The intermediate populations, if highly variable, could be evidence of hybridization and introgression between two distinct taxa, either current or ancient or both. The implication is that two distinct species linked by hybrid swarms are present, and is very likely if the intermediates are highly variable.

3. The intermediate series could represent a "genotypically rich" (Stebbins,1950,p.281) ancestral race from which the other extremes evolved. The implication is that two well-differentiated but closely related "sister" species exist, flanking the taxon from which they segregated.

4. The intermediates could be one or more relicts of glacial or "environmental" refugia that have preserved and perhaps "highlighted" ancient clinal races through extinction of morphological forms and stochastic processes like genetic drift (Stebbins,1950,eg. pp.280-282; Franklin,1964; Wright,1976, p.36; Dobzhansky*et al.*,1977). Such an occurrence would imply the existence of only one, variable gene pool from which two or three "modern" species have segregated or are segregating, as suggested by hypothesis 3., above. or

5. Each entire complex could represent a single species so that the pattern of variation is simply ecotypic or clinal variation within a gene pool whose variability is correlated with environmental parameters throughout that species' range.

The *Abies* section *Nobilis* consists of three varieties that belong to two tree species that grow in the western United States from Washington south through central California (Hitchcock and Cronquist,1973; Cronquist*et al.*,1972; Hitchcock*et al.*,1969; Munz and Keck,1959). The taxonomic relationships within the section *Nobilis* are not clear; *A.magnifica* A.Murray var.*shastensis* Lemm. is intermediate in some morphological characters that are used to distinguish between *A.magnifica* A.Murray var. *magnifica* and *A.procera* Rehd. Four "operational" taxa are referred to in this thesis. These taxa are *A.procera*; northern *A.magnifica* var.*shastensis* from the area of range sympatry between the two "typical" species; *A.magnifica* var.*magnifica*; and southern *A.magnifica* var.*shastensis*. This last taxon occurs at the southern edge of the current range of the *Abies* section *Nobilis*.

Yeatman(1967) stresses the importance of including the evolutionary and migratory history of a species in any evaluation of its genetic variation, particularly if that variation is associated with geographic origin. The migratory history and evolution of the *Abies* section *Nobilis* is somewhat unclear for two major reasons. First, the paleobotanical history of the western United States is complicated because it is below the southernmost extension of continental glaciation, because it has been subjected to many different geologic processes, and because of the complexity and diversity of both extant and past floras. Secondly, fossil evidence for the section is minimal, particularly in the area of current northernmost occurrence.

The objective of this thesis was three-fold. Firstly, the *Abies* species pair that comprises the *Abies* section *Nobilis*, as it occurs today in the western United States, was examined. A summary of the topography and paleobotanical history of the area, followed by a

description of the taxonomic considerations relevant to the *Abies* section *Nobilis* and a review of the flavonoid systematics in the genus *Abies* as a whole are used to orient the reader.

Secondly, the needle flavonoids present in the *Abies* section *Nobilis* were determined. Flavonoid profiles are generally accepted as reflecting phylogenetic affinity among closely related species (Levy,1983; Mabry,1973), and chemical diversity in closely related plant groups is often related to ecological selection (Harborne,1975; Mabry,1973; Levin,1971; Grant,1963). Flavonoids have been implicated as defensive agents in plants, as light screens and in control of plant growth, development and photosynthesis (McLure,1979; Harborne,1975; Levin,1971; etc.), so generally speaking they are important to the physiological functioning and hence to the survival of an individual plant.

Flavonoid class, hydroxylation, methylation, glycosylation and acylation are usually under genetic rather than environmental control, and are often determined by single genes (Mabry,1973; Levin,1971), so that variation in flavonoid profiles can be a reliable indication of genetic differences between closely related taxa. Mabry(1973) maintains that a particular flavonoid structural theme almost always persists in all populations of a taxon or populations of closely related taxa. Levin(1971) qualifies this statement with the point that the more important a chemical constituent is to a plant species, the more reliable that constituent is as an indicator of phylogenetic affinities, in both interpopulational and interspecific comparisons. McLure(1979) suggests that flavonoids produced last in the biosynthetic sequence, for example glycosides, are most likely to be susceptible to quantitative or qualitative changes by physiological "manipulations".

Finally, with these points in mind, variability in the flavonoids of

sampled trees was assessed. Illumination of the patterns of species and population differentiation in the *Abies* section *Nobilis* was attempted assuming that one or more of the five hypotheses outlined above best explains the variation present in the section.

LITERATURE REVIEW

CURRENT PHYSIOGRAPHY OF THE WESTERN UNITED STATES OF AMERICA

The Pacific mountain system today dominates the west coast of North America from south-eastern Alaska south through Mexico (Figure 1). Two lines of mountains run through Washington and Oregon; the Coast Ranges are south of the Olympic Mountains (Mts.) in northwestern Washington, and the Cascade Range runs roughly parallel to the Coast Range, inland about 240 km. To the east of the Cascade Mts. are the Columbia Plateau of Washington and Montana in the north, and to the south the Great Basin desert that covers large parts of eastern Oregon, western Idaho, western Utah and almost all of Nevada. The Columbia Plateau and the Great Basin are bordered farther east by the Rocky Mountain Complex. The Coastal and Cascade Mts. merge in southwestern Oregon at the Klamath, Siskyou and Yolla Bolly Mts. of southwestern Oregon/northwestern California. Just south of Mt. Shasta in the Cascade Range of north-central California, the mountains split again across the Great Valley of California, into the massive Sierra Nevada range inland and the Coast Ranges of central and southern California at the Pacific Ocean. In southern California, two lesser mountain ranges, the Peninsular and the Transverse Mts., complete this enclosure of the Great Valley.

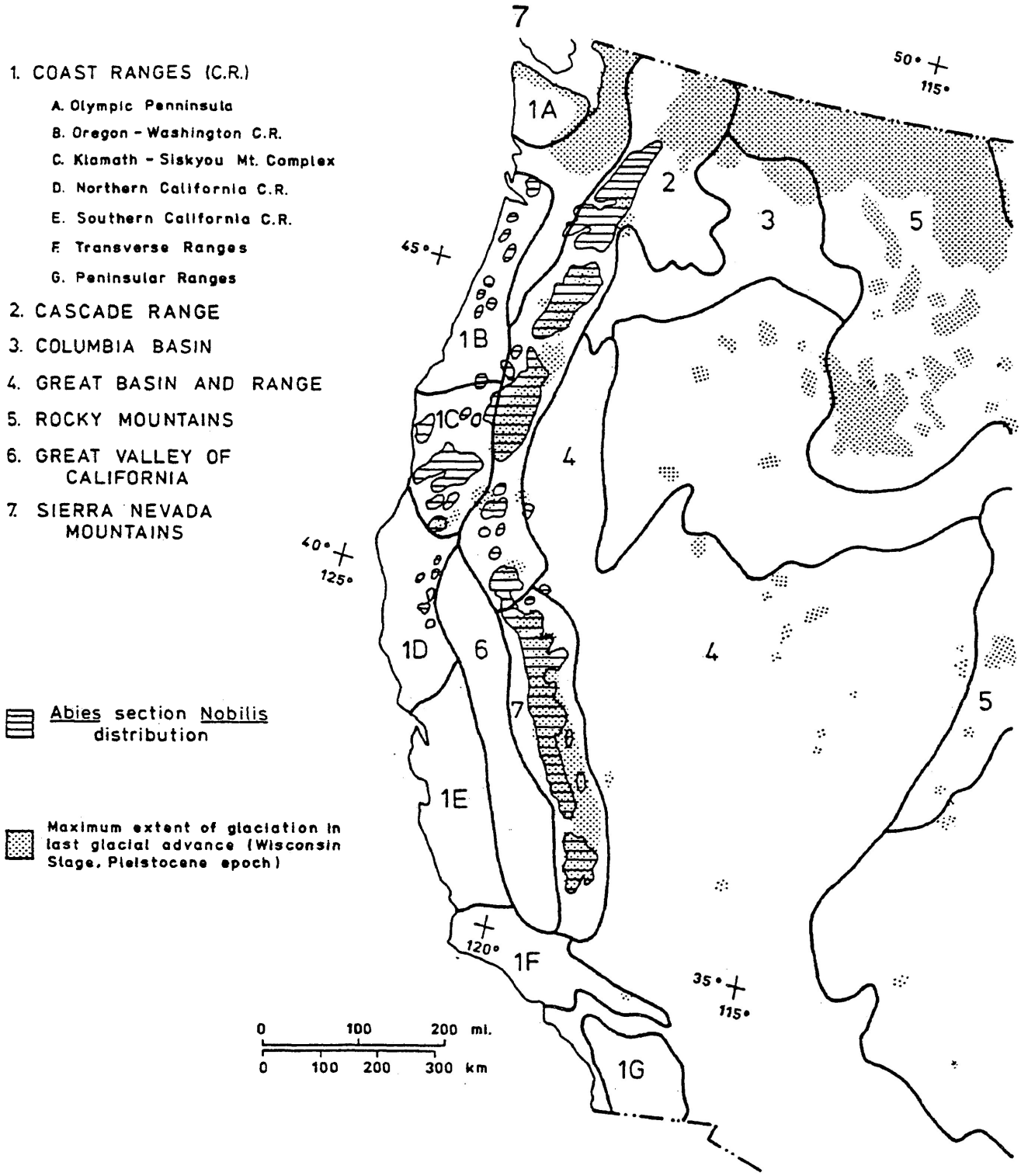


FIGURE 1. Map showing the physiographic regions, the mountain ranges and the extent of Wisconsin glaciation in the western United States with the current distribution of the *Abies* section *Nobilis*. (Heckard,L.R. and Hickman,S.C. 1984. The phytogeo-graphical significance of Snow Mt.,North Coast Ranges,Calif. Madrono 31:30-47; Little, E.L. 1971. Atlas of United States trees,V.1.Conifers and important hardwoods. Misc.Pub. 1146,U.S.Dept.Agr. Forest Service,Wash.,D.C.; Heusser,C.J. 1960. Late Pleistocene environments of North Pacific North America. Am.Geog.Soc.Spec.Pub.35.)

THE FOSSIL RECORD OF *ABIES* SECTION *NOBILIS*

The fossil record of Coniferales begins in the late Paleozoic age, before the start of the Mesozoic age (Arnold,1947; Table 1). Conifers are considered to have reached a developmental "climax" by the early Cretaceous period, by which time the "typical" members of Pinaceae including *Abies* had segregated from transitional forms no longer extant (Stockey,1981; Stebbins,1950,pp.518-519; Arnold,1947). The fossil record in northwestern North America from ten to two or three million years before present (ybp) is very poor (Wolfe,1969). There are no fossil floras at all for northwestern California, including the refugial Klamath Mts. region (Heckard and Hickman,1984). However, recovered fossils of woody plant species indicate that very little morphological change has taken place in most surviving woody plant species since the middle of the Cenozoic age, ie. since about the Oligocene epoch, twenty-six to seven million ybp (Axelrod,1976).

The most useful fossils in the study of the origins of the *Abies* section *Nobilis* are macrofossils consisting of cone scales and their attached cone scale bracts. *Abies* pollen can be distinguished by its size and morphology from other conifer species, but of western true firs, only *A.lasiocarpa* can be reliably identified to species (Sercelj and Adam,1975). Reports of fossil pollen of *A.procera* in Washington (Hansen,1943), should therefore be treated with caution. No other fossil occurrence of *A.procera* has been published, although Becker(1961) attributes some of the Oligocene Ruby Floras from southwest Montana simply to the section *Nobilis*. Modern cone scales of *A.procera* with attached and flattened bracts can be very similar to *A.magnifica* var. *shastensis* (Liu,1971); with bract detached, fossil scales of *A.procera*

TABLE 1. Geologic time scale. (Arms,K. and Camp,P.S. 1982.Biology, 2nd ed. Saunders College Publishing,Toronto.)

APPROX.YEARS BEFORE PRESENT (millions)	ERA	PERIOD	EPOCH
	Cenozoic	Quaternary	Recent
			Pleistocene
2.5		Tertiary	Pliocene
7			Miocene
26		Oligocene	
38		Eocene	
53		Paleocene	
65	Mesozoic	Cretaceous	
135		Jurassic	
195	Triassic		
225	Paleozoic	Permian	

would be difficult to distinguish from *A.magnifica* var. *magnifica.*

Almost all of the reliable *Abies* section *Nobilis* fossils are cone scales that have been identified as either *A.laticarpus* MacGintie, the fossil equivalent of *A.magnifica* var. *magnifica* which has included bracts, or as *A.klamathensis* Axelrod, the fossil equivalent of *A.magnifica* var. *shastensis*, which has exserted bracts. Most of these *Nobilis* fossils occur in Oligocene, Miocene or Pliocene floras in the following localities :

1. Oligocene - southwestern Montana (Axelrod,1976; 1964; Becker,1969; 1961);
2. Miocene - southern Idaho (Axelrod,1976; 1964),

north-central and southeastern Oregon (Axelrod,1976; 1964; Chaney,1959; Chaney and Axelrod,1959), southwestern Nevada, northeastern Colorado and northeastern California (Axelrod,1964; Chaney,1959; Chaney and Axelrod,1959);

3. Pliocene - southern Oregon (Chaney,1944).

The floras with which these *Abies* section *Nobilis* fossils are associated are fairly similar to modern montane and subalpine forests of western North America (Axelrod,1976;1964; Becker,1961; Chaney,1959; Chaney and Axelrod,1959). The oldest reported fossil occurrence for the section has been identified as *A.klamathensis*, and was found in an Eocene flora in northeastern Nevada (Axelrod,1976; 1964).

The following brief paleohistory of the Pacific Northwest and California outlines the changes in forest composition of that area from the beginning of the Cenozoic age through to the Recent epoch. It is based on a review of the literature, and emphasizes the montane and subalpine forests with which the *Abies* section *Nobilis* has probably evolved.

PALEOHISTORY OF THE FORESTS OF WASHINGTON, OREGON AND CALIFORNIA

The climate became progressively humid over the course of the Paleocene and Eocene epochs so that subtropical vegetation growing close to the equator migrated as far north as 67 degrees north (N) latitude, displacing temperate forests that included the *Abies* section *Nobilis* to the north and inland (Axelrod,1976; 1968; 1958; Whittaker,1961;

Chaney,1947). The climate cooled and became drier through the Oligocene and Miocene epochs (Axelrod,1976; Whittaker,1961), and the northern, temperate forest migrated south. By the end of the Miocene epoch, it reached as far south in North America as central California. Warm temperate forests with modified subtropical elements became restricted to coastal Oregon and northern California; subalpine and montane forests that contained mostly deciduous broadleaf species grew on upland sites in this area (Whittaker,1961; Axelrod,1976). The interior, including the Columbia Plateau, the Great Basin and Nevada, was higher in elevation and had a cooler climate than the coast during the Oligocene, Miocene and Pliocene epochs, as evidenced by a fossil record composed of species adapted to cooler climates, including taxa similar to *A.magnifica* (Axelrod,1976; 1968; 1958; Wolfe,1969). Subalpine conifer forests of *Abies*, *Chamaecyparis* Spach, *Larix* Mill., *Picea* A.Dietr., *Pinus* L., *Pseudolarix* Gord., *Pseudotsuga, Sequoiadendron* Buchholz, *Thuja* L. and *Tsuga* (Endl.)Carr. came to cover the current range of *A.procera* and *A.magnifica* except the modern Sierra Nevada, as well as what is now the Columbia Plateau (Axelrod,1968; 1958). A trend to increased dryness has persisted through major temperature fluctuations, including episodic glaciation, over the rest of the Cenozoic age (Whittaker,1961; Axelrod,1958). This trend to increasingly reduced, more variable and more seasonal precipitation has probably caused most of the forest migration in western North America since the Eocene, south of continental glaciation (Heckard and Hickman,1984; Stebbins,1982; Raven and Axelrod,1978; Axelrod,1976).

The Rocky Mountain uplands of Nevada and Idaho have been high enough to support subalpine and alpine taxa since about the Oligocene period (Stebbins,1982). These interior forests appear to be ancestral to

the montane and subalpine forests growing today both in the central Rocky Mts. and in the Sierra Nevada (Axelrod,1976), although only the latter contains extant members of the *Abies* section *Nobilis.*

The Cascade Mts. were a belt of low, scattered volcanic peaks until the Miocene (Whittaker,1961); they were not high enough to support montane or subalpine vegetation until the late Miocene or early Pliocene (Axelrod,1976; Daubenmire,1969; Wolfe,1969; Chaney,1959). At that time, the whole Cascade belt was uplifted, worsening dessication of the interior of Washington and Oregon (Whittaker,1961). Partial uplift of the Sierra Nevada during the middle Pliocene probably allowed subalpine forests to invade the north-central Sierra Nevada from the interior (Axelrod,1976). High montane habitat was created sequentially near the end of the Pliocene, first in the Klamath ranges, then in the Oregon Coast ranges and finally in the Sierra Nevada (Heckard and Hickman,1984; Axelrod,1976; Daubenmire,1969; Wolfe,1969). The northern California Coast ranges were uplifted about the same time, but were subjected to additional deformation during the Pleistocene (Axelrod,1966), and are not considered to have been able to support *A.magnifica* or other upper montane species and communities during the pre-Pleistocene coastal migrations (Heckard and Hickman,1984).

Mixed conifer forest fossils appear first in the Sierras at the southern end of the range, northeast of Bakersfield, Calif., during the Miocene period, but no representatives of the *Abies* section *Nobilis* have been reported in published floras (Axelrod,1976). Subalpine conifers appear in the central and northern Sierras during the Pliocene, and again, no *A.magnifica* -like fossils have been documented from these forests.

As the summer dry period lengthened and as growing season

temperatures became more extreme, subalpine conifers were apparently eliminated from the temperate mixed conifer forests of the Sierra Nevada and bordering regions (Axelrod, 1976). By the late Pliocene, temperate forests were restricted to higher elevations in California (Axelrod,1958), and were completely eliminated from the central lowlands east of the Sierras (Axelrod,1976).

Over the course of the Miocene and Pliocene epochs, as the interior temperate conifer-broadleaf forest was reduced, many taxa migrated coastward to the Klamath region, displacing much of the mixed deciduous broadleaf forest at higher coastal elevations (Axelrod,1976; Chaney,1947). Some extant boreal species like *Picea englemannii* Parry and *Abies lasiocarpa* (Hook.)Nutt. appear to have migrated into the Pacific Northwest from farther north after the middle Miocene, possibly in response to glacial advance during the Pleistocene epoch (Axelrod,1976; Wolfe,1969).

This diverse, multi-origin coastal forest is represented today by the forests of the Klamath-Siskyou mountain complex. The Klamath-Siskyou region is cited as both a refuge for relict species (Heckard and Hickman,1984; Axelrod,1976; Stebbins and Major,1965; Whittaker,1961; etc.) and as a centre for outward migration to both the north and the south (Heckard and Hickman,1984; Wells,1983; Axelrod,1976; Whittaker,1961; Chaney,1959). Whittaker(1961) reports that today all of the western North American forest types occur together in the Klamath region, with species that are restricted to the subalpine in the Sierra Nevada occurring at lower elevations in this area (Axelrod,1976). The Klamath-Siskyou region is climatically and floristically similar to the very diverse late Miocene conifer forest of the Great Basin, the Columbia Plateau and the Cascade Range (Wolfe,1969; Whittaker,1961; Chaney,1944), and has been protected

from the extreme climatic fluctuations that accompanied the glacial advances and retreats during the Pleistocene (Axelrod,1976). According to Wolfe(1969), about 50 percent of the late Miocene conifer species have survived here, including *A.magnifica* var.*magnifica* and var.*shastensis*.

Continental glaciation (Figure 1) in the Pacific Northwest reached as far south as Washington and northern Idaho, and receded from there completely by about 10 500 years ybp (Daubenmire,1969; Heusser,1960). Wells(1983) maintains that the southeastern corner of Oregon was a subarctic landscape during the last glacial period. South of the Columbia River, Washington, there were areas of discontinuous glaciation along the Cascade Mts., in the Klamath Mts. and in the Sierra Nevada (Little,1971; Heusser,1960). The northern half of the Sierra Nevada was heavily glaciated (Axelrod,1981), while glaciation in the southern half of the range was minimal (Stebbins,1982). The extent of glaciation throughout most of the current range of the *Abies* section *Nobilis* suggests that much of this modern distribution has been achieved since the last glacial retreat (Wells,1983; Heusser,1960). Stebbins(1982) and Axelrod(1976) assert that some migration south along the Cascade-Sierran axis must have taken place during glacial advances, probably into the Klamath Mountain complex and into refugia in the high ridges and plateaus of the southern Sierra Nevada. Montane and subalpine forest which apparently did not include members of the *Abies* section *Nobilis* migrated south from the Sierra Nevada into the Transverse and Peninsular ranges during the early Pleistocene, and elements of these forests have grown in southern California at varying altitudes ever since (Axelrod,1966).

The climate in the Pacific states has fluctuated since the last glacial advance between a cool, humid regime and one that is drier and warmer

(Huesser,1960), so that existing plant distributions including that of the *Abies* section *Nobilis* is probably not static (Wells,1983; Huesser,1960). The last glaciers were followed by an xerothermic period between 8 500 and 3 000 ybp. During this xerothermic, mesic forests were restricted to the interior and the south, and coastward and northern occurrences of associated species like *A.procera* were favoured (Axelrod,1981; 1976; Schofield,1969).

Just prior to the present, the climate cooled somewhat, which has triggered the opposite migration trends to some extent (Axelrod,1966). Many current Sierra Nevada plant species are probably either relatively recent immigrants or new ecotypes (Heckard and Hickman,1984; Axelrod,1976; 1966). Wells(1983) identifies *A.magnifica* in the Sierra Nevada as a relatively recent northern immigrant from the Klamath region. Migration from the east is ruled out because of the much less equable climate in the Great Basin and the Rocky Mts. that has eliminated all but the hardiest montane conifers from that area.

Over the course of the Cenozoic era, variable folding and uplifting and volcanic activity contributed to a fluctuating environment. The Eocene saw major volcanic activity in western Washington, eastern Oregon, north-central Idaho and northwestern Nevada and adjacent California (Axelrod,1968). Vulcanism during the late Cenozoic created specific areas that were raised above the general level of the Casade crest from British Columbia to California. Daubenmire(1969) feels that the ejecta from Glacier Peak (14 000 ybp) and Mt. Mazama (Crater Lake, 8 600 ybp) in particular caused major ecological disruptions, especially in the interior of Oregon and Washington that are now occupied by xeric vegetation (Whittaker,1961).

The diversity of species growing together in the montane and

subalpine forests of the Oligocene, the Miocene and the Pliocene epochs was much greater than that in any equivalent forests growing today. In addition, montane and subalpine conifer species were not as restricted to specific altitudes as they are today. Axelrod(1976) suggests that this diversity occurred because the taxa growing earlier in the Cenozoic era had broader ecological amplitudes than those modified taxa that survived through to the present day. Alternatively, climatic and topographic diversity has increased considerably since the Eocene epoch, and surviving species have probably adapted accordingly (Stebbins,1982; Axelrod,1981). The uniform mesophytic forest pattern predominant before the Pliocene has changed to strongly-zoned plant community patterns consistent with the steep climatic gradients in the mountains and valleys of the Pacific coast states (eg. Whittaker,1961; Axelrod,1958). It is in this drastically changing environment that the *Abies* section *Nobilis* has survived and evolved.

DISTRIBUTIONAL AND TAXONOMIC BACKGROUND OF THE *ABIES* SECTION *NOBILIS*

The genus *Abies* section *Nobilis* consists of two generally recognized species, *A.procera* and *A.magnifica*, with the latter species including both the typical variety and *A.magnifica* var. *shastensis* (Shasta red fir). Colville(1897) and Lamb(1912) considered the Shasta variety to be a species, *A.shastensis* Lemm., and Liu(1971) recognized it as the hybrid *Abies* x *shastensis* Lemm., emend.Liu. Typical representatives of the two species are easily distinguished by cone and needle morphology (Table 2), except where their natural distributions overlap. *A.procera* ranges from about 48 degrees 30 minutes N latitude through the Cascade Mts. of Washington and Oregon down to about 41 degrees N latitude in the Klamath-Siskyou Mts. of northwestern California (Hitchcock and Cronquist,1973; Liu,1971). *A.magnifica* ranges from 43 degrees 35 minutes N latitude in the Western Cascades of southwestern Oregon west into the Klamath-Siskyou Mts. and east, then south down through the Sierra Nevada Mts. of California to about 35 degrees 40 minutes N latitude (Liu,1971; Griffin and Critchfield,1972; Figure 2).

The intermediate taxon *A.magnifica* var.*shastensis* (Table 2) occurs both where the ranges of the two typical species overlap in the Western Cascades of Oregon and the Klamath-Siskyou Mts. of southwestern Oregon and northwestern California, and at the southern limit of the range of *A.magnifica* (Griffin and Critchfield,1972; Schopmeyer,1974). The taxonomic status of the intermediate trees in the area of sympatry and in the North Coast mountains of California is not clear. This uncertainty has led also to uncertainty about the taxonomic and phylogenetic affinity of the southernmost populations of the variety

TABLE 2. Morphological and silvical features of *Abies procera* Rehd., *A.magnifica* var.*magnifica* A.Murr. and *A.magnifica* var.*shastensis* Lemm.

CHARACTER	*A.PROCERA*	*A.MAGNIFICA* V. *MAGNIFICA*	*A.MAGNIFICA* V. *SHASTENSIS*
NEEDLES	flattened (5), groove on upper surface (2,5)	rhomboidal (5)	rhomboidal, somewhat flattened (5)
CONES	exserted & tightly reflexed bracts with long awns(2,5)	bracts shorter than cone scales, shorter awn (5)	bracts partly exserted, may or may not be reflexed (2,5)
	bract enlarged at apex (1)	bract acute at apex (1)	bract enlarged at apex (1) or acute (5)
BARK	thinner ridges, flakier bark (2,5)	fewer,thick ridges (2,5)	thick ridges (5)
	inner bark red-brown (5)	inner bark bright cinnamon-red (5)	inner bark deep brown (5)
SEEDS	29 800/kg (6)	14 100/kg (6)	16 100/kg (6)
COTY-LEDONS	5 to 6, occ.4 to 7 (5); 5.4 (3)	7 to 8 (5) 8.8 (3)	7.4 (4)
DATE OF POLLEN RELEASE	June to early July (6)	late May to early June (6)	mid- to late June (6)
HEIGHT AT MATURITY	43 to 70m (6)	30 to 49m (6)	30 to 49m (6)
SHADE TOLERANCE	low to intermediate (8)	intermediate (8)	variable (8)
SUCCESS. ROLE	pioneer (8)	climax (8)	variable (8)

TABLE 2 . (Continued).

CHARACTER	*A.PROCERA*	*A.MAGNIFICA* V. *MAGNIFICA*	*A.MAGNIFICA* V.*SHASTENSIS*
DISTRI-BUTION	Central Cascade & Coast Ranges Wash. & Ore.(7); NW.Calif. (6)	Sierra Nev. Mts. & N.Coast Range, Calif.;S.Cascade Ranges, Ore. & N. Calif.; adj. Nev.(6)	S.Cascade Mts., Ore.; Klamath Mts. & Coast Ranges of N.Calif.; S. Sierra Nevada, Calif. (6);
		increases E. to W. in the S. Sierra Nevada (7)	Bract exsertion in N.Calif., & to the S.
ELEVATION	900 to 2200m; as low as 60m in Coast Ranges of Wash. & Ore. (5,7)	1800 to 2750m; as low as 1350m (5,7)	1500 to 3050m (5)

(1) Lamb,W.H.1912. A synopsis of the red firs. Soc. Am. Foresters, Proc.7,184-186.

(2) Parker,E.L.1963. The geographic overlap of noble fir and red fir. Forest Sci. 9:207-216.

(3) Silen,R.R.,Critchfield,W.B. and Franklin,J.F.1965. Early verificationof a hybrid between noble and California red firs. Forest Sci.11:460-462.

(4) Franklin,J.F. and Greathouse,T.E.1968. Seed origin studies noble - California red fir species complex. Western Forestry and Cons. Assoc., W.Forest Nursery Council Proc. 1968:11-16.

(5) Liu,T.S.1971.A monograph of the genus *Abies*. Dept.Forestry, College of Agric., Nat. Taiwan Univ., Tapei, Taiwan, China. 608pp.

(6) Schopmeyer,C.S.,ed.1974. Seeds of woody plants in the United States. Forest Service, U.S.Dept.Agric. Hdbk.450. 883pp.

(7) Zavarin,E.,Critchfield,W.B., and Snajberk,K.1978. Geographic differentiation of monoterpenes from *Abies procera* and *A.magnifica*. Biochem. Syst.and Ecol. 6:267-278.

(8) See text for explanation and references.

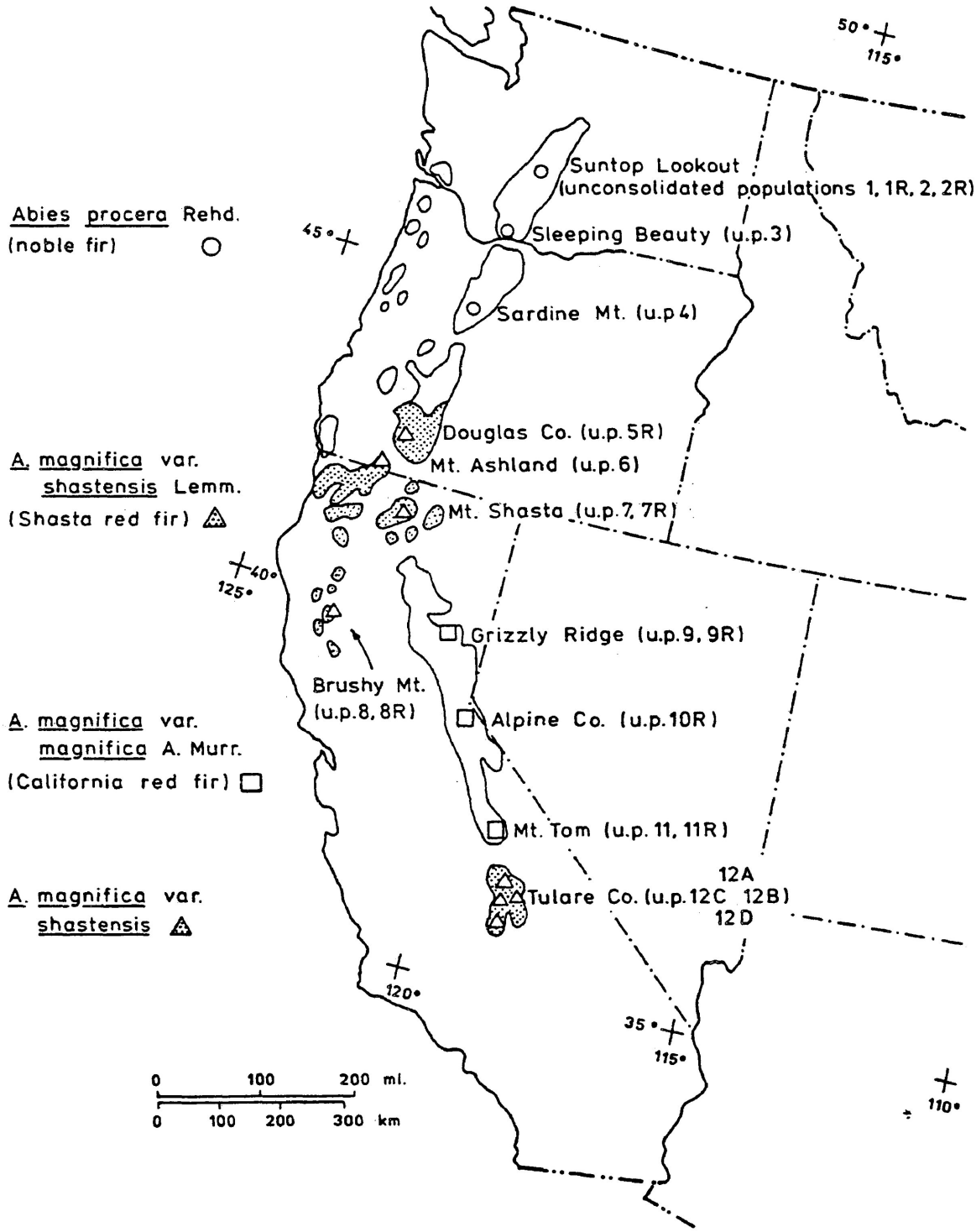


FIGURE 2. Map of Washington, Oregon and California showing species' distributions and sample population locations. (Little,E.L. 1971. Atlas of United States trees, V.1. Conifers and important hardwoods. Misc. Pub. No.1146, U.S.Dept.Agr. Forest Service,Wash.,D.C.)

shastensis.

Parker(1963), Franklin(1964; 1982) and Ustin(1976) give succinct reviews of the differences of opinion concerning the identity, occurrence and origin of the three taxa that are now recognized taxonomically. In the area of range overlap, Parker(1963) and Franklin(1964) both found trees exhibiting various combinations and degrees of bract exsertion and needle grooving growing with typical *A.procera.* However, Parker(1963) concluded that these trees formed almost pure stands of somewhat variable *A.procera*, with an almost pure forest of *A.magnifica* var.*shastensis* occurring only on Mt. Shasta in California, not ranging into Oregon or Washington at all. Franklin(1982) considers *A.magnifica* var.*shastensis* to be highly variable, where *A.procera* is substantially variable only in silvical characteristics like seedling growth; he feels that *A.procera* is quite uniform in cone and leaf morphology. Franklin(1982) therefore concludes that the *Abies* section *Nobilis* fir populations in southern Oregon and northern California are

> "distinct from noble fir and perhaps clinally related with a strong latitudinal gradient to [*A.magnifica* var.*magnifica*] found farther south in the Sierra Nevada."(p.67)

Despite this distinctness, he does not classify these populations as *A.magnifica* var.*shastensis*, because he feels that the "classical characteristics of cone and needle morphology suggest a close relationship to noble fir". Franklin(1982) rules out hybridization between *A.magnifica* and *A.procera* as a source of this variability on the basis of terpene data collected by Zavarin*et al.* (1978), which did not support the existence of hybrid swarms in the region of sympatry. This terpene data

showed the transitional populations to be more like *A.magnifica* in their terpene composition, but more variable than that species; the transitional populations showed variability on the same level as *A.procera.*

Ustin(1976) looked principally at variation in *A.magnifica* and southern occurrences of the exserted bract variety. She discovered a sharp discontinuity in the occurrence of exserted bracts at the Kings River watershed in Tulare County, California (36 degreees 31 minutes N latitude). Trees to the south of the watershed had exserted bracts like the variety *shastensis*, while those to the north had hidden bracts like the typical variety of *A.magnifica.*

All three taxa - *A.procera*, *A.magnifica* var.*magnifica* and *A.magnifica* var.*shastensis* - occupy similar ecological niches (Fowells,1965; Franklin and Dyrness,1973). They are high-elevation species growing in upper montane conifer or subalpine forests (Table 2). However, *A.magnifica* tends to be a climax species of intermediate shade tolerance, whereas *A.procera* is considered to be a pioneering species of low to intermediate shade tolerance (Fowells,1965; Hodges,1962). *A.procera* is often a pioneering species following fire in the northern part of its range (Franklin and Dyrness,1973), but has been reported as a codominant climax species with *Tsuga heterophylla* (Raf.)Sarg. and *Pseudotsuga menziesii* (Mirb.)Franco in the Oregon Coast Ranges (Merkle,1951). Franklin(1964,p.8) suggests that there may be ecotypic variation with regard to shade tolerance in *A.procera.*

A.magnifica var.*shastensis* exhibits variable successional behaviour from transitional through climax in the area of range sympatry (Franklin and Dyrness,1973,p.158), and from pioneer after fire through climax in the Sierra Nevada (Barbour and Majors,1977,p.574). This variable successional behavior could also be interpreted as ecotypic

variation in shade tolerance and seedbed requirements, especially given the habitat diversity of mountainous regions (Axelrod,1981,p.865).

According to Fowells(1965), *A.procera* grows on a wide range of soils, as long as the moisture supply is adequate. This restriction probably accounts for its scarcity in the geologically young "high Cascades", as mentioned by Franklin(1964). Franklin(1964) suggests that the prevalence of coarse pumice soils which are very dry is limiting the growth of *A.procera* in this area. Franklin(1964), Fowells(1965) and Gessel and Oliver(1982) all postulate that noble fir is extending its range farther north in Washington, because northernmost populations of this species are very vigorous.

A.magnifica grows best on glacial morraines or on unglaciated but deep soils in the Sierra Nevada (Fowells,1965). Farther north in southern Oregon, it is found also on clay loams and pumice soils, soils which have very different moisture-holding capacities. The southernmost occurrence of this species is probably limited by the hot, dry summer season which prevents seedling establishment (Axelrod,1976,p.29). The northernmost extension of its range may be limited by competition from ecologically and successionally similar high-elevation species like *A.amabilis* (Dougl.)Forbes and *A.concolor* (Gord. and Glend.)Lindl. Axelrod(1976) suggests that the montane conifers like *A.magnifica* that grow today in the south-central Sierra Nevada may be recently adapted to the relatively severe "Mediterranean" climate which has short, hot, dry summers and long, cool, wet winters.

The pattern of variation in the whole *Abies* section *Nobilis* complex, the range disjunction of *A.magnifica* var.*shastensis*, and the intermediate morphological and silvical characteristics of this variety have

led to a number of recent investigations into the origin and systematics of the section.

Silen, Critchfield and Franklin(1965) attempted successful artificial crosses of *A.procera* and *A.magnifica.* They found no significant genetic barriers to interbreeding between the two species. According to Fowells(1965) and Schopmeyer(1974), the time of pollen shedding for both species can be about the same (late May to early June; Table 2), so hybridization in natural stands is possible if the species' ranges do indeed overlap.

Franklin and Greathouse(1968) found a clinal gradation in seed weight and cotyledon number from north to south through the *A.procera / A.magnifica* var.*shastensis / A.magnifica* complex. Using both morphological (bracts, cone scales and cotyledon number) and chemical (terpene) characters, Ustin(1976) supplemented this work by examining the variation in *A.magnifica* in the southern Sierra Nevada, well south of the putative range of sympatry with *A.procera.* The "strong disjunction" she discovered between the occurrence of cones with exserted, to the south, versus cones with hidden bracts, to the north, of the Kings River watershed was interpreted as geographical rather than as ecotypic variation. This interpretation implies that genetic drift due to isolation of small populations is more important than adaptation to different habitats. Clinal variation tending toward exserted bracts was exhibited on both sides of the disjunction, but with a much steeper gradient to the south compared to the north through the range of typical *A.magnifica.* The pattern of variation in both bract length and seedling cotyledon number was more similar to *A.magnifica* var.*shastensis* from the area of range sympatry to the north between the two typical species than to populations of *A.magnifica* var.*magnifica* from the southern Sierra Nevada. In

addition, both characters appeared to vary together; the shorter the cone scale bract, the larger the number of seedling cotyledons (Ustin,1976).

However, all of the populations that Ustin(1976) examined exhibited similar monoterpene composition with little within- or between-population variation, so that the southern populations of *A.magnifica* var.*shastensis* were more similar to the typical variety of *A.magnifica* than they were to the northern populations of *A.magnifica* var.*shastensis* (cf. Zavarin and Snajberk, 1965). She comments that although the northern and southern populations of *A.magnifica* var.*shastensis* are different chemically, they are morphologically more similar to each other than they are to *A.magnifica* var.*magnifica*, so that the southern populations could be isolated, "relict stands of what may once have been a continuous range" (Ustin,1976,p.53) of *A.shastensis* Lemm. whose "distinguishing" characteristics have been "highlighted" by genetic drift.

Zavarin, Critchfield and Snajberk(1978) examined cortical oleoresin from thirty-five localities representing all four operational taxa, and essentially confirmed Ustin's findings. They found that

> "the populations segregated latitudinally into three related clusters - above 44 degrees [N latitude] (*A.procera*), between 44 degrees and 40 degrees (transitional) and below 40 degrees (*A.magnifica*). *A.magnifica* from [the] southern Sierra Nevada and *A.magnifica* from Mtn. (sic)Shasta [in the area of sympatry] differed in a number of parameters, with southern Sierra Nevada populations being chemically much closer to typical *A.magnifica* from [the] central and northern Sierra Nevada."(p.267).

Dr.W.H. Parker and Dr.J Maze (unpub. data), sampled 22 populations of mature and/or immature trees along a north-south transect

that covered the distribution of the three taxa in question, excluding the southernmost populations of *A.magnifica* var.*shastensis*. The results of their analysis of morphological characters were somewhat inconclusive. Principal components analysis (PCA) of their data showed two patterns of variation, depending upon the type of morphological evidence used. Populations of *A.magnifica* var.*shastensis* clustered between populations of the two typical species when cone characters were analyzed. In PCA of immature and mature needle characters from the same trees, *A.magnifica* var.*shastensis* clustered with typical *A.magnifica*. In contrast, Franklin(1982) and Parker(1963) concluded that populations of northern *A.magnifica* var. *shastensis* were more similar to *A.procera* than to *A.magnifica* var.*magnifica*. Canonical variates analysis (CVA) by Parker and Maze (unpub. data) using the same cone data had populations of *A.magnifica* var.*shastensis* and a population of *A.magnifica* from north-coastal California clustered closer to *A.procera* than to *A.magnifica* ; CVA analysis of the same needle data from mature trees had *A.magnifica* var.*shastensis* clustered with the typical variety (like the PCA of needle data); CVA of the same immature needle data had one population of *A.magnifica* var.*shastensis* clustered with *A.procera*, and two with *A.magnifica*. One population in particular, collected from Mt. Ashland, California, exhibited a range of morphological attributes intermediate between *A.procera* and *A.magnifica* var.*magnifica* (W.H.Parker, pers.comm.).

Cladistic analysis by Dr. Maze (pers.comm.) of this same data has led Dr.Maze to conclude that *A.magnifica* var.*shastensis* has evolved more than once, and therefore is paraphyletic. He is also of the opinion that sample populations of *A.magnifica* var.*shastensis* are not more variable than populations of either *A.procera* or *A.magnifica*, and that

both typical *A.magnifica* and the variety *shastensis* exhibit about the same amount of variability. He further concludes that clinal variation rather than hybridization accounts for most of the variability in the *Nobilis* complex.

FLAVONOID SYSTEMATICS IN *ABIES*

Flavonoids have been used extensively as taxonomic characters in angiosperms, and are particularly useful as a guide to taxonomic relationships when hybridization and /or introgression is suspected (Harborne*et al.*,1975). Flavonoids are ubiquitous secondary plant products that can be found in the wood, leaves and reproductive structures of all photosynthesizing plants (Geissman and Crout,1969). They are considered by some to be a fundamental adaptation by plants to terrestrial habitats, limiting damage caused by ultra-violet light (Lowry*et al.*,1980; Harborne*et al.*,1975).

Research into the flavonoids, particularly leaf flavonoids, of most conifer genera is incomplete or lacking (Niemann,1979). Extensive work has been published on the leaf flavonoids of various *Larix* Mill. species (eg. Niemann,1980; 1976; 1975; 1969; Niemann and Baas,1978a; 1978b; Niemann and Koerselman-Kooy,1977; Niemann and Miller,1975; Medvedeva and co-workers,1972-1974, as cited in Niemann,1979). Almost all of this literature deals with identification of the flavonoids and other phenolics present in individual species. Only quantitative variation in the occurrence of specific flavonoids is apparent in *Larix*, both within (Niemann and Baas,1978a) and between species (Niemann,1976). The

kind of flavonoids identified in *Larix* species to date are fairly similar to those so far identified in *Abies* species. Both genera contain a number of phenolic glycosides including flavonol, flavone and C-glycosyl flavonoid derivatives. Two rare flavonols, syringetin and laricytrin, commonly occur in both *Abies* and *Larix* (Niemann,1979; Parker*et al.*,1984; 1979).

Seasonal variation in flavonoid composition was found in *L.leptolepis* (Sieb. and Zucc.)Gord. (Niemann,1976) in three of six compounds monitored over the growing season. Working with *A.balsamea* (L.)Mill., Cleveland(1979) found occassional quantitative variation in the occurrence of one flavonoid, rhamnosylvitexin, in samples collected from dormant trees compared to those collected during the growing season. Samples taken from the lower crown of dormant trees sometimes exhibited greater concentrations of rhamnosylvitexin compared to samples taken from the upper crowns of the same dormant tree. Only irregular variation in occurrence was found in physiologically active trees by crown position. Cleveland(1979) also found some variation according to age of leaves sampled, with the current season's foliage containing aglycones not found in older leaves.

Identification of the leaf flavonoids of two European species of *Abies* have been reported by Medvedeva*et al.* (1974b, as cited in Niemann,1979). Aglycones, 3-O and 7-O-glucosides of kaempferol and quercetin, isorhamnetin-3-O-glucose and apigenin-8-C-glucorhamnose (-8-C-rutinose) were isolated from *A.nephrolepis* Maxim. and *A.sibirica* Ledeb.

Of North American *Abies* species, only *A.amabilis* (Dougl.)Forbes (Parker*et al.*,1979), *A.balsamea* and *A.lasiocarpa* (Parker and Maze,1984; Parker*et al.*,1984; Cleveland,1979) have been subjected to comprehensive, published flavonoid analyses. These

analyses in general have been based on both massed foliage samples from different trees of the same species and on surveys of individual trees from geographically separated populations. *A.amabilis* has a flavonoid profile distinct from both *A.balsamea* and *A.lasiocarpa*, although all three contain various combinations of 3-O-glucoside, 3-O-galactoside, 3-O-rhamnoside and 3-O-rutinoside derivatives of the six flavonol aglycones myricetin, laricytrin, quercetin, kaempferol, isorhamnetin and syringetin. *A.amabilis* also contains dihydroquerciten (taxifolin), rhamnosylvitexin and two other partially identified rhamnosyl-C-glycosyl derivatives of apigenin not consistently present in either *A.balsamea* or *A.lasiocarpa*.

A.balsamea and *A.lasiocarpa* have very similar flavonoid profiles. However, *A.balsamea* from the central part of its range contains diglycosides, flavones, dihydroflavonols and at least one flavanone (naringenin) that are found rarely in samples of *A.lasiocarpa* west of the Rocky Mountain Crest in western Alberta. *A.lasiocarpa* contains acetylated glycosides of the six flavonols listed above that are present occasionally in *A.balsamea* in trace amounts. On the basis of both morphological and flavonoid population surveys, *A.balsamea* and *A.lasiocarpa* appear to intergrade where their ranges overlap in western Canada (Parker and Maze,1984; Parker*et al.*,1984; 1981; 1979).

Parker and Maze(1984) emphasize the importance of using as many kinds of taxonomic characters as possible when interpreting the systematics of *Abies*. Therefore, any population variation in flavonoid profiles would best be interpreted in conjunction with the results of other chemical and morphological systematic studies of the same taxa.

MATERIALS AND METHODS

SITES AND COLLECTIONS

Foliage samples from 21 populations of the *Abies* section *Nobilis* complex were collected along a north-south transect through Washington, Oregon and California. Site codes, descriptions, locations, collectors and date of collection are specified in Table 3. Samples of *A.procera* from the Oregon and Washington Coast Ranges could not be included due to time and budget constraints. Otherwise, samples from the whole range of the section, which consists of four operational taxa - *A.procera*, northern *A.magnifica* var.*shastensis*, *A.magnifica* var. *magnifica*, and southern *A.magnifica* var.*shastensis* - were collected (Little,1971). Figure 2 shows sampling locations.

Populations 1 through 5R were collected from the published range for *A.procera* in the Cascade Mts. of Washington and Oregon (Little,1971). Four of these were mature tree populations and three, immature. Five populations, 6 through 8R; three mature and two immature, were collected from the area of range sympatry for *A.procera* and *A.magnifica*, in the Klamath-Siskyou Mts. of southwestern Oregon/northwestern California and in the northern Coast Range of California. This is the northernmost region where *A.magnifica* var.*magnifica* occurs (Liu,1971), and the area where *A.magnifica*

Table 3. Site codes, locations, descriptions, collector, collection date, sample size and taxon hypothesized by location and by visual evidence (1).

SITE CODE	SITE LOCATION, NAME AND DESCRIPTION	TAXON(1)	CONSOLIDATED POP. #(2)
1	SunTop Lookout High: Sun Top Mt.,Pierce Co.,Wash. Mature dense second growth forest with little understory, established after a fire. Aba,Abp,Pnm,Ptm,Tgh(3). 1 438m alt.; 47 03'N,121 36'W. Collection (Coll.) 1 (4).	P10 p10	1
1R(5)	SunTop Lookout High Regeneration: In open spots under mature forest,above.Coll.1.	PR5 pr5	1
2	SunTop Lookout Low: As pop.1, above. Mature open forest that had been thinned. Abp,Ptm,Tgh. 975m alt., same lat.long. as pop.1 above. Coll.1.	P7 p7	1
2R	SunTop Lookout Low Regen.: Understory of open, mature forest, above. Coll.1.	PR5 pr5	1
3	Sleeping Beauty: Sleeping Beauty Ridge, Skamania Co.,Wash. Mature old growth forest. Aba,Abp,Ptm,Tgh. 1 523m alt.; 46 07'N, 127 38'W. Coll.1.	P10 p10	2
4	Sardine Mountain: Sardine Mt.,Williamette Nat.For.,Marion Co.,Ore. Mature old-growth forest. Aba,Abp,Tgh. 1 676m alt.; 44 46'N, 122 14'W. Coll.1.	P10 p10	3
5R	Douglas County: Umpqua and Rogue River Nat.For., Douglas Co.,Ore. Understory/ regeneration. ____m alt.; 43 N, 123 20'W. Coll.2.	PR10 pr10	4
6	Mount Ashland: Rogue River Nat. For., Jackson Co.,Ore. Mature old growth forest. Abc,AbN,Pnl,Ptm. 1 676m alt.; 42 06'N, 122 45'W. Coll.1.	S8 p4,s4	5

Table 3 . (Continued).

SITE CODE	SITE LOCATION, NAME AND DESCRIPTION	TAXON(1)	CONSOLIDATED POP.#(2)
7R	Siskyou County: Shasta Nat. For.,Siskiyou Co.,Calif. Regeneration/understory. _____m alt. 41 30'N, 122 30'W. Coll.2.	SR10 mr10	6
7	Mount Shasta: E side of Mt. Shasta, Shasta-Trinity Nat.For.,Siskiyou Co., Calif. Dense mixed old- and second-growth forest 1 633m alt.;41 22'N, 122 30'W. Coll.1.	S10 s5,m5	6
8	Brushy Mountain: Mendocino Nat. For., Glen Co.,Calif. Open old growth forest. Abc, AbN. 2 035m alt., 39 43'N, 122 45'W. Coll.1.	M/S10 s10	7
8R	Brushy Mt. Regen.: As above; understory under open canopy. Coll.1.	SR9 sr9	7
9	Grizzly Ridge: Plumas Nat. For.,Plumas Co., Calif. Open mature second-growth forest. Abm,Pnj. 2 193m alt.; 39 58'N; 120 45'W. Coll.1.	M10 m10	8
9R	Grizzly Ridge Regen.: As above, understory under open canopy. Coll.1.	M10 m10	8
10R	Alpine County: El Dorado Nat. For., Amador and Alpine Co.,Calif. Regeneration/under-story. _____m alt.;38 35'N,120 00'W. Coll.2.	MR10 mr10	9
11	Mount Tom: Sierra Nat. For.,Fresno Co.,Calif. Mature old-growth forest with extensive un-derstory of various heights. Abm,Pnj. 2 717m alt.;37 25'N, 119 08'W. Coll.1	M10 s5,m5	10
11R	Mt. Tom Regen.: As above, understory trees.	MR9 ?r9	10

Table 3 . (Continued).

SITE CODE	SITE LOCATION, NAME AND DESCRIPTION	TAXON(1)	CONSOLIDATED POP.#(2)
12A	Jordan Peak: Sequoia Nat. For.,Tulare Co., Calif. Advanced regeneration/understory at edges of clear-cut. AbN,Pn. 2 387m alt.; 36 11'N, 118 30'W. Coll.3.	SR10 sr10	11
12B	Sherman Peak: Sequoia Nat. For.,Tulare Co.,Calif. Advanced regeneration under selection cut. AbN. 2 783m alt.; 36 00'N, 118 22'W. Coll.3.	SR10 sr10	11
12C	Bone Meadow: Sequoia Nat. For.,Tulare Co., Calif. Understory and regeneration of open second-growth forest. Abc,AbN,Pn. 2 134m alt.; 36 00'N, 118 36'W. Coll.3.	SR10 sr10	11
12D	Tobias Pass: Sequoia Nat. For.,Tulare Co., Calif. Understory of mature forest to either side of alpine meadow.Abc,AbN,Pn. 2 298m alt.; 35 51'N, 118 32'W. Coll.3.	SR10 sr10	11

(1)

Upper case letters denote identification according to published ranges for the three taxa (Little,E.L.,1971.Atlas of United States trees V.1.Conifers and important hardwoods. Misc.Pub.No.1146,U.S.D.A. Forest Service, Wash. D.C.; Liu,T.S,1971.A monograph of the genus *Abies* .Dept.Forestry,College of Agric., Nat.Univ.Taiwan, Tapei,Taiwan, China.608pp.).

Lower case letters denote occular identification by collector:

P,p - *Abies procera* Rehd. (noble fir)
S,s - *A.magnifica* var.*shastensis* Lemm. (Shasta red fir)
M,m - *A.magnifica* var.*magnifica* A.Murr. (California red or red fir)

Numbers indicate number of individuals analyzed for that population, or sample size.

(2)

Populations were consolidated by location for some of the data analyses.

NOTES CONTINUED NEXT PAGE

Table 3 . (Continued).

(3)
Codes for tree species growing in the sampled stand:
Aba - *Abies amabilis* (Dougl.)Forbes
Abc - *A.concolor* (Gord.&Glend)Lindl.
Abm - *A.magnifica* var.*magnifica*
AbN - *Abies* section *Nobilis*
Abp - *A.procera*
Pn - *Pinus* L. species
Pnj - *P.jeffreyi* Grev.&Balf.
Pnl - *P.lambertiana* Dougl.
Ptm - *Pseudotsuga menziesii* (Mirb.)Franco.
Tgh - *Tsuga heterophylla* (Raf.)Sarg.

(4)
Collection 1 : made by Drs.W.H.Parker and J.Maze, August 1978.
Collection 2 : made by Dr. J. Maze, August 1980.
Collection 3 : made by F.E.Bennett, October 1981.

(5)
R = Regeneration or understory, immature populations.

var.*shastensis* is traditionally located (Franklin,1964). Five populations, 9 through 11R; two mature and three immature, were collected from stands in the Sierra Nevada Mts. of California, where the *A.magnifica* var.*magnifica* that best conforms to the species description grows (Griffin and Critchfield,1972). Finally, four populations, 12A through 12D; all immature individuals, were collected from disjunct stands in Tulare County, California at the southernmost occurrence of the noble firs. This is the other area reported to contain *A.magnifica* var.*shastensis* (Griffin and Critchfield,1972).

Needles from both the apex of mature, cone-bearing trees (see Parker*et al.*,1981, for method of collection) and from the lower crown of immature or understory trees were collected from most of the localities sampled north of Tulare County, California. Only lower crown needle samples of immature trees were collected from the southernmost

populations of *A.magnifica* var.*shastensis* in Tulare County, California.

All of the collections used in this study except this last collection of four populations were taken late in the growing season, before dormancy had set in. The collections from Tulare County, California, were taken in late October, about two months after all of the others, just before the beginning of the dormant season.

On the basis of studies by Cleveland(1979) and Niemann(1976), no major within-tree needle variation was expected between needles from the cone-bearing apical crown branches and healthy needles from the lower crown or between collections taken during the growing season and those taken at the beginning of the dormant season. However, differences in the variability of leaf morphology between mature and immature *A.procera* has been reported (Maze and Parker,1983). Some variation in flavonoid patterns between juvenile and mature herbaceous plant species has been found (Asker and Frost,1970), but no comparisons between the flavonoids of mature and immature conifer species have been reported. As a precaution, therefore, immature and mature samples collected from the same locations were examined first as separate populations in order to expose any flavonoid variability due to age of the population sampled.

Five to ten sample trees were chosen randomly at each collection site for each population (Table 3). The 21 populations were numbered consecutively from north to south with immature, understory or regeneration populations designated by an additional capital letter in their code. A second code was assigned to the populations as they were consolidated by site (Table 3), resulting in 11 consolidated populations. Each population was also named by location.

Voucher specimens are stored at Lakehead University, Thunder

Bay, Ontario (LKHD) and at the University of British Columbia, Vancouver, British Columbia (UBC).

FLAVONOID CHARACTERIZATION

The use of flavonoids in a systematic study of variation in a group of closely related taxa is a two-step process. Initially, characteristic flavonoids of the taxa under investigation must be determined. Only then can any variation in the characteristic pattern of flavonoid occurrence be documented through a population survey.

Massed foliage samples from the northernmost populations collected (SunTop Lookout) were used to characterize the flavonoids of typical *A.procera*. Most flavonoids are very stable compounds, and dried herbarium specimens are commonly used for flavonoid surveys (Harborne,1975, p.1065). Needles were stripped from air-dried and pressed branches, weighed, and 1.159kg of needles were set to soak in 100 percent methanol for 48 hours. The extract was filtered, and the needles set to soak a second time in fresh 100 percent methanol. Both extracts were combined and flashed dry, then dissolved in boiling water with diatomaceous earth and filtered to help separate the flavonoids from unwanted extractives (Parker*et al.*,1979). This partially cleaned fraction was dried, dissolved in 20 percent aqueous methanol and run through a Poragel (Waters Associates, Inc., Maple St., Milford Mass. 01757) liquid chromatography column with a solvent sequence of 20 percent aqueous methanol through 100 percent methanol.

Individual compounds were then isolated from the fractions

decanted from this large chromatography column using preparative one-dimensional polyamide (MN-Polyamid-DC6.6, Macherey, Nagel and Co., W.Germany; distributed by Brinkman Instruments (Canada) Ltd., 50 Galaxy Blvd., Unit 1, Rexdale, Ontario M9W 4Y5) thin-layer chromatography (TLC) and crystalization techniques. The purified flavonoids were identified using ultaviolet (UV) spectroscopy, sugar hydrolysis, co-chromatography with standards and, wherever possible/necessary, proton magnetic resonance (PMR) spectroscopy (Mabry*et al.*,1970).

In this study, it was difficult to get enough of certain trace compounds to permit absolute identification. Other compounds were unidentifiable due to lack of expertise and/or time. The identity of these compounds is still under investigation.

POPULATION FLAVONOID SURVEY

Approximately one-gram samples of the needles from each tree were set to soak for 48 hours in 100 percent methanol. Half of each sample consisted of freshly flushed or the current year's needles, and half consisted of the previous year's needles. Cleveland(1979) determined that some aglycones occur in greater amounts in new foliage of *A.balsamea.* He recommended using both current and the previous year's foliage to characterize the flavonoids in species of this genus.

Each raw extract was filtered free of needles, flashed dry and dissolved in boiling water. It was then run through a 2cm deep by 2.5cm diameter G-25 Sephadex (Pharmacia Fine Chemicals AB, Upsala,

Sweden) column with 20 percent aqueous acetone followed by sufficient 100 percent acetone to clear any remaining flavonoids off of the column. Both fractions (20 percent acetone and 100 percent acetone) were flashed dry and stored separately in 80 percent methanol in the dark, at 2 to 4 degrees Celsius.

Each fraction was spotted on separate polyamide TLC plates and run through an aqueous solvent in the first dimension - 80:10:5:5; water: n-butanol: acetone: acetic acid - and an organic solvent in the second dimension - 50:15:15:20; chloroform: isopropanol: methyl ethyl ketone: acetic acid - (Parker*et al.*,1979). After drying, each plate was checked for any yellow-flourescing compounds under 366nm long-wave UV light. The colour and absorption properties of flavonoid compounds under these conditions is diagnostic; yellow-flourescing compounds are flavonols with a free 3-position hydroxyl (Mabry*et al.*,1970). The plates were then sprayed with diphenyl boric acid ethanolamine chelating reagent (Aldrich Chem.Co., Milwaukee Wisconsin 53233) to visualize trace compounds and to provide further diagnostic colour reactions of resolved compounds.

A sketch of each TLC plate was made over a light table within two days of spraying to preserve the two flavonoid patterns of each individual; one of the 20 percent aqueous acetone fraction and one of the 100 percent acetone fraction. Chromatography packing materials tend to retain flavonoids, especially in aqueous solvents; only those compounds containing water-soluble sugars are easily eluted with aqueous solvents. Fractions eluted with 20 percent aqueous acetone include the more soluble di- and tri-glycosides. Fractions eluted with 100 percent acetone usually contain only aglycones or aglycones and monoglycosides of flavonoid compounds. The flavonoid survey was carried out using these "maps" of the TLC plates.

In order to help eliminate possible artifacts of handling and extraction for the flavonoid identification, two one-gram needle samples for each of ten trees of one unconsolidated population - Siskyou County, coded as 5R - were extracted separately. Using TLC plate "maps", the flavonoids of each tree for each extraction were compared to check for differences of occurrence between extractions. In addition, one set of ten extractions, each from a different tree, were spotted on two different sets of TLC plates and the plates for each tree compared to check for differences of occurrence between TLC plates of the same extract. Compounds that were inconsistent in their appearance were eliminated from the final population survey.

TABULATION OF RESULTS AND ANALYSIS

The results of the mass extraction and the population survey were summarized in a composite flavonoid "map" that included all of the consistently-appearing compounds that were observed in all of the sample trees. Each spot or compound on the composite map was numbered and then the individual trace maps were reviewed. Each spot was numbered when it occurred on an individual map. Then each population was tabulated, by tree, for the occurrence of each compound. These results were then coded 0(absent), 1(present in trace amounts) or 2(present), and summarized by population. Only those flavonoid compounds that occurred consistently, as determined by the duplicated extractions, were considered for numerical and cladistic analysis. Non-varying compounds were not used to look at the pattern of variation

between and within populations (Sneath and Sokal,1973).

Twenty-four naturally occurring, variable compounds were chosen as chemical characters. These included compounds which had no obvious pattern of occurrence. Frequency histograms of these variable compounds were drawn for all 21 populations to check for irregularities between mature and immature populations from the same locality. Histograms were also drawn for populations consolidated by location to check for overall trends in occurrence.

The presence/trace/absence of these 24 compounds in both the 21 mature and immature populations and in the 11 populations consolidated by location were subjected to principal components analysis (PCA) and discriminant analysis (DA) using a Vax 11/780 computer and the Statistical Package for the Social Sciences (SPSS; Nie*et al.*,1975). A graphics algorithm developed by Dr. W.H. Parker for the Apple IIe microcomputer was used to ordinate the sample trees and populations on PCA axes in two dimensions. Both sets of population data were subjected to clustering analysis using the weighted pairs group method (WPGM; Sokal and Sneath,1963), as developed in another algorithm by Dr. Parker for the Apple IIe.

Finally, both sets of data were recoded to 0(absent), 1(present) for cladistics analysis by Dr. Jack Maze using the "Wagner 78" algorithm (Farris and Mickevich,1983; Farris*et al.*,1970) at the computing centre at the University of British Columbia. This recoding was necessary because of constraints built into the algorithm. If the population average occurrence for a given variable was less than the overall average, the character state assigned to that population for that variable was 0(absent). If the population average was greater than or very close to - ie. within 1 percent of - the overall average, the state assigned was 1(present). Two

hypothetical ancestors, one with all variable compounds present and one with all variable compounds absent were used to determine the most parsimonious (Eldredge and Cracraft,1980) representation of the recoded data.

RESULTS AND ANALYSIS

FLAVONOID IDENTIFICATION

Identification of all of the flavonoid compounds in a given taxon is challenging, particularly if there are trace compounds present. Harborne(1975) suggests identifying all of the more variable compounds in a taxon before applying the data to systematic studies. This is primarily to avoid overestimates of the flavonoid characters available. Overestimation could be due to glycosylation with only a few sugars of all of the aglycones present in a taxon or to artifacts formed during extraction and purification by breakdown of less stable compounds. In addition, naturally-occurring but non-flavonoid compounds can be mistaken for flavonoids.

Compound identification is useful in determining what biosynthetic pathways are present in a group of plants. While parallel evolution can result in the same biosynthetic pathways, and consequently the same secondary plant products in unrelated plant groups, the occurrence of unique compounds in a taxon of uncertain affinity can help to determine its taxonomic status and/or phylogenetic background.

Preliminary study of the flavonoid patterns of the two varieties of *A.magnifica* indicated that most of the flavonoid compounds present in the species complex occur either in *A.procera* or in other species of

Abies already characterized (Parker*et al.*,1979; 1984). The composite flavonoid "map" for the *Abies* section *Nobilis* complex is reproduced in Figure 3.

Table 4 lists all of the compounds on the map by spot number, and indicates the diagnostic techniques used to identify them. Variable compounds used in the population survey are indicated by asterisks. Not all of the compounds used for the numerical analyses have been completely identified. Appendix V contains available UV spectral data.

Sixty-four probable flavonoid compounds occur in this species complex. A few of these compounds were unstable, and broke down during purification. Some compounds occurred only in trace amounts, so that not enough material was collected for complete analysis. Some compounds run to the same position on TLC plates as other compounds in the developing solvents used, complicating purification and complete identification of overlapping compounds.

As in other *Abies* species analyzed to date (Parker*et al.*,1979; 1984), the flavonoids of the noble firs consist primarily of flavonol glycosides of myricetin, laricytrin (3'-methyl myricetin), quercetin, kaempferol, isorhamnetin and syringetin. attached sugars that have been identified are glucose, galactose, rhamnose and rutinose (rhamnosyl glucose); generally these sugars are attached to hydroxyl ions at the 3-position (Figure 4). Combinations of these six simple flavonols and four sugars as monoglycosides and diglycosides account for approximately twenty-four of the spots on the composite flavonoid map (Table 4, Figure 3), most of them occurring consistently in all taxa of the section.

Other flavonoids partially identified in the noble firs so far are :

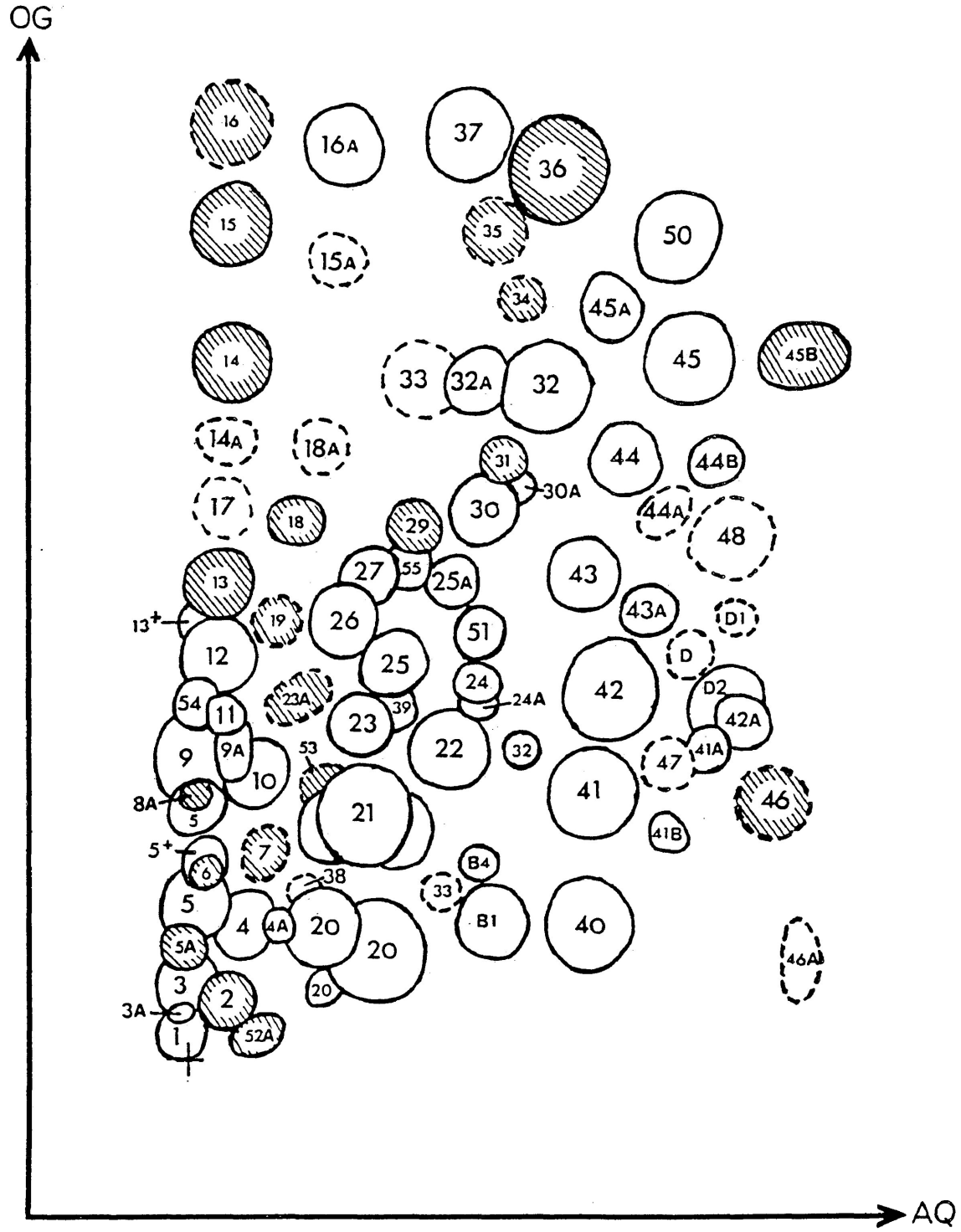


FIGURE 3. Composite TLC "map" of all spots exhibited by methanol extracts of needles of sampled trees. A dotted outline means that the compound is usually present in trace amounts; a solid outline means that the compound is usually present in more than trace amounts. Shaded spots are variable compounds that were used in the analyses. AQ=aqueous solvent front; OG=organic solvent front.

TABLE 4. List of all "spots" exhibited by methanol extracts of needles of sampled trees. Compounds are grouped by glycosylation class, which usually correlates to location on the polyamide chromatograph along the aqueous developer front. Asterisks indicate the compounds used in the variation analyses.

SPOT NO.	COLOUR BEFORE REAGENT SPRAY(7)	COLOUR AFTER SPRAY	ANALYSES	TENTATIVE ID.	FREQUENCY
PROBABLE AGLYCONES					
1/3A		oj,gr,y	UV	dhfl. or (6)	(4),(5)
* 2	y	oj			variable
3		y	UV	dhfl.?	constant (absent in scattered indv.only)
4		y			constant, usu.tr.
* 4A		gr			variable
5/5+		gr	xtal.,UV, PMR	dhfl.?	constant, runs to same place as 5A
* 5A		y	xtal.,UV	dhfl.?	variable
* 6	y	oj			variable
* 7		oj			variable, usu.tr.
8		gr			(4)
* 8A		y			variable
9		gr	xtal.,PMR, UV	acetyl deriv.?	constant
1O		gr	xtal.,PMR, UV	ap-deriv.?	constant
11	y	oj	UV,hydrol.	ap-aglyc.?	(4)
12	y	gr	UV	acetyl deriv.?	(4), runs to same place as 54; both together are const.
*13		gr	xtal.,PMR,UV	acetyl deriv.?	variable

TABLE 4. (Continued).

SPOT NO.	COLOUR BEFORE REAGENT SPRAY(7)	COLOUR AFTER SPRAY	ANALYSES	TENTATIVE ID.	FREQUENCY
13+		gr			(4)
*14		gr	xtal.,PMR,UV	acetyl deriv.?	variable
14A		gr			(4)
*15		gr	UV	acetyl deriv.	variable, occ.(5)
15A		gr	UV	chr-deriv.?	(4)(5),tr.
*16		gr-y-br			variable, usu.tr.
16A		y			(4)
17		gr			(4), usu.tr.
*18		gr			variable
18A		y	UV	dhfl.?	(4)
*19		gr	UV	vit-deriv.?	variable
*23A		oj			variable
*52A		oj			variable
54		gr			(4), see 12
PROBABLE MONOGLYCOSIDES					
2O		oj	UV,(1),(2)	my-glu/gal/rham	constant
21		y	UV,(1),(2)	qu-glu/gal/rham	constant
22		oj	(1),(2)	lc-glyc.	constant
23		oj	(1),(2)	lc-glyc.	constant
24		gr	UV,(1),(2)	kp-glyc.	constant
24A		bm			(4)

TABLE 4. (Continued).

SPOT NO.	COLOUR BEFORE REAGENT SPRAY(7)	COLOUR AFTER SPRAY	ANALYSES	TENTATIVE ID.	FREQUENCY
25/26/27		gr	UV,(1),(2)	kp-glyc.	complex constant, sugars var.
25A		gr		kp-glyc.	(4)
*29		oj	(3)	my-acetyl?	variable
3O		y	(1),(2)	ir-glyc.	constant
3OA		gr			(4)
*31		oj	(3)	lc-acetyl?	variable
32		y	UV,(1),(2)	sg-glyc.	constant
32A		gr	UV,(3)	7-O-glyc.-ap?	(4),(5)
33		gr	(3)	kp-acetyl?	(4), usu.tr.
*34		y	(3)	qu-acetyl?	variable, occ.(5)
*35		gr	(3)	ir-acetyl?; 7-O-glyc.-tricin?	variable
*36		y	UV,hydrol. (3)	sg-acetyl or 3,5-dihidroxy-3',4',5'-trimethoxyflavone?	variable
39		y	UV		(4)
45A		bm	UV,(2)	ng-glyc.	(4),(5)
51		oj			(4)
*53	y	y		qu-7-O-glyc.	variable, usu.tr.
PROBABLE DIGLYCOSIDES					
4O		oj	(1),(2)	my-3-O-rut	constant
41		y	(1),(2)	qu-3-O-rut	constant

TABLE 4. (Continued).

SPOT NO.	COLOUR BEFORE REAGENT SPRAY(7)	COLOUR AFTER SPRAY	ANALYSES	TENTATIVE ID.	FREQUENCY
41A		oj			(4)
41B		y			(4)
42		oj	(1),(2)	lc-3-O-rut	constant
42A		bm			(4)
43		gr	(1),(2)	kp-3-O-rut	constant
43A		r	(2)	dhfl.	(4),(5)
44		gr	(1),(2)	ir-3-O-rut	constant
44A		bm			(4)
44B		y			(4)
45		y	UV,(1),(2)	sg-3-O-rut	constant
*45B		y	(3)	dhfl.	variable
*46		y-oj	UV,(2)	tax-glyc.	variable, tr.
46A		oj	(2)	(4), tr.	
*47/D2		gr	(3)	rhamnosylvitexin	variable
*48		gr	UV,(2)	kp-3,7-O-diglyc.	variable
D, D2		gr			(4)
NON-FLAVONOID COMPOUNDS					
B1,B2,B4		b			
B3,B5		p			
28,37,38,50		p			
55		b			

SEE NOTES NEXT PAGE

TABLE 4. (Continued).

(1)
Parker,W.H.,Maze,J. and McLachlan,D.G. 1979. Flavonoids of *Abies amabilis* needles. Phytochem.18:5O8-51O.

(2)
Parker,W.H.,Maze,J.,Bennett,F.E.,Cleveland,T.A. and McLachlan,D.G.1984.Needle flavonoid variation in *Abies balsamea* and *A.lasiocarpa* from western Canada. Taxon:33:1-12.

(3)
unpublished data, including Cleveland,T.A. 1979. Needle flavonoids of *Abies balsamea* : identification and effects of needle age and crown position. H.B.Sc. Thesis,Lakehead Univ.,Thunder Bay,Ont.

(4)
frequency cannot be determined because identification is uncertain; the compound may run to the same location as another spot, or trace amounts may be hard to see, so compound was not used as a character.

(5)
frequency cannot be determined because compound is inconsistent within individuals, so is not a reliable character.

(6)
compound is probably not a flavonoid, so was not used as a character.

(7)
the spray reagent used to visualize trace amounts and to assist in identifications through colour reactions was diphenyl boric acid ethanolamine (Aldrich Chem.Co.,Milwaukee Wisc. 53233).

ABBREVIATIONS:

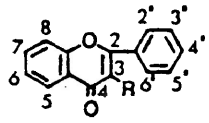
Colour
b=flourescent blue; brn=brown; gr=green; oj=orange;p=flourescent purple; r=red; y=yellow

Analysis
hydrol.=acid hydrolysis of compound; PMR=proton or nuclear magnetic resonance spectroscopy; UV=ultraviolet spectroscopy; xtal.=crystalization of pure compound.

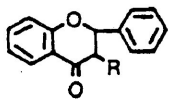
Identification
agly.=aglycone; ap=apigenin; chr=chrysin; deriv.=derivative; dhfl.=dihydroflavonol; diglyc.=diglycoside; gal=galactose; glu=glucose; glyc.=glycoside; ir=isorhamnetin; isoflav.=isoflavonol; kp=kaempferol; la=laricytrin; my=myricetin; ng=naringenin; non-F.=non flavonoid compound; qu=quercitin; rham=rhamnose; rut=rutinose; vit.=vitexin.

Frequency
const.=constant; indiv.=individual; occ.=occasionally;tr.=always present in trace amounts; usu.tr.=usually present in trace amounts; var.=variable.

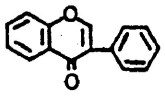
A. BASIC ARRANGEMENT



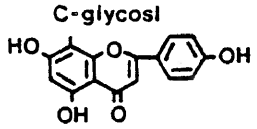
B. FLAVANONE, DIHYDROFLAVONOL



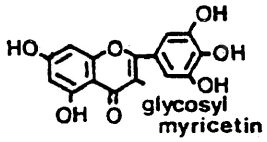
C. ISOFLAVONE



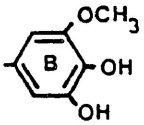
D. 8-C-GLYCOSYLATED FLAVONE



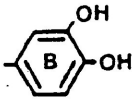
E. 3-O-GLYCOSYLATED FLAVONOL



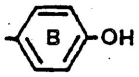
F. R=OH, LARICYTRIN



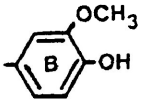
G. R=OH, QUERCETIN



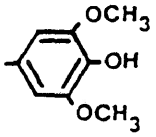
H. R=OH, KAEMPFEROL
R=H, APIGENIN



I. R=OH, ISORHAMNETIN



J. R=OH, SYRINGETIN
R=H,TRICIN



K. R=H, CHRYSIN

NOTES : **A.** Basic flavonoid phenolic ring arrangement and numbering scheme. Flavones, R=H; flavonols, R=OH.

B. Flavanones and dihydroflavonols have no double bond between 2- and 3-position of C-ring.

C. Isoflavones have the B-ring attached at position 3- instead of position 2-.

D. Two 8-C-glycosylated flavones occur sporadically in the *Abies* section *Nobilis*. Vitexin, glycosyl=glucose; rhamnosylvitexin, glycosyl=rhamnoglucose.

E. - K. Most of the flavonoids of *Abies* species are 3-O-glycosylated flavonols of myricetin, laricytrin, quercetin, kaempferol, isorhamnetin and syringetin.

H., J., K. A few simple flavones also occur, which may or may not be glycosylated at the 7-position.

FIGURE 4. Base skeletons of some flavonoids that occur in the *Abies* section *Nobilis*.

1. three flavones, apigenin, chrysin and tricin, and several monoglycoside derivatives, probably 7-O-monosides;
2. a flavanone, naringenin, and at least one monoglycoside of naringenin;
3. two C-glycosyl flavones, rhamnosylvitexin and vitexin;
4. glycosylated dihydroflavonols, including dihydrokaempferol and taxifolin (dihydroquercetin) - the presence of aglycones of these two compounds in the flavonoids of the section has not been confirmed;
5. a 7-O-glycosylated derivative of quercetin;
6. approximately 26 partially-identified compounds that will be referred to as aglycones because of their low mobility in the aqueous solvent; and
7. five acetylated derivatives of the flavonols myricetin, quercetin, laricytrin, isorhamnetin and syringetin.

POPULATION SURVEY

Table 5 lists the population codes for the frequency histograms of variable compounds that were used in a visual survey of flavonoid variation by population. Frequency histograms prepared for variable flavonoids that are not referred to directly in this section are presented in Appendix I. Histograms were drawn for both the 21 immature and mature populations and the 11 populations consolidated by location. Six of eleven locations were sampled for both mature and immature tree populations - SunTop Lookout High (unconsolidated population codes

1,1R), SunTop Lookout Low (2,2R), Mt.Shasta/Siskyou County (7,7R), Brushy Mt. (8,8R), Grizzly Ridge (9,9R) and Mt.Tom (11,11R) - two populations from each operational taxon except southern *A.magnifica* var.*shastensis*.

TABLE 5. Population codes for frequency histograms and principal components ordinations of variable compounds.

UNCONSOL. POP.CODE	CONSOL. POP.CODE	POPULATION IDENTIFICATION
A.procera		
1	1	Suntop Lookout High,Wash. - mature
2	1	Suntop Lookout High,Wash. - immature
3	1	Suntop Lookout Low,Wash. - mature
4	1	Suntop Lookout Low,Wash. - immature
5	2	Sleeping Beauty,Wash. - mature
6	3	Sardine Mt.,Ore. - mature
7	4	Douglas Co.,Ore. - immature
Northern *A.magnifica* var.*shastensis*		
8	5	Mt. Ashland,Ore. - mature
9	6	Mt. Shasta,Calif. - mature
10	6	Mt. Shasta,Calif. - immature (Siskyou Co.)
11	7	Brushy Mt.,Calif. - mature
12	7	Brushy Mt.,Calif. - immature
A.magnifica var.*magnifica* (Calif.)		
13	8	Grizzly Ridge - mature
14	8	Grizzly Ridge - immature
15	9	Alpine Co. - immature
16	10	Mt. Tom - mature
17	10	Mt. Tom - immature
Southern *A.magnifica* var.*shastensis* (Calif.)		
18	11	Tulare Co., Jordan Peak - immature
19	11	Tulare Co., Sherman Peak - immature
20	11	Tulare Co., Bone Meadow - immature
21	11	Tulare Co., Tobias Pass - immature

Only the five acetylated monoglycosides and the 7-O-monoglycoside of quercetin are variable compounds based on the flavonol glycosides. All but one of the other variable compounds are glycosides of dihydroflavonols, unidentified aglycones or are possibly acetylated flavonoid derivatives. The exception, 45B (see Figure 3), has not yet been identified, and occurs in only three consolidated populations (see Figure 5.F). There is some evidence that it also is a dihydroflavonol (Cleveland,1979).

One of the five variable acetylated monoglycosides has been tentatively identified as syringetin-3-O-acetyl-glucosyl-galactoside (spot 36). Three of them - the acetylated myricetin (spot 29), laricytrin (spot 31) and spot 36 - are present in almost all individuals of consolidated populations 1 through 7 (Figure 5.A). This includes all populations of *A.procera* and the 3 consolidated populations sampled from the area of range sympatry between *A.procera* and *A.magnifica* var.*magnifica*, designated as northern *A.magnifica* var.*shastensis.* Frequency histograms for both consolidated and nonconsolidated populations illustrate the break in occurrence of the myricetin, laricytrin and syringetin acetyls at the Douglas County population in Oregon. The quercetin (spot 34) and the isorhamnetin (spot 35) acetyl derivatives are usually present in trace amounts which limits their reliability somewhat; nevertheless they were used in the numerical analyses. Like the myricetin, laricytrin and syringetin acetyls, spot 35 does not occur in populations south of Brushy Mt., consolidated population 7, ie. in populations of *A.magnifica* var.*magnifica* or southern *A.magnifica* var.*shastensis* (Figure 5.B).

Spot 34 does not follow this trend; it occurs in all populations except

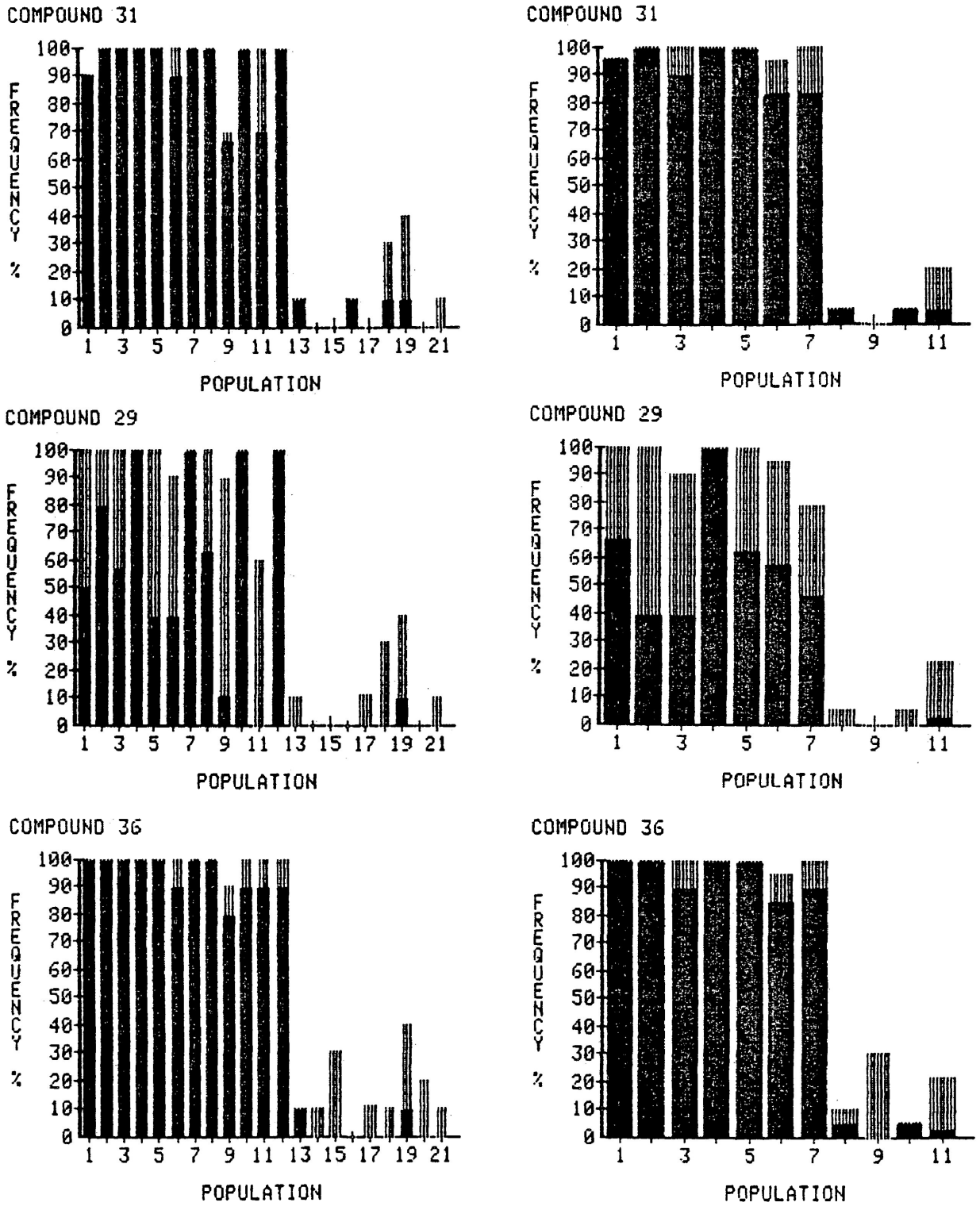


FIGURE 5.A. Frequency histograms of occurrence for the myricetin (spot 29), laricytrin (31) and syringetin (36) acetyls. Unconsolidated populations are on the left and consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

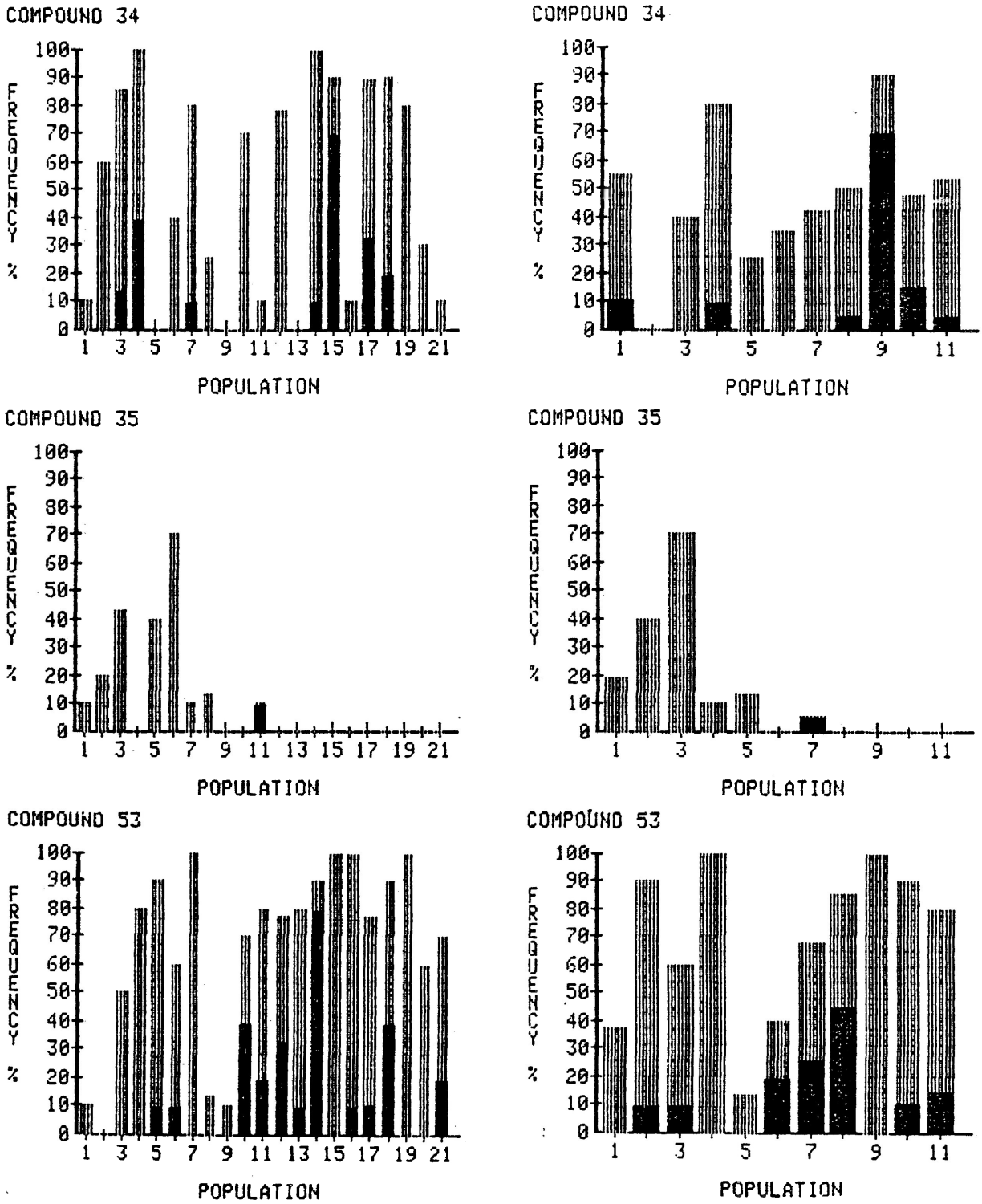


FIGURE 5.B. Frequency histograms of occurrence for the quercetin (spot 34), isorhamnetin (spot 35) and the quercetin-7-O-glycoside(spot 53). Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

Sleeping Beauty, consolidated population 2, but not in the same proportion of individuals for each population (Figure 5.B). Greatest percent occurrence is in the Alpine County, California immature population of *A.magnifica* var.*magnifica*. This lack of discernible pattern may be due to missing the occurrence of trace amounts of this compound when it is actually present.

The quercetin-7-O-monoglycoside (spot 53) resembles the quercetin acetyl in its variability, with no easily discernible pattern of occurrence apparent (Figure 5.B). It occurs in all populations, but in 10 to 100 percent of the individuals in each population. Both spots 53 and 34 show some discrepancy in occurrence between mature and immature trees from the same location. In the *A.magnifica* var.*magnifica* population collected from Grizzly Ridge, serious discrepancy in occurrence between immature and mature trees occurred only in this acetylated monoglycoside of quercetin, spot 34. No mature trees sampled from this location exhibited spot 34, while 100 percent of immature trees contained at least trace amounts of this compound. In addition, the acetylated monoglycosides of myricetin (spot 29) and quercetin (spot 34) were present in 100 to 78 percent of immature trees and only 60 to 10 percent of mature trees respectively, from the North California Coast Range population of *A.magnifica* var.*shastensis* at Brushy Mt.

None of the aglycones found in this species complex have as yet been adequately identified, not even those chosen for the numerical analyses. UV and PMR spectroscopy results for these compounds indicate some similarities to very simple flavones or to dihydroflavonols. Rf values are similar to flavonol p-coumarylglycosides found by Niemann and Baas(1978) in *Larix decidua* Mill.

Four aglycones that are very mobile in the organic solvent (Rf=0.46

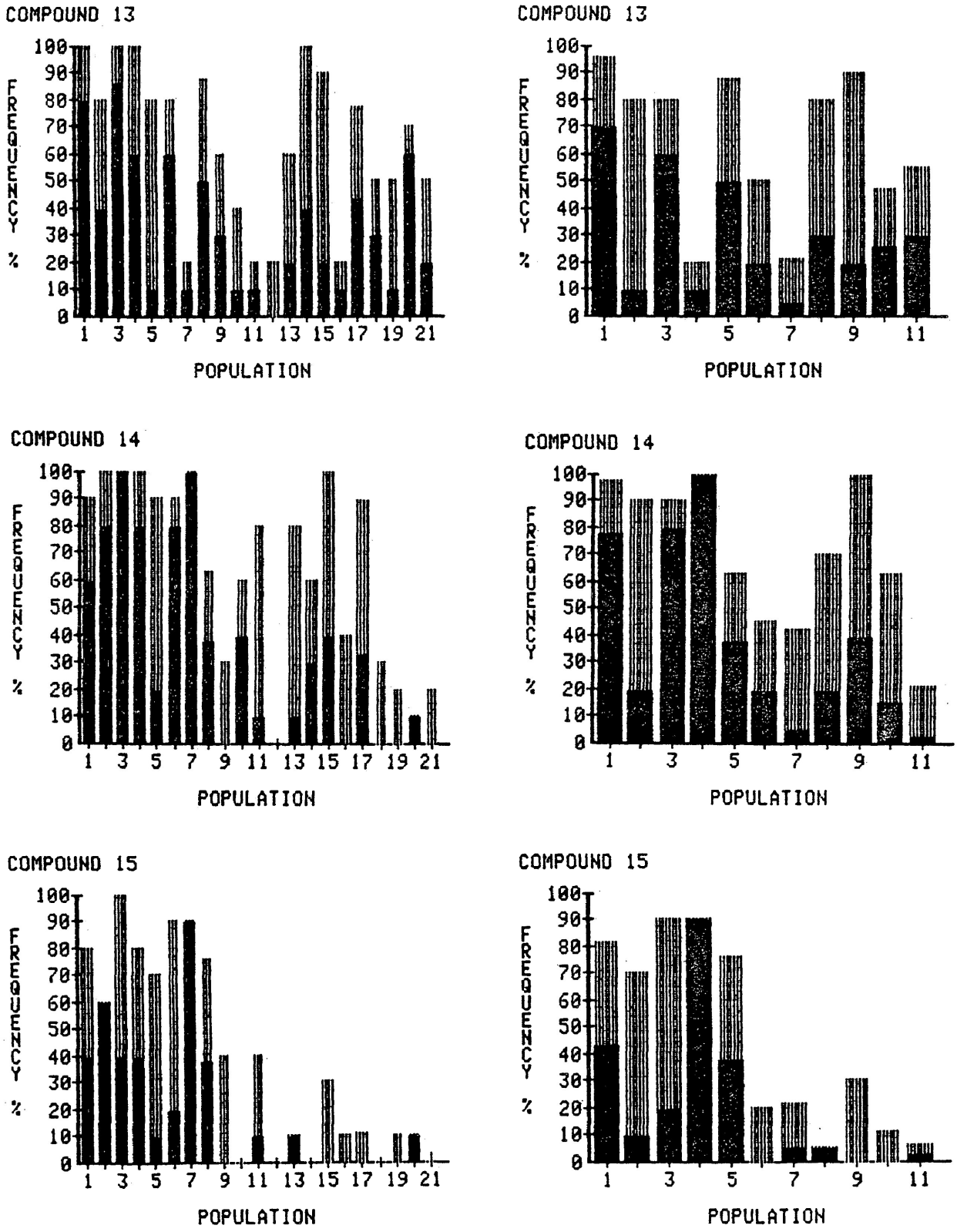


FIGURE 5.C. Frequency histograms of occurrence for the three "upper" aglycones, spots 13, 14 and 15. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

to 0.90) have been retained for the variation analyses. The pattern of occurrence in these four compounds, spots 13, 14, 15 and 16, is not as clear-cut as in the acetylated monoglycosides (Figure 5.C). Spot 14 is absent in more than 50 percent of the individuals of consolidated populations 5, 6, 7 and 11, the populations of *A.magnifica* var.*shastensis*, both north and south. It tends to occur in greater quantities in a greater proportion of individuals of populations of *A.magnifica* var.*magnifica* and *A.procera*, especially in the latter. Spot 15 occurs in 60 to 90 percent of all populations of *A.procera* and in only 5 to 40 percent of populations of *A.magnifica* var.*magnifica* and *A.magnifica* var.*shastensis* north and south (Figure 5.C). Spot 13 has an irregular pattern of occurrence similar to the quercetin-7-O-glycoside - spot 53 - and the acetylated quercetin derivative - spot 34 (Figure 5.B).

There is some discrepancy in occurrence comparing mature to immature trees in spots 14 and 15, particularly for populations of northern *A.magnifica* var.*shastensis*. In the *A.magnifica* var.*shastensis* populations at Mt.Shasta/Siskyou County and at Brushy Mt., the occurrence of upper aglycones 14 and 15 varied inconsistently between immature and mature populations. These two upper aglycones did not occur at all in the immature trees from the California North Coast Range site, while present in 80 to 40 percent, respectively, of the mature trees from that location.

A number of aglycones that run between Rf=0.10 and 0.46 in the organic solvent have the opposite pattern of occurrence; they tend to occur in the more southerly populations sampled, especially consolidated population 11, the population of southern *A.magnifica* var.*shastensis*. Fifteen of these aglycones were chosen for the numerical analyses; approximately eleven additional aglycones were excluded because they

tended to overlap each other and could not be reliably picked out on the TLC plates. No compounds that occurred exclusively in all populations from Tulare County, California were used as characters because their retention could be due to the season of sample collection (Cleveland,1979).

Aglycones 5A, 8A and 23A were present only in immature populations of some of the populations south of Douglas County (population 5R), Alpine County (population 10R) and Jordan Peak (population 12A), respectively (Figure 5.D). None of these populations had companion populations of mature trees, so no conclusions can be drawn correlating presence of these aglycones to age of sample trees. Only spot 5A was present in more than trace amounts in more than 30 percent of the trees of any population. It was actually present in more than trace amounts in 70 to 100 percent of trees sampled from Tulare County, California, the four populations of southern *A.magnifica* var.*shastensis*.

The two dihydroflavonol glycosides used as characters in the numerical analyses have been identified in other *Abies* species (Parker*et al.*,1984). Dihydrokaempferol-galactoside (48) and taxifolin-galactoside (46) were diagnostic characters for the *A.balsamea* /*A.lasiocarpa* complex in western Canada. In the *Abies* section *Nobilis* complex, these dihydrokaempferol and taxifolin glycosides tended to occur in a higher proportion of individuals in consolidated populations south of Sardine Mt., population 3 (Figure 5.E). In the Mt.Shasta/Siskyou County populations, the dihydrokaempferol and the taxifolin diglycosides occurred in 80 to 90 percent of immature trees and only 20 percent of mature trees. A similar discrepancy in occurrence was apparent at the Mt.Tom site for those two compounds.

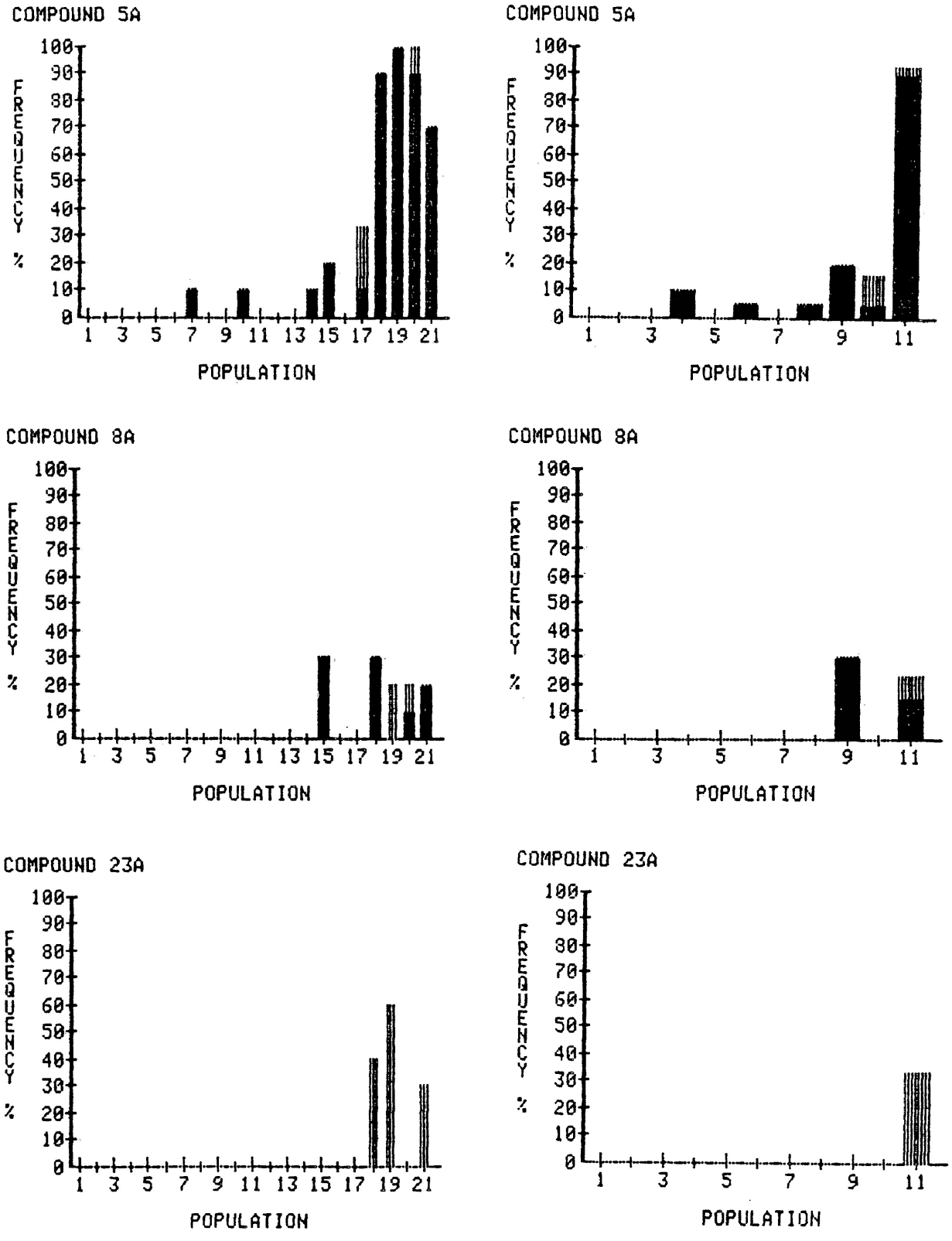


FIGURE 5.D. Frequency histograms of occurrence for the unidentified "lower" aglycones, spots 5A, 8A and 23A. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

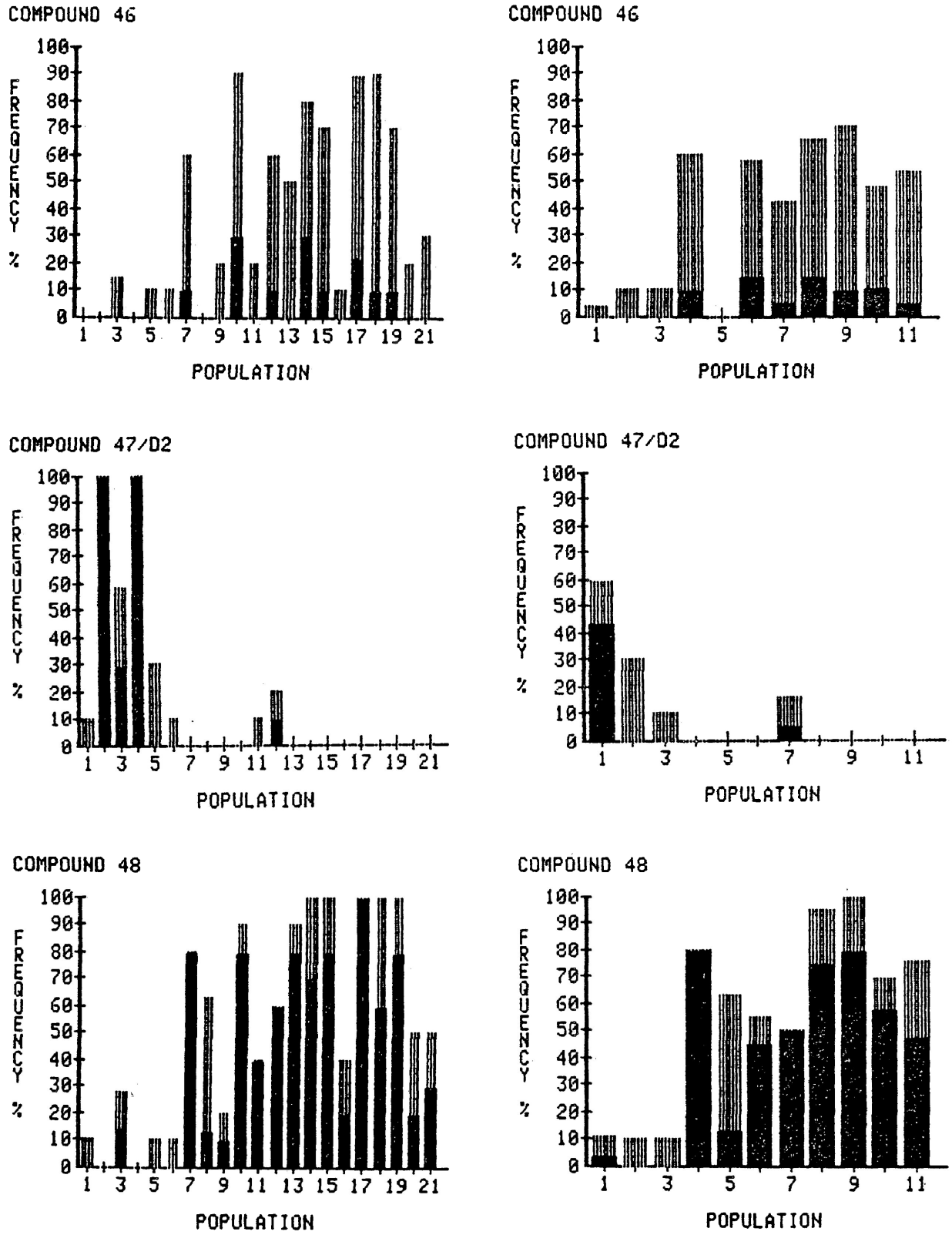


FIGURE 5.E. Frequency histograms of occurrence for the taxifolin glycoside (spot 46), rhamnosylvitexin (spot 47) and the dihydrokaempferol glycoside (spot 48). Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

A third compound, rhamnosylvitexin - spot 47 - is a C-glycosylated derivative of the flavone apigenin (see Figure 4), and has been identified in populations of *A.balsamea* east of Manitoba (Cleveland,1979), but not in western populations. Rhamnosylvitexin tends to occur in a higher percentage of individuals in populations of *A.procera* north of Sardine Mt., particularly in immature populations, which are sampled from the lower crown (Figure 5.E).

Rhamnosylvitexin was found in 10 to 60 percent of the individuals in 4 consolidated populations - 3 of *A.procera* and 1 from the Klamath Mts. population of northern *A.magnifica* var.*shastensis*, Brushy Mt.(population 7). However, it occurs in more than trace amounts in 100 percent of the immature SunTop Lookout trees, and in only 35 percent of the mature trees from that same location. Cleveland(1979) found that the occurrence of rhamnosylvitexin varied according to crown position. It was present in only trace amounts in the middle and upper crown of dormant *A.balsamea* while occurring in moderate amounts in the lower crown of mature trees. Mature population specimens for this study were all taken from the apex of sample trees while immature trees were sampled from lower parts of the crown, so variation in the occurrence of rhamnosylvitexin between populations from the same location may be accounted for by crown position.

Spot 45B, which has an Rf similar to the taxifolin and dihydrokaempferol glycosides in both the aqueous (AQ) and the organic (OG) solvents (Rf=0.84 OG; 0.72 AQ), also occurs only in immature populations, and in a very disjunct fashion (Figure 5.F). It occurs in more than trace amounts in 2 individuals of the SunTop Lookout High regeneration population (1R), 7 percent of the consolidated SunTop Lookout population. It also occurs, in more than trace amounts, in 6 of 10

individuals of Alpine County (population 10R), a population to the south in the range of *A.magnifica* var.*magnifica*. It does not occur in either the mature or immature populations of the next more southerly population of *A.magnifica* var.*magnifica*, Mt.Tom (11,11R), but re-occurs in more than trace amounts in 18 of 40 individuals collected from the four populations in Tulare County, California. Interestingly, percent occurrence of spot 45B in each of the 4 populations collected from the disjunct stands of southern *A.magnifica* var.*shastensis* in Tulare County ranged from 10 percent to 90 percent, although these populations were all collected at the same time from the same crown position and from immature trees.

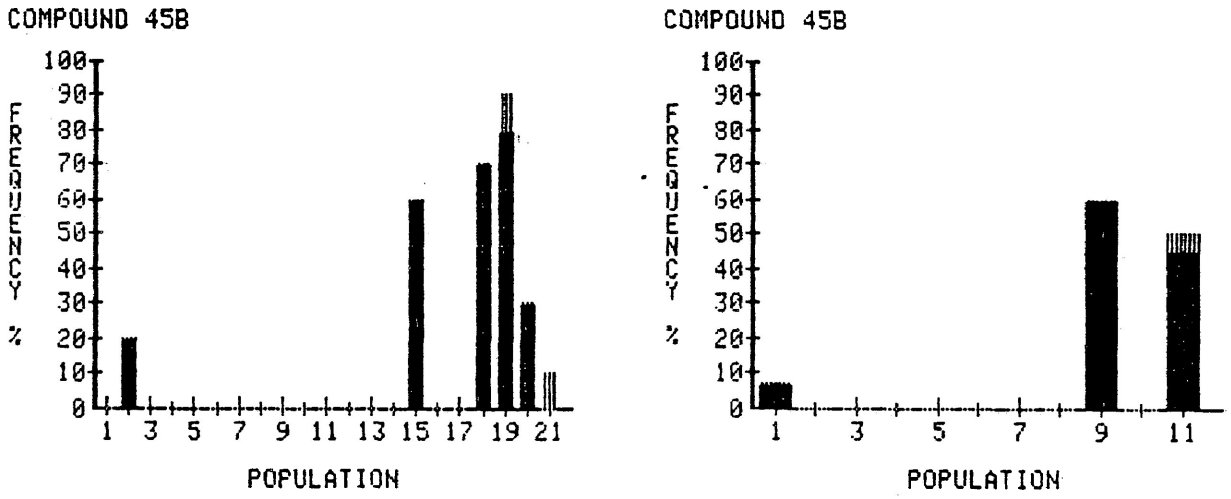


FIGURE 5.F. Frequency histograms of occurrence for the unidentified dihydroflavonol, spot 45B. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are in Table 5. Light shading = present in trace amounts; dark shading = present in more than trace amounts.

In summary, 24 naturally occurring, reliably traced and variable flavonoid compounds were selected for numerical analysis of the flavonoid data (Figure 3). A Basic Data Matrix (BDM) of the occurrence of each variable compound in each tree, organized by population, is contained in Appendix II.

Five possibly acetylated monoglycosides tend to occur only in populations of *A.procera* and northern *A.magnifica* var.*shastensis*. Four unidentified flavonoids that are very mobile in the organic solvent and not at all in the aqueous solvent likewise tend to occur either in populations of *A.procera* or in populations of *A.procera* and northern *A.magnifica* var.*shastensis*. Fifteen aglycones with low mobility in the organic solvent have a less clear-cut pattern of occurrence, but tend to occur mostly in populations south of Sardine Mt., Oregon (consolidated population 3), at approximately latitude 44 degrees N. They are particularly prominent in the southernmost populations sampled, those consisting of southern *A.magnifica* var.*shastensis*. Two dihydroflavonol glycosides, monoglycosides of dihydrokaempferol and taxifolin (dihydroquercetin) likewise tend to occur more in populations south of Sardine Mt., with highest frequency of occurrence in the immature population of *A.procera* from Douglas County, the consolidated population of *A.magnifica* var.*magnifica* from Grizzly Ridge and the immature population from Alpine County. These two compounds were found in 80 to 90 percent of immature trees from the Mt.Shasta/Siskyou County populations and only 20 percent of immature trees. The only other populations that showed this serious a discrepancy between immature and mature trees from the same location for these compounds were those collected from Mt.Tom. A C-glycosylated flavone, rhamnosylvitexin, occurs exclusively in populations north of Sardine Mt. and in 3 of 19 individuals of northern

A.magnifica var.*shastensis* from the North California Coast Range population. A further possible dihydroflavanol occurs only in populations of southern *A.magnifica* var.*shastensis,* one population of *A.magnifica* var.*magnifica* and in two individuals of *A.procera.*

NUMERICAL ANALYSES

Three different approaches to analyzing the coded flavonoid data numerically were used in this study. The first two, principal components analysis (PCA) and cluster analysis (CA) examine interdependence among entries of a non-partitioned data matrix (Green,1978); they make no assumptions about the underlying structure of the data. Both PCA and CA are used to "simplify" a multivariate data set to facilitate interpretation of the variability within it (Green,1978; Sneath and Sokal,1973). Where CA may simplify the data set by "reducing" the number of objects or cases in it, PCA focuses on the variables in the data set. The first step in both procedures is to produce a matrix of correlation coefficients or similarity coefficients; CA uses the correlations between objects or cases and PCA usually uses the correlations between variables. The third approach, discriminant analysis (DA), emphasizes the analysis of independent and dependent structures (Green,1978), and uses a data set defined by subfiles or groupings.

It should be noted that the use of presence/absence data violates assumptions of normality for both the PCA and DA. This occurs because

coded data such as that used for this study represent essentially qualitative characters. However, the use of coded data in numerical taxonomic analyses of this sort has proven itself useful (Sneath and Sokal,1973; Harborne, Mabry and Mabry, 1975).

Principal Components Analysis

Initial examination of a matrix of product-moment correlation coefficients between all 24 variable compounds showed both positive and negative correlations. (Appendix III includes a complete summary of product-moment correlations greater than | 0.40 | .) All of the negative correlations except a correlation of -0.52 between spots 5A and 7 had an absolute value that was smaller than 0.40. Four sets of compounds exhibited correlation coefficients with each other greater than or very close to | 0.50 |. These compounds were :

1. three aglycones that run low in the organic solvent; spots 5A, 7 and 52A.
2. two aglycones that run high in the organic solvent; spots 14 and 15.
3. one aglycone that runs to the middle of the organic front, and two dihydroflavonol glycosides; spots 18, 46 (taxifolin) and 48 (dihydrokaempferol) respectively.
4. three of the acetylated monoglycosides; spots 29 (myricetin), 31 (laricytrin) and 36 (syringetin).

A number of other correlations of less than | 0.50 | but more than | 0.40 | were noted; these included :

1. spots 23A with 5A and 52A; these are all unidentified aglycones that run between Rf=0.10 and Rf=0.49 in the organic solvent;

2. the unidentified dihydroflavonol 45B with 5A, 7, 52A - all aglycones with little mobility in the AQ solvent - and 18, an aglycone with slightly more mobility in the AQ solvent;

3. spot 5A with the acetylated laricytrin, spot 31, and the acetylated syringetin, spot 36;

4. aglycone spot 7 with the acetylated laricytrin and syringetin;

5. the "high-running" aglycone 15 with the acetylated myricetin, laricytrin and syringetin; and

6. the acetylated quercetin, spot 34 with the taxifolin glycoside, spot 46.

Incomplete identification makes interpretation of these correlations difficult. All of these compounds were treated as individual variables; only the acetylated monoglycosides of myricetin, laricytrin and syringetin together and the taxifolin and dihydrokaempferol glycosides together showed correlations of between | 0.72 | and | 0.93 |. These correlations may assist in additional compound identification because different derivatives of a single flavonoid compound tend to co-occur in species of this genus (W.H.Parker,pers.comm.).

The product-moment correlation matrix between variables was calculated and a PCA produced using the SPSS subprogram FACTOR. Using the correlation matrix, PCA constructs a new set of variables that are mathematical transformations of the original data set. A series of mutually orthogonal linear composites are derived from the transformed variables or scores. The first linear composite of scores, called a

component, accounts for the greatest amount of variability in the data; it is the single "best" summary of linear relationships exhibited by the data (Nie*et al.*,1975; Green,1978). Subsequent components account for sequentially less variability until all of the variability in the data set is accounted for. Unless two or more variables are perfectly correlated, the number of components produced will be equal to the number of variables; in this study, 24.

As can be seen from Table 6, it takes 15 components or dimensions to account for 89.1 percent of the variation in the data. Only 25.0 percent and 9.4 percent of the variation in the data was accounted for by the first and second components respectively.

The eigenvalues associated with each linear component represent the amount of variability accounted for by that component. In this study, an eigenvalue of less than 1/24th or 0.0417 is assumed to be due to sampling error or random variation. A common method of reducing the number of components to consider when interpreting the trends in data sets with a large number of variables is to discard components with eigenvalues of less than 1 (Nie*et al.*,1975; Green,1978). If this is done, then 7 components are retained that account for 64.8 percent of the variability in the data (Table 6).

Other taxonomic studies in the genus *Abies* using PCA have found much higher proportions of the variability in the data set accounted for by the first two components. These values ranged from 56.2 percent (33.4 plus 22.8 percent) for cone morphology (Parker*et al.*,1981) to 62.6 percent (43.9 plus 18.7 percent) for flavonoid data (Parker*et al.*,1984) of populations of *A.balsamea* and *A.lasiocarpa*. A study of 19 taxa in the family of the herbaceous Limnanthaceae (Parker and Bohm,1979) reports PCA results more like those found in this study for the *Abies* section

Nobilis , with relatively small proportions of the variability accounted for by all components. This correspondence of PCA results suggests that the section *Nobilis* has more diverse elements than the section *Balsamea* Engelm. (Parker,1976).

TABLE 6. Results of principal components analysis.

COMPONENT	EIGENVALUE	% VARIATION	CUMULATIVE %	
1	5. 998 90	25.0	25.0]	} COMPONENTS
2	2. 260 14	9.4	34.4]	} USED FOR
3	2. 124 25	8.9	43.3]	} GRAPHS
			]	
4	1. 613 32	6.7	50.0]	COMPONENTS
5	1. 328 27	5.5	55.5]	USED FOR
6	1. 162 65	4.8	60.4]	VARIMAX
7	1. 060 78	4.4	64.8]	ROTATION
8	0. 943 86	3.9	68.7	
9	0. 866 17	3.6	72.3	
10	0. 833 81	3.5	75.8	
11	0. 788 99	3.3	79.1	
12	0. 723 91	3.0	82.1	
13	0. 626 23	2.6	84.7	
14	0. 541 12	2.3	87.0	
15	0. 508 10	2.1	**89.1**	
16	0. 453 12	1.9	91.0	
17	0. 419 03	1.7	92.7	
18	0. 377 37	1.6	94.3	
19	0. 371 60	1.5	95.8	
20	0. 294 56	1.2	97.1	
21	0. 286 49	1.2	98.3	
22	0. 216 76	0.9	99.2	
23	0. 141 22	0.6	99.8	
24	0. O59 36	0.2	100.0	
	24. 000 01			

To facilitate interpretation of the relationships between the variables, PCA was supplemented by rotation of these 7 components using the rotation method VARIMAX supplied by the SPSS subprogram FACTOR. VARIMAX maximizes the variance of the squared loadings or eigenvalues in each column of a matrix of correlation coefficients between the original variables and the principal components. When this is done, the first rotated dimension does not necessarily account for the greatest amount of variance, so it is no longer a principal component, it is simply a factor (Green,1978, p.379). However, all variables then load highly on only one factor, which helps to simplify assessment of the variables.

The three acetylated flavonols, myricetin, laricytrin and syringetin account for most of the variability in the rotated factor 1. The upper aglycone number 15 and rhamnosylvitexin (spot 47) have the next highest loadings on this factor. Referring back to the frequency histograms for the variable compounds, these are all compounds that tend to occur more in populations of *A.procera* and/or northern *A.magnifica* var.*shastensis* (Figures 5.A, 5.C and 5.E).

The lower aglycones 52A, 5A, 23A, and 7, and the dihydroflavonol 45B account for most of the variability in factor 2; they tend to occur more in populations south of Sardine Mt. or typical *A.procera*, especially in the southern populations of *A.magnifica* var.*shastensis* (Figures 5.D, 5.F and Appendix I).

The taxifolin glycoside 46, the dihydrokaempferol glycoside 48, the quercetin acetyl 34 and the upper aglycone 18 account for most of the variability in factor 3; they likewise tend to occur in populations south of Sardine Mt., with highest frequency of occurrence in one population of *A.procera* and two of *A.magnifica* var.*magnifica* (Figures 5.B, 5.E and Appendix I).

Upper aglycones 13, 16, 14 and 15 account for most of the variability in factor 4; they tend to occur in populations of *A.procera* and northern *A.magnifica* var.*shastensis* (Figure 5.C and Appendix I). Aglycones 6, 8A and 2 account for most of the variability in factor 5 (Figure 5.D and Appendix I); aglycone 19 in factor 6 (Appendix I); and aglycone 53 and the quercetin acetyl (spot 34) in factor 7 (Figure 5.B).

PCA results cannot be applied directly to observing relationships between groups of objects because PCA is a numerical technique that explores the relationship between variables. To help visualize the relationships between populations based on the PCA of variables, the first three original component eigenvalues or loadings were converted to component scores using standardized character states. The first component accounted for 25.0 percent of the total variability, the second component accounted for 9.4 percent, and the third for 8.9 percent (Table 6). The standardized components are referred to as axes and the standardized component scores are used as co-ordinates to draw 2-dimensional graphs. PCA graphs or ordinations are particularly useful when most of the variation is accounted for in the first two or three components/factors/axes. Even though this was not the case in this study, a few interesting points were highlighted.

Plots of all individuals (Figure 6) produced fairly random scatters with a slightly ellipsoid shape. A number of individuals overlapped each other, with perhaps 12 separate concentrations of individuals; sample size made it difficult to identify concentrations as populations. Three plots - axis 1 versus axis 2, axis 1 versus axis 3 and axis 2 versus axis 3 - were drawn for the 21 mature and immature populations and the same three plots for the 11 consolidated populations (Figures 7 and 8). Each figure shows

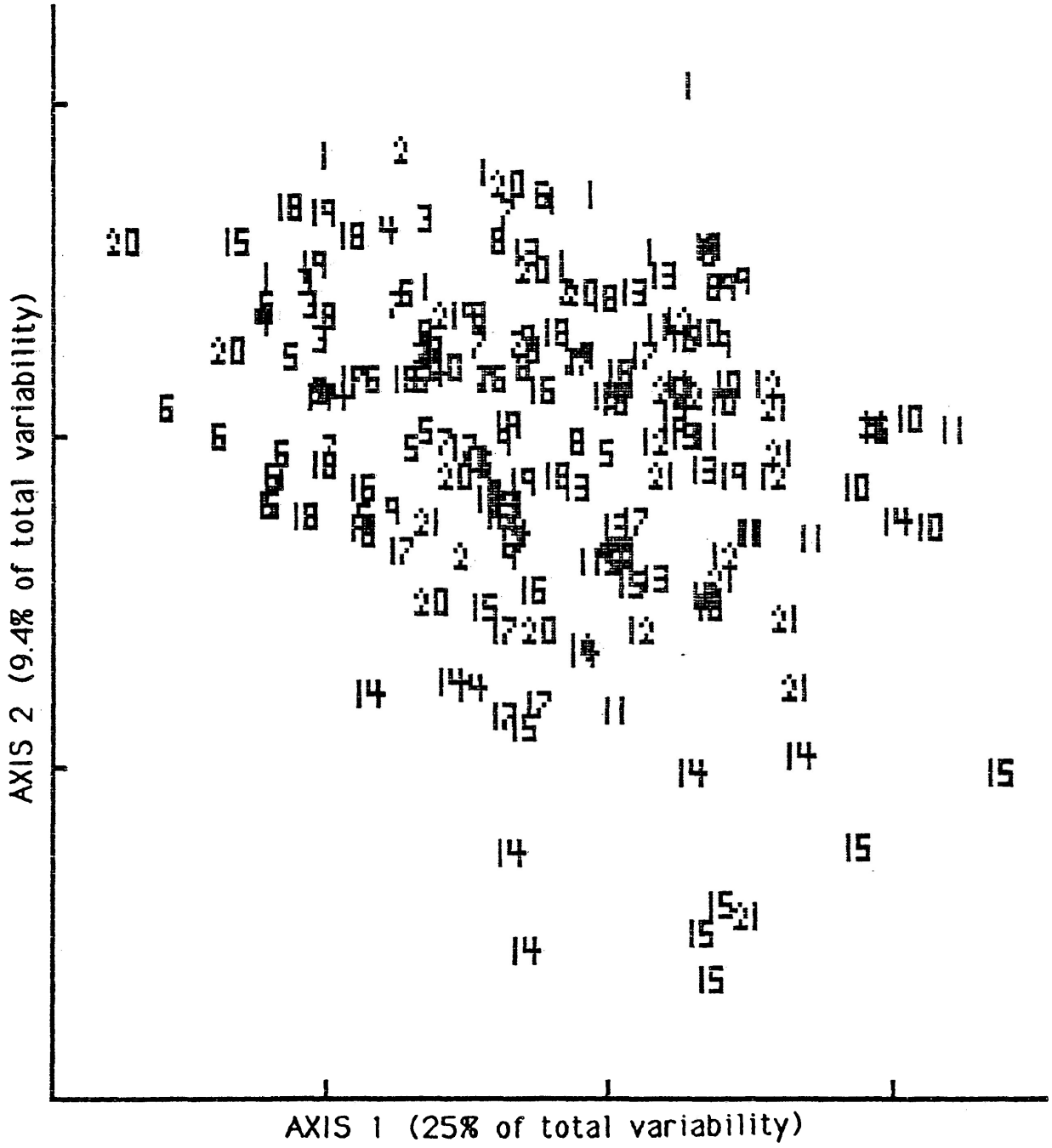


FIGURE 6.A. Principal Components Analysis scatterplot of individuals, axis 1 versus axis 2, as identified by population codes. Population codes are in Table 5.

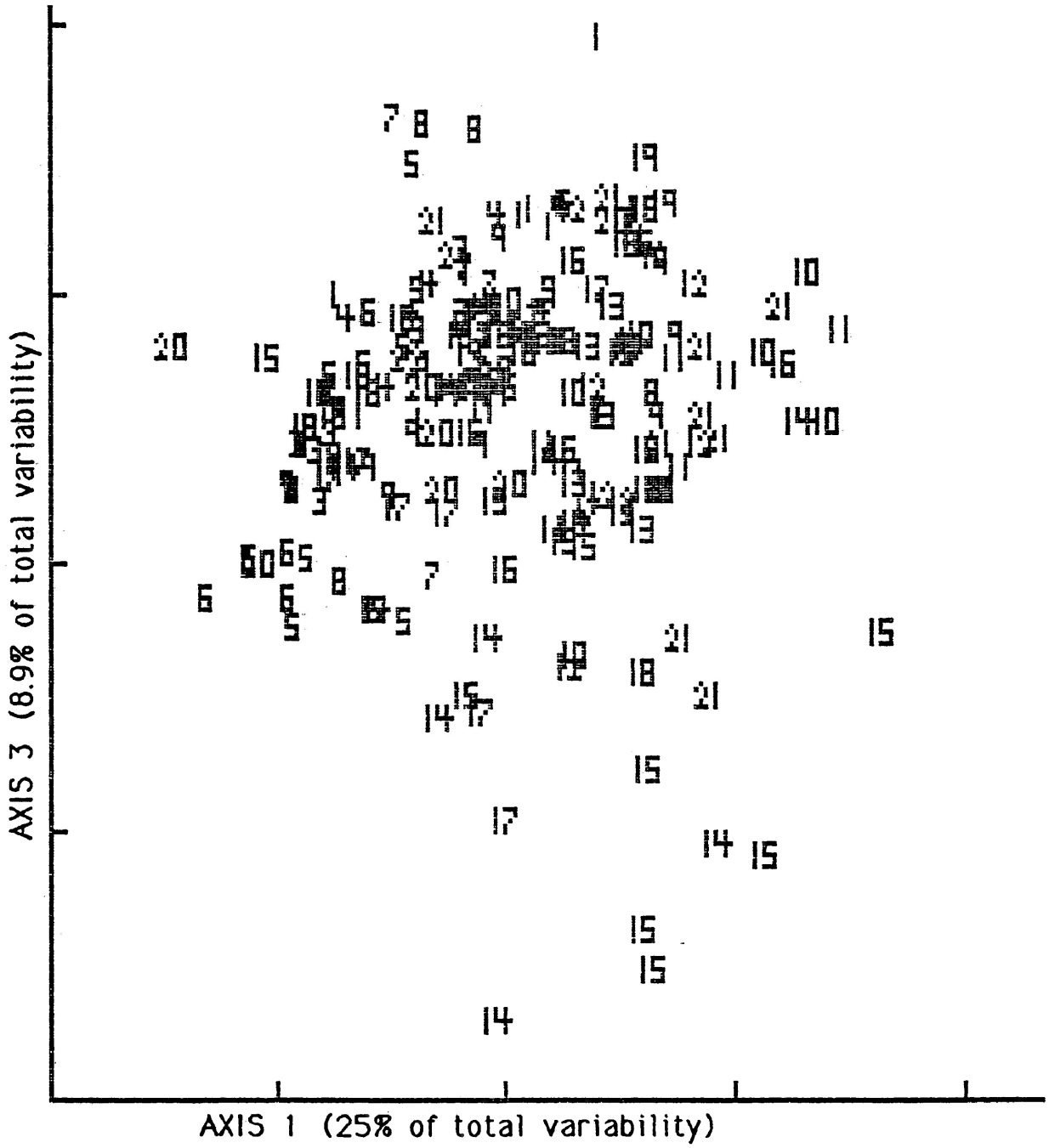


FIGURE 6.B. Principal Components Analysis scatterplot of individuals, axis 1 versus axis 3, as identified by population codes. Population codes are in Table 5.

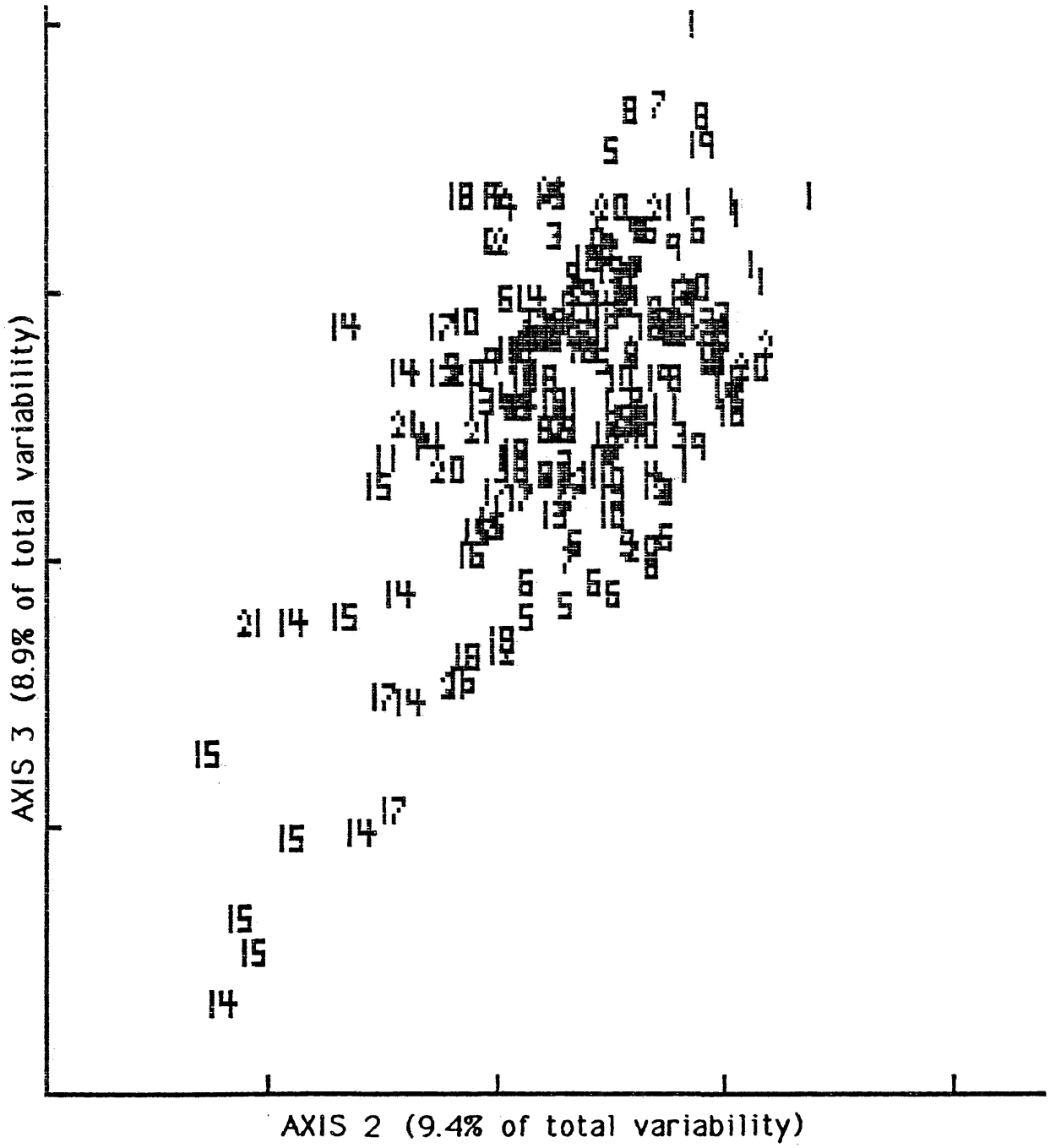


FIGURE 6.C. Principal Components Analysis scatterplot of individuals, axis 2 versus axis 3, as identified by population codes. Population codes are in Table 5.

population centroids and standard deviations along each axis for each population. The 21 populations tended to cluster fairly tightly, with the centroids of populations 9R and 10R - Grizzly Ridge regeneration and Alpine County, respectively - offset together from the rest of the populations. The position of these two immature populations on the PCA ordinations is probably taxonomically insignificant. The one to one correlation of the second and third axes in Figure 7C suggests a clinal pattern of variation because uncorrelated components are apparently correlating.

Further interpretation is difficult because of standard deviation overlap along all three axes. However, the populations of *A.procera* and *A.magnifica* tend to group at opposite sides of the ordinations with populations of northern *A.magnifica* var.*shastensis* from Mt.Ashland and Mt.Shasta in between. The population of southern *A.magnifica* var.*shastensis* appears to group with the typical variety.

The plots for the 11 consolidated populations were a little clearer (Figure 8), although standard deviations were larger. *A.magnifica* var.*magnifica* consolidated population 9, Alpine County, was consistently offset on all axes, and clustered loosely with the other two *A.magnifica* var.*magnifica* populations and the Klamath region northern *A.magnifica* var.*shastensis* population 7. This semi-isolation of an immature population may not be taxonomically significant. Consolidated populations 1, 2, 3, 4 and 5 - and to a lesser extent 6 - tended to cluster closely together; these are the populations of *A.procera* and two of the three populations of northern *A.magnifica* var.*shastensis*. Population 11, the southern *A.magnifica* var.*shastensis*, clustered with this group on all three graphs. Again, overlap between populations within this group, as indicated by overlap of

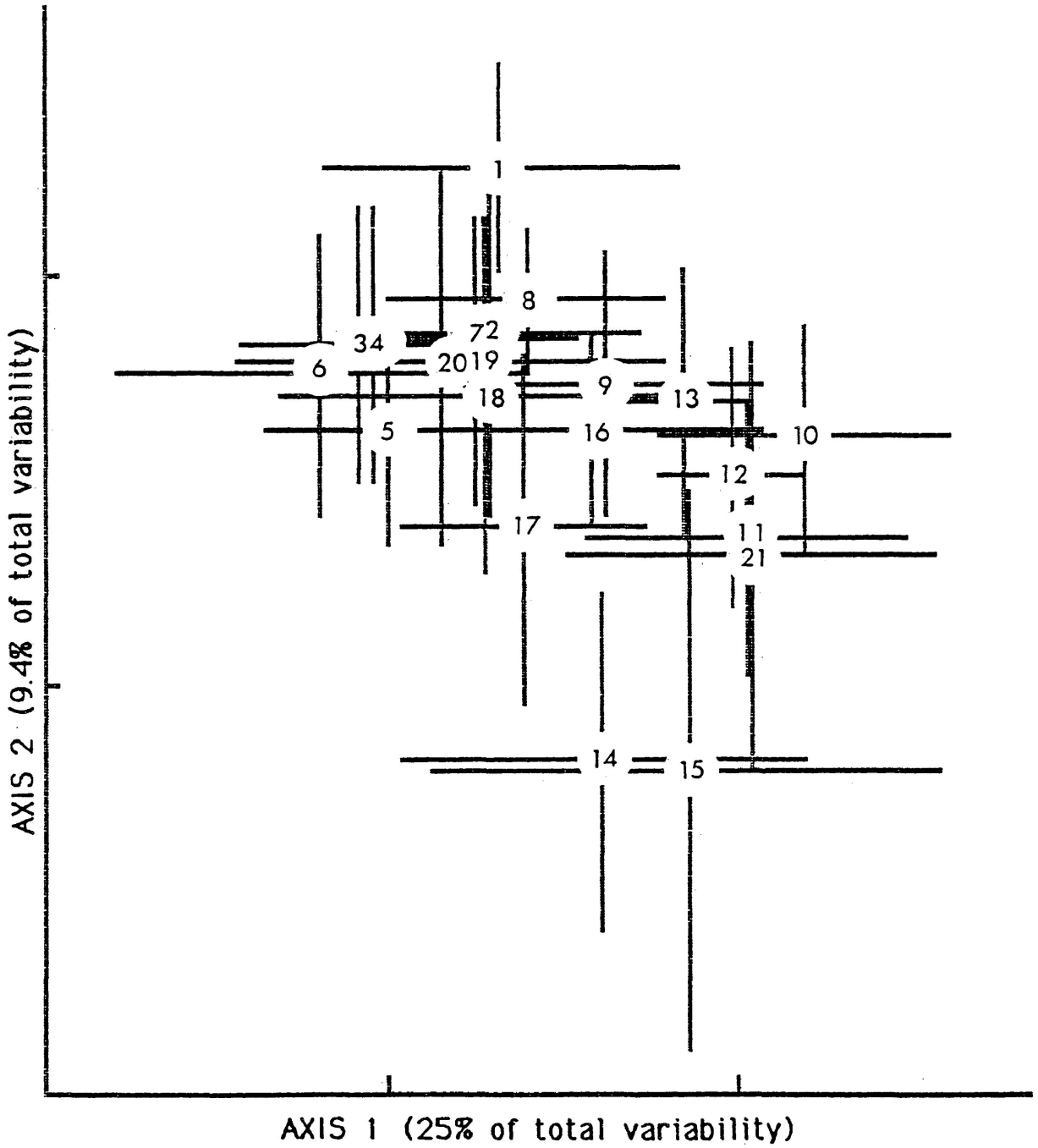


FIGURE 7.A. Principal Components Analysis ordination of 21 unconsolidated populations, axis 1 versus axis 2. Population codes are in Table 5.

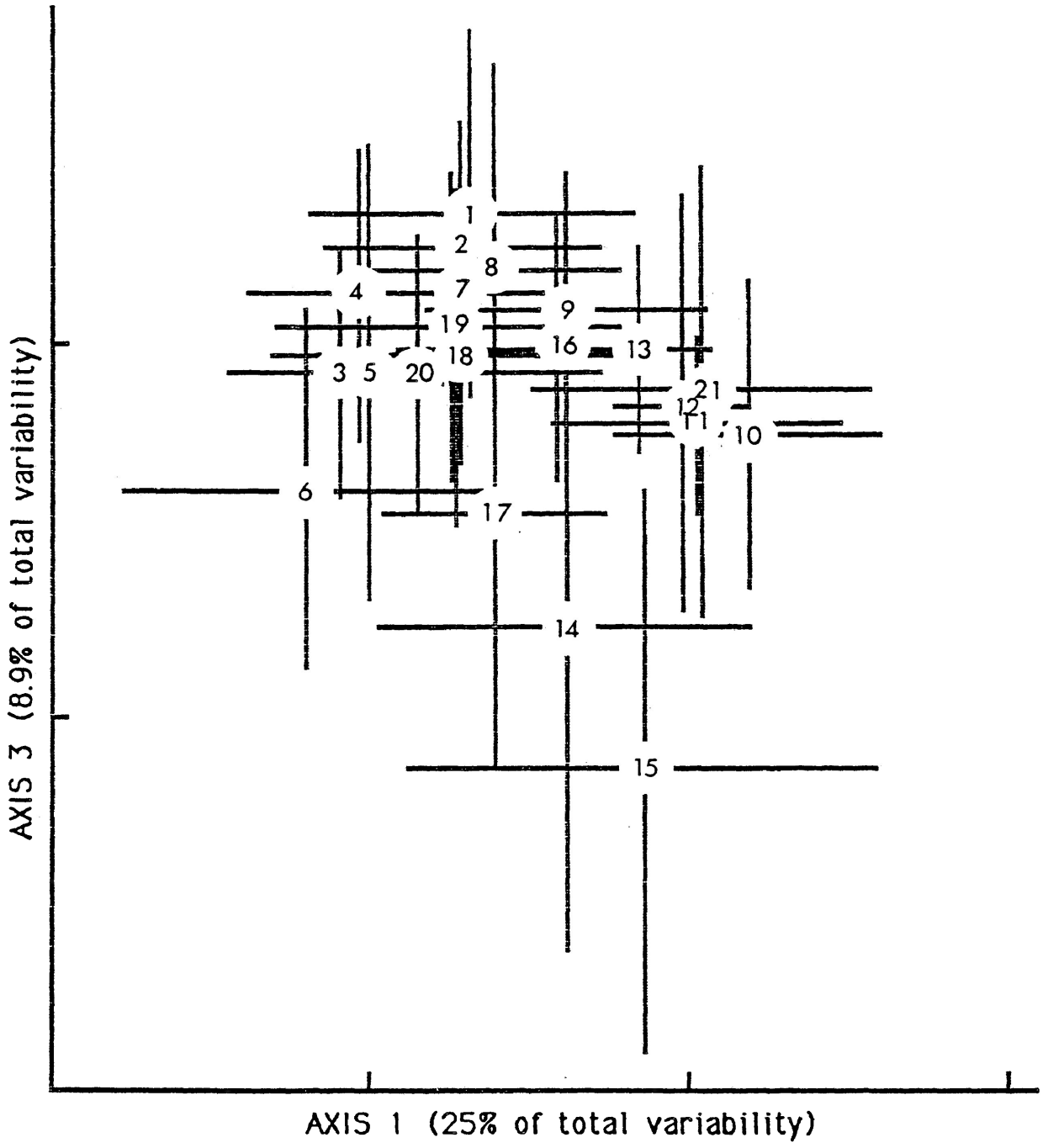


FIGURE 7.B. Principal Components Analysis ordination of 21 unconsolidated populations, axis 1 versus axis 3. Population codes are in Table 5.

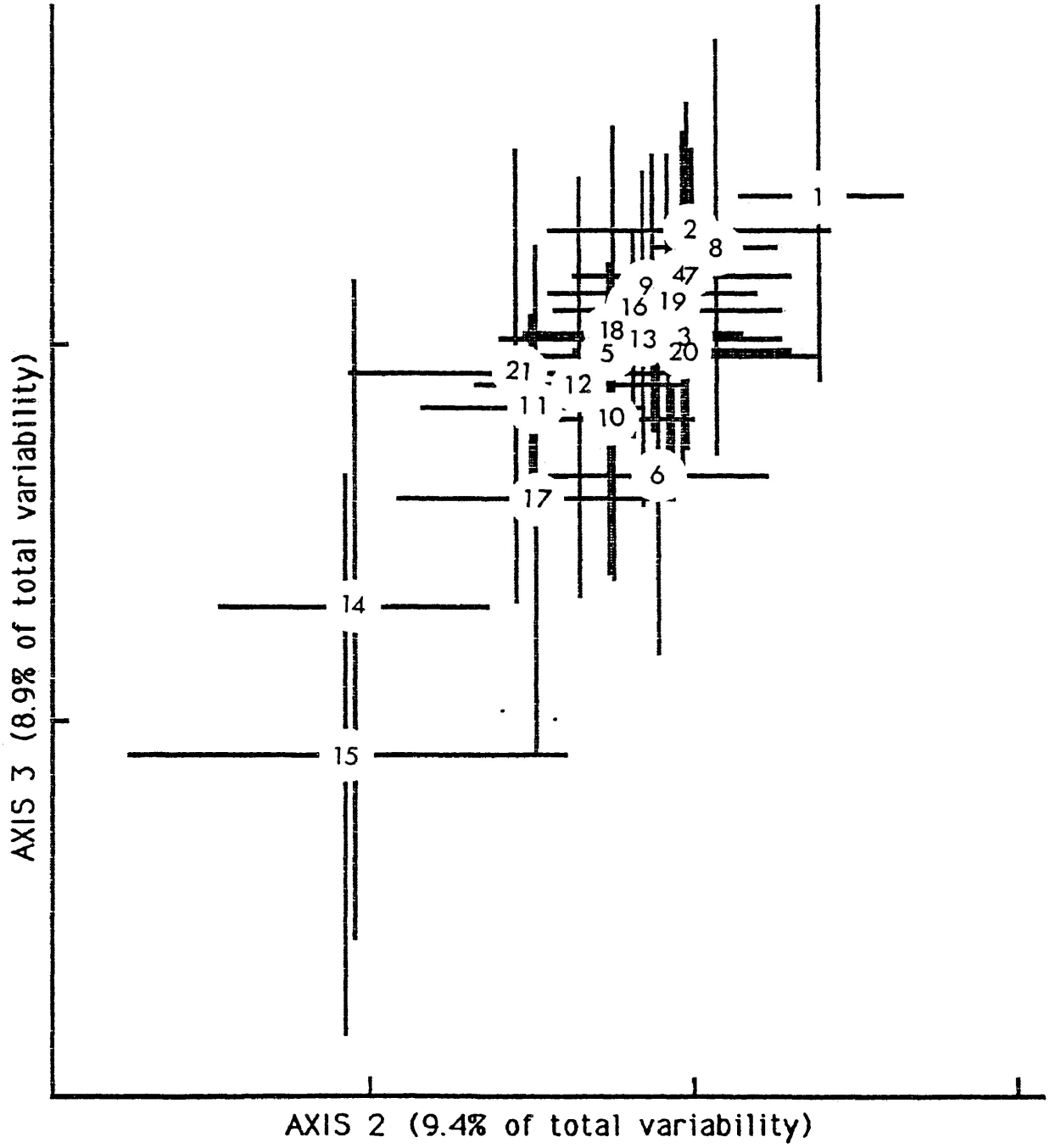


FIGURE 7.C. Principal Components Analysis ordination of 21 unconsolidated populations, axis 2 versus axis 3. Population codes are in Table 5.

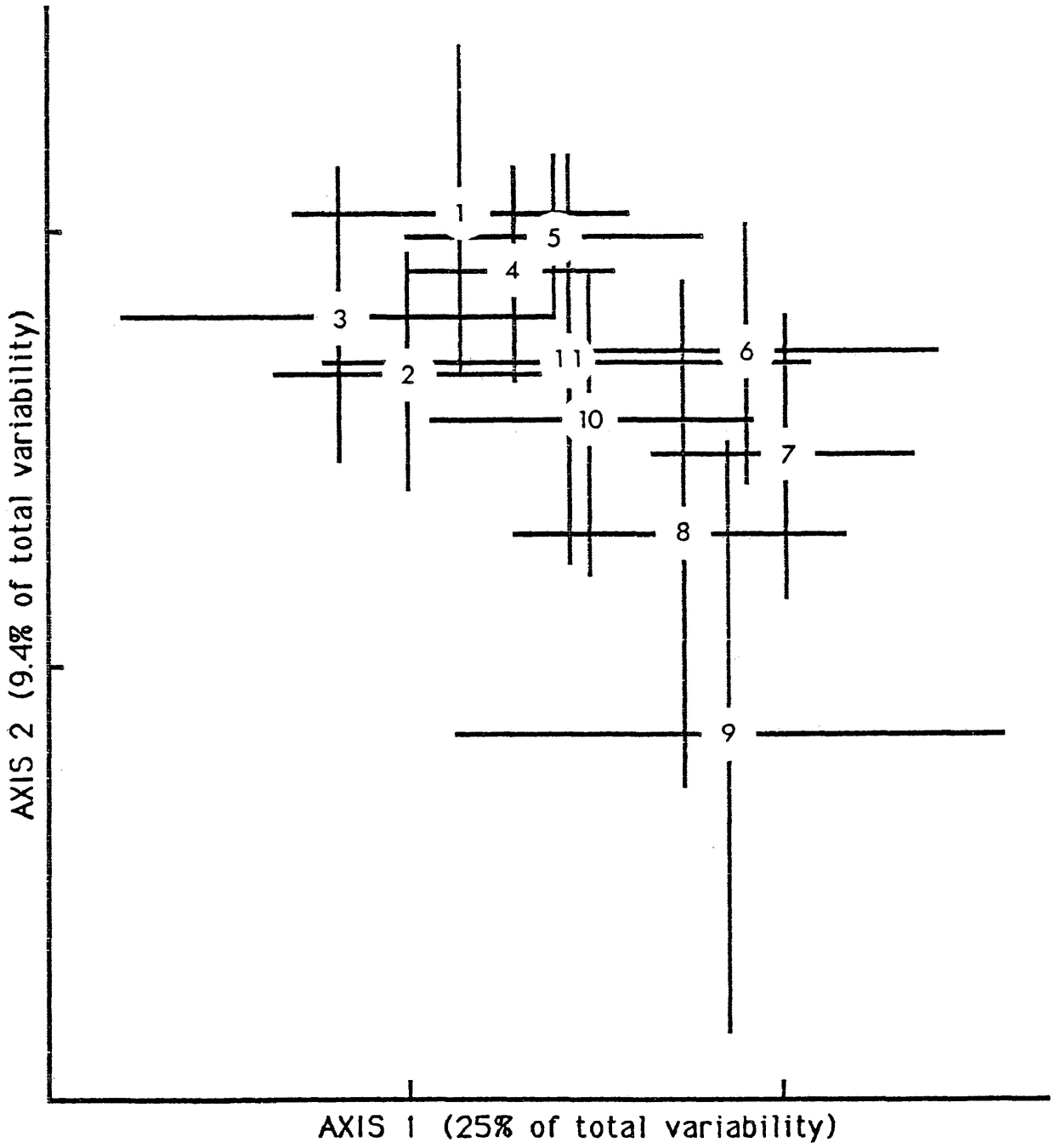


FIGURE 8.A. Principal Components Analysis ordination of 11 populations consolidated by location, axis 1 versus axis 2. Population codes are in Table 5.

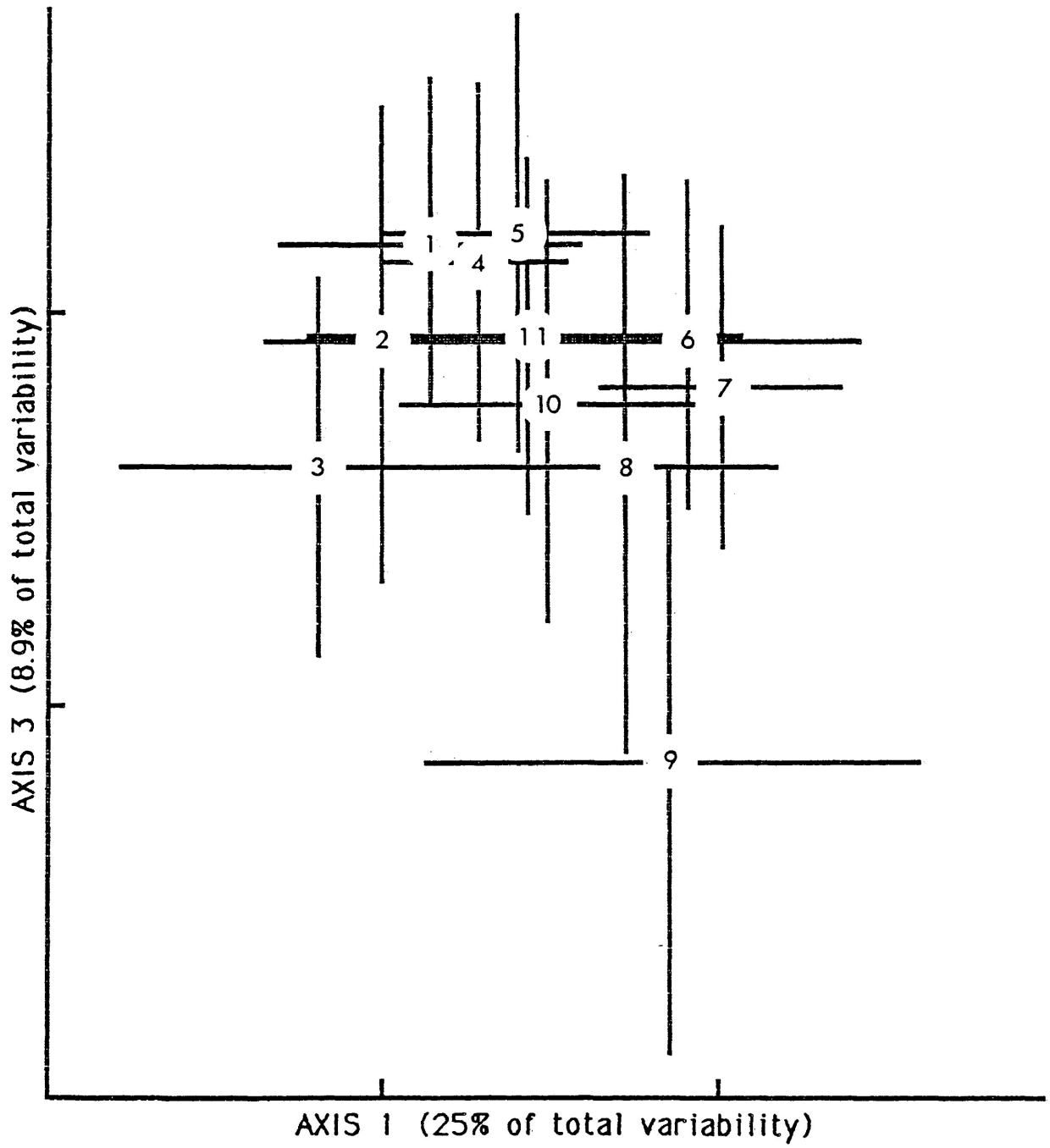


FIGURE 8.B. Principal Components Analysis ordination of 11 populations consolidated by location, axis 1 versus axis 3. Population codes are in Table 5.

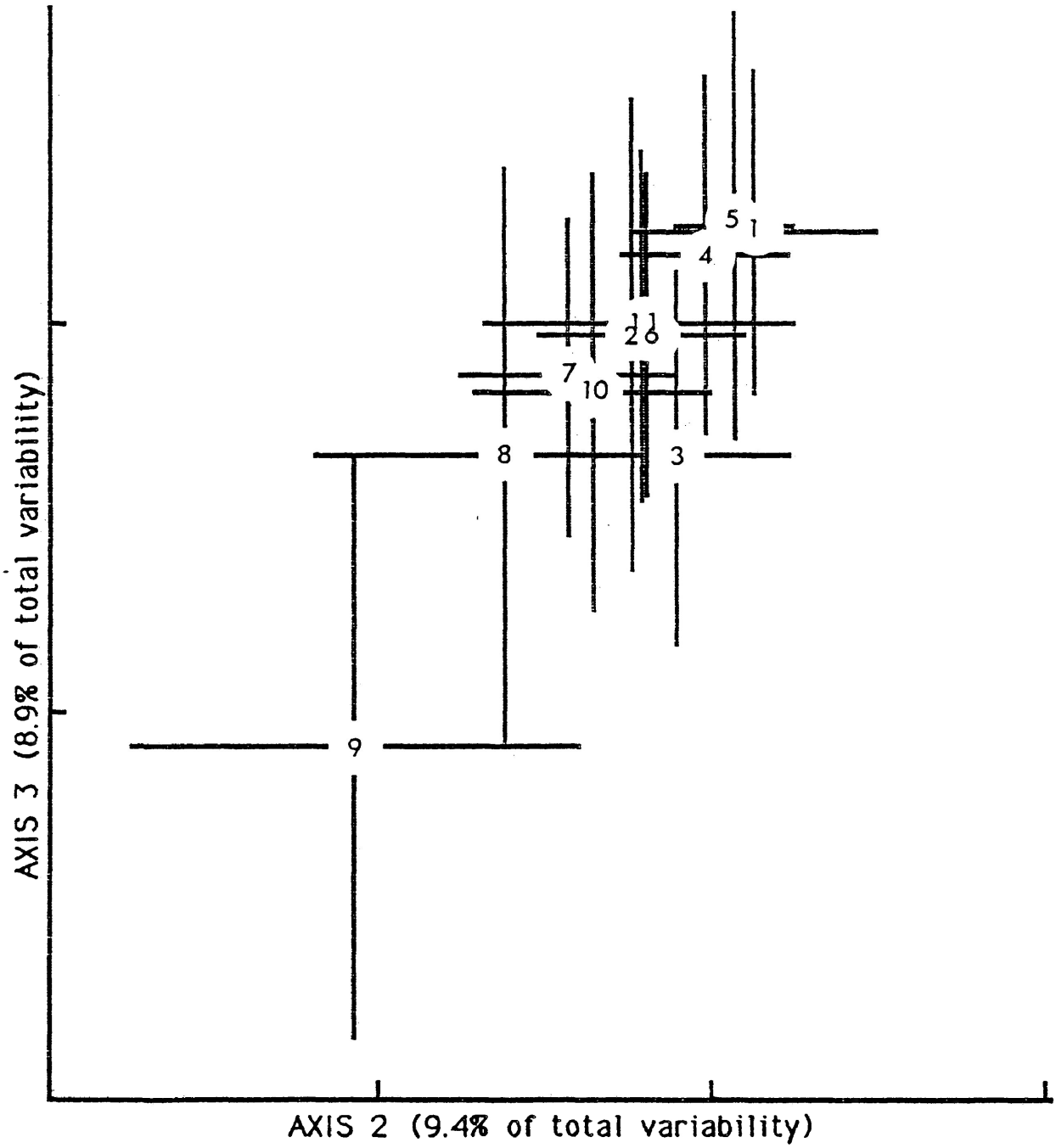


FIGURE 8.C. Principal Components Analysis ordination of 11 populations consolidated by location, axis 2 versus axis 3. Population codes are in Table 5.

standard deviations around population centroids was extensive. However, populations of *A.procera* tended to have less within-population variation than the others.

In summary, correlations between 19 of 24 compounds were not high enough to justify discarding any of the variables. PCA results of flavonoid data suggested that consolidated populations of *A.procera* tended to be less variable than consolidated populations of the other three taxa. Also, the consolidated population of southern *A.magnifica* var.*shastensis* appeared to group closer to *A.procera* and northern *A.magnifica* var.*shastensis* than to typical *A.magnifica.*

Cluster Analysis

Cluster analysis (CA) may simplify the interpretation of a large set of data by sequentially reducing the number of cases or objects in that data set. This "reduction" is achieved by grouping the objects into clusters on the basis of similarities or correlations. CA is a classic taxonomic tool because it leads to the construction of hierarchically- linked dendrograms that follow the same format as most modern taxonomic classifications. In general, however, it is not possible to test the statistical significance of a given cluster (Green,1978, p.431), so the reliability of the technique is not demonstrable. It is also not always possible to fit the clusters to classical taxonomic units like section, genus, species or variety, especially if one is dealing with variation at the lower levels of classification.

Green(1978) suggests that cluster analysis be used to formulate rather than to test a classification. He recommends using techniques like discriminant analysis to assign new members to groups perhaps originally formulated by a cluster analysis that has been supplemented by further

research. With this recommendation in mind, the results of the cluster analysis of the flavonoid data in this study serve to illuminate rather than to define the pattern of variation in the *Abies* section *Nobilis* species complex.

Sneath and Sokal(1973) recommend referring to the cases or objects subjected to CA as operational taxonomic units (OTU's). The OTU for a given study is the object of lowest taxonomic rank. In this study, because of the memory limitations of the microcomputer used for the cluster analysis, the OTU was a population, either consolidated (11 populations) or not consolidated (21 populations) by location. The data set consisted of average character states for each of the 24 flavonoid variables taken over each of either 11 or 21 populations (Apendices I and II).

The clustering algorithm applied to the flavonoid data in this study used correlations rather than distance measures, and was based on the weighted pair-group method (WPGM) as described by Sokal and Sneath(1963,Appendix A.3). The first step in the CA, as in the PCA, was the computation of a matrix of product-moment correlations. However, the correlations in CA are between OTU's for all 24 variables rather than between variables for the 193 individuals. The "mutually highest correlation" (Sokal and Sneath ,1963) or the correlation between any two OTU's which is higher than the correlation of these OTU's with any other OTU, is found first. These two OTU's form the first cluster. This step is taken for any pair-group clustering method; it is a pairwise comparison. Pairwise comparisons are the best method of cluster admission when variable populations rather than populations or single OTU's with relatively low amounts of differentiation are used (Sneath and Sokal,1973,p.216).

In weighted clustering, a correlation matrix is then recalculated

using the remaining OTU's, and the OTU most highly correlated to the first cluster is allowed to join that group. Each successive cluster added has the same weight as the whole previous cluster it is added to. In unweighted clustering, the original correlation matrix is used to determine which OTU or cluster is added at each step. Weighted clustering is preferred to unweighted when sample sizes are very different, as is the case in this study. Given disparate sample sizes, weighted clustering results in a less biased estimate of intercluster distance because it weights smaller samples or populations more than they would be weighted otherwise (Sneath and Sokal,1973,p.228). It should be noted that average distances between clusters are higher than they would be if unweighted clustering were used.

Successive correlations are generally lower, which yields the hierachical form of the resultant dendrogram. Occasionally a small inversion in the correlations can take place; the correlation of a new cluster with the next potential cluster or OTU to be added may be higher than the correlation between the immediately preceeding cluster and the next potential cluster. This may be due to unequal cluster size, and will not be considered a problem for the purposes of this study since the inversions apparent in the dendrograms are small (Sokal and Sneath,1963; Sneath and Sokal,1973).

The initial dendrograms for the two WPGM cluster analyses performed on the flavonoid data are reproduced in Figures 9 and 10. Figure 9 shows the dendrogram from the cluster analysis performed on the 21 immature and mature populations of the *Abies* section *Nobilis*. Two large groups that are negatively correlated to 0.699 result. One contains all of the populations of *A.procera* and two of the five populations of northern *A.magnifica* var.*shastensis*, as well as three of

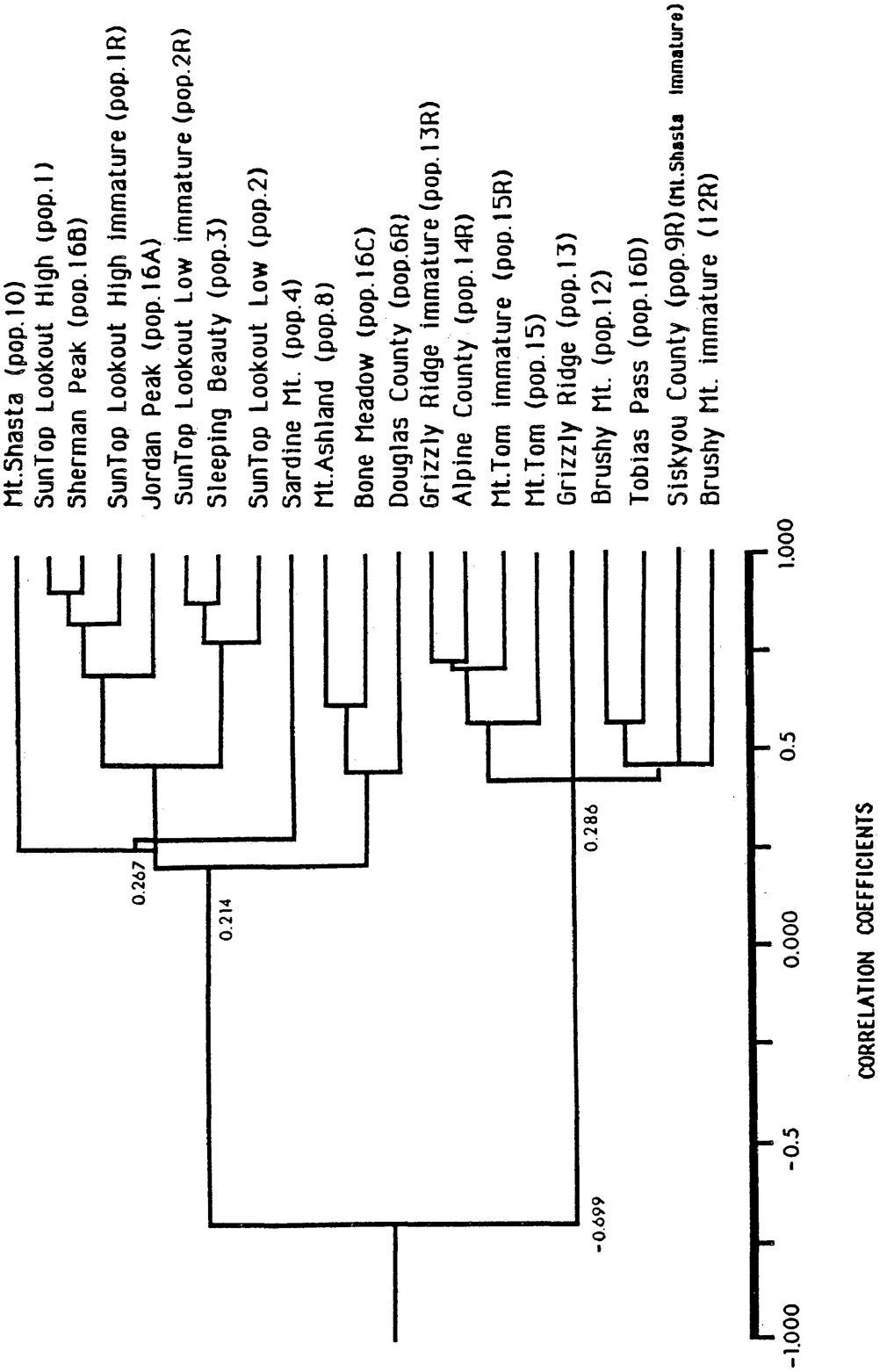


FIGURE 9. Dendrogram illustrating the results of the Weighted Pair-Group Method cluster analysis of flavonoid data for 21 nonconsolidated populations.

the four populations of southern *A.magnifica* var.*shastensis*. The other group contains all of the populations of *A.magnifica* var. *magnifica*, the regeneration population collected from Mt. Shasta and the Brushy Mt. populations from western California, all of which are populations of northern *A.magnifica* var.*shastensis* ; as well as the remaining two populations of southern *A.magnifica* var.*shastensis*.

Within the *A.procera* grouping, four clusters are evident :

1. Mt. Shasta mature population 7, which is initially isolated, joins the *A.procera* group at a low correlation of +0.267;
2. Sardine Mt. mature population 4, which joins the *A.procera* group at about the same low level of correlation as Mt. Shasta (+0.286), also sits by itself - this is where the only "reversal" in the dendrogram occurs - Sardine Mt. is slightly more highly correlated with the other northernmost population of *A.procera* and two populations of southern *A.magnifica* var.*shastensis* when the Mt. Shasta mature population is added to that group;
3. Mt. Ashland northern *A.magnifica* var.*shastensis* mature population 6, which may contain two of the operational taxa (W.H.Parker,pers.comm.) and Bone Meadow southern *A.magnifica* var.*shastensis* immature population 12C join together at a correlation of +0.623; and Douglas County immature population 5R, which consists of *A.procera*, clusters with them at a correlation of about +0.458;
4. SunTop Lookout Low, both immature and mature populations (2,2R), and Sleeping Beauty (3) all of which are northernmost populations of *A.procera*, cluster together at a fairly high correlation of +0.784; the two southern *A.magnifica* var.*shastensis* populations Sherman Peak (12B) and Jordan Peak (12A) cluster closest to SunTop

Lookout High at correlations above +0.705; these two groups join together at a correlation of +0.476.

Three clusters are evident in the *A.magnifica* var.*magnifica* grouping :

1. the Grizzly Ridge mature population 9 of *A.magnifica* var.*magnifica* stands alone; it joins the rest of the group at a fairly low correlation of +0.432;

2. the Brushy Mt. mature population 8 and the southern *A.magnifica* var.*shastensis* population 12D from Tobias Pass join together at a correlation of +0.575; they join the Mt. Shasta regeneration population 7R (Siskyou County) and the Brushy Mt. regeneration population 8R, both populations of northern *A.magnifica* var.*shastensis*, at correlations of +0.467 and +0.461 respectively;

3. the *A.magnifica* var.*magnifica* populations Mt. Tom (11,11R), Alpine County (10R) and the Grizzly Ridge regeneration (13R) cluster together at correlations above +0.578. The regeneration populations in this cluster grouped closer together than the Mt. Tom regeneration population did with its companion mature Mt. Tom population.

Figure 10 reproduces the results of the cluster analysis of the 11 consolidated populations. This figure is a little easier to interpret because differences between immature and mature populations are removed. Segregation of the populations into the same two large groupings is again evident, with the stems of the two clusters joining at a slightly greater negative correlation of 0.789, compared to when the larger number of OTU's was used.

Looking again first at the group including *A.procera*, both of the

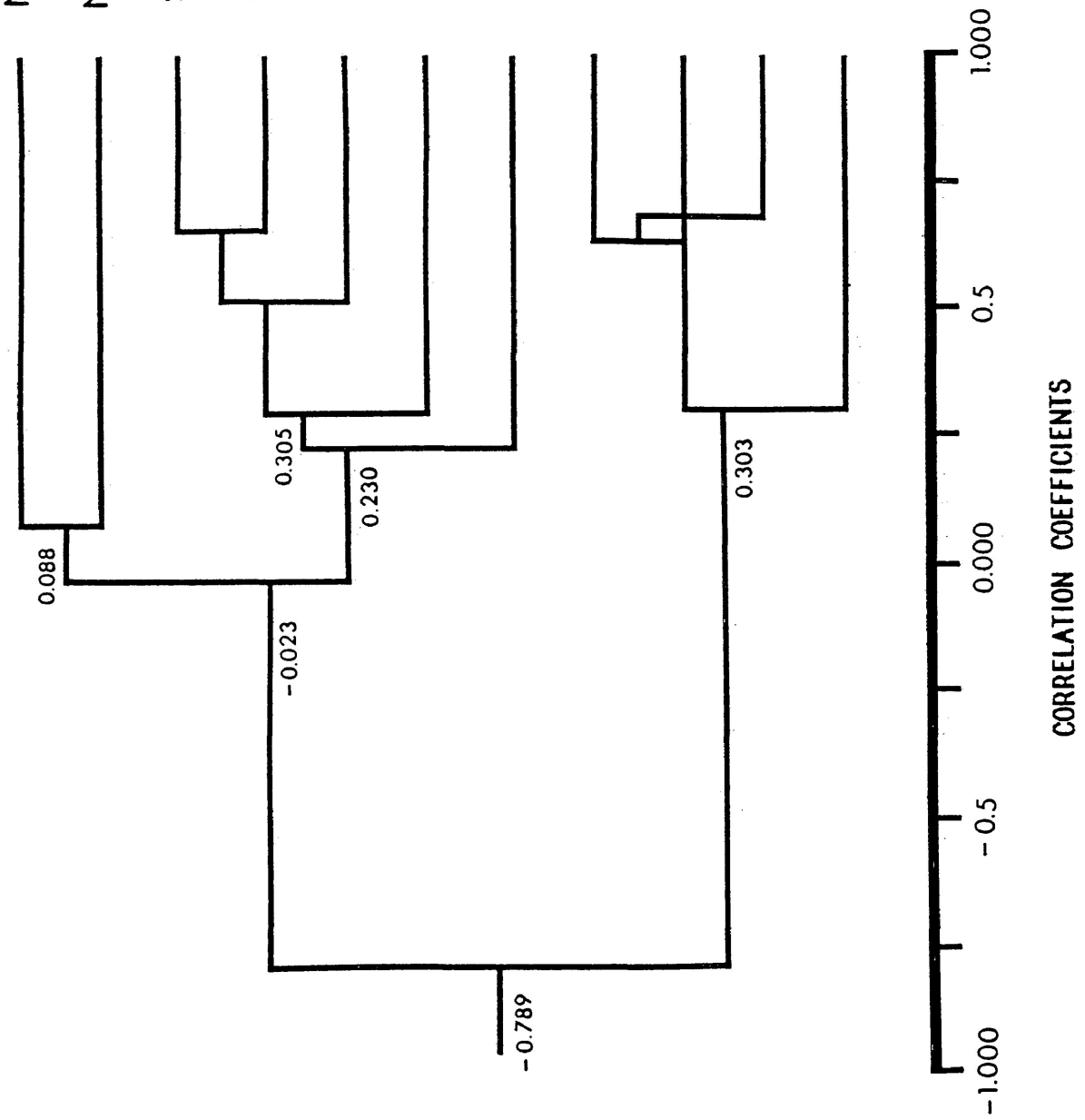


FIGURE 10. Dendrogram illustrating the results of the Weighted Pair-Group Method cluster analysis of flavonoid data for 11 populations consolidated by location.

Klamath Mts. populations of northern *A.magnifica* var.*shastensis* cluster together, and are distinct both from each other and from the other the four populations of southern *A.magnifica* var.*shastensis*. The other group contains all of the populations of *A.magnifica* var. *magnifica*, the regeneration population collected from Mt. Shasta and the Brushy Mt. populations in this group. These two populations cluster together at a correlation of +0.088, and the two join the rest of the group at a correlation of -0.023. SunTop Lookout and the consolidated populations of southern *A.magnifica* var.*shastensis* from Tulare County cluster together at a fairly high correlation (+0.662), followed by Sleeping Beauty, Douglas County and finally Sardine Mt. at successively smaller positive correlations.

In the other major grouping that consists mostly of populations of *A.magnifica* var.*magnifica*, the Brushy Mt. population of northern *A.magnifica* var.*shastensis* from the Coast Ranges of California joins the other three populations at a correlation of +0.303, close to the level that Sardine Mt. joins its first cluster. The *A.magnifica* var.*magnifica* populations of Mt. Tom, Alpine County and Grizzly Ridge cluster closest together of all of the populations, at a correlation of +0.680. This is where the only "reversal" in this dendrogram takes place; Grizzly Ridge and Alpine County cluster together at a correlation of +0.647, and Mt. Tom is added at the slightly higher correlation.

In summary, the four populations of southern *A.magnifica* var.*shastensis* are divided, grouping with a cluster consisting mostly of populations of *A.procera* and a cluster consisting mostly of populations of *A.magnifica* var.*magnifica*. When consolidated, the four populations

of southern *A.magnifica* var.*shastensis* cluster with *A.procera* at a fairly high level of correlation. The California Coast Range population of northern *A.magnifica* var.*shastensis* (Brushy Mt.) clusters with populations of *A.magnifica* var.*magnifica*, but the other populations of northern *A.magnifica* var.*shastensis* tend to cluster with *A.procera*. These three populations each appear to be much more distinct from the larger clusters than is the population of southern *A.magnifica* var.*shastensis*. Finally, the Sardine Mt. and Douglas County populations of *A.procera* are slightly different from the other *A.procera* populations, clustering closer to the populations of northern *A.magnifica* var.*shastensis*. This last point is in accordance with the work of Zavarin*et al.*(1978), which found "transitional populations" south of about 44 degrees N latitude.

A final supplementary cluster analysis was performed on all of the populations consolidated by their operational taxon identification into four OTU's - *A.procera*, northern *A.magnifica* var.*shastensis*, *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* (Figure 11). Southern *A.magnifica* var.*shastensis* clustered closest with *A.procera* ; *A.magnifica* var.*magnifica* and northern *A.magnifica* var.*shastensis* also clustered together at a lower correlation. These two large clusters joined at a very high negative correlation of 0.966. A negative correlation of this magnitude is unusual, but may be partly explained by the use of coded data. Sokal and Sneath(1963) note that standardized data tends to show negative correlations. While the data for cluster analysis in this study were not standardized, the data matrix used for the cluster analysis was recoded to 0(absent) and 1(present in more than trace amounts). In the case of a large number of present/absent characters delimiting clusters, one cluster

could be considered "the opposite" of another, which leads to very high negative correlations not normally expected for biological data.

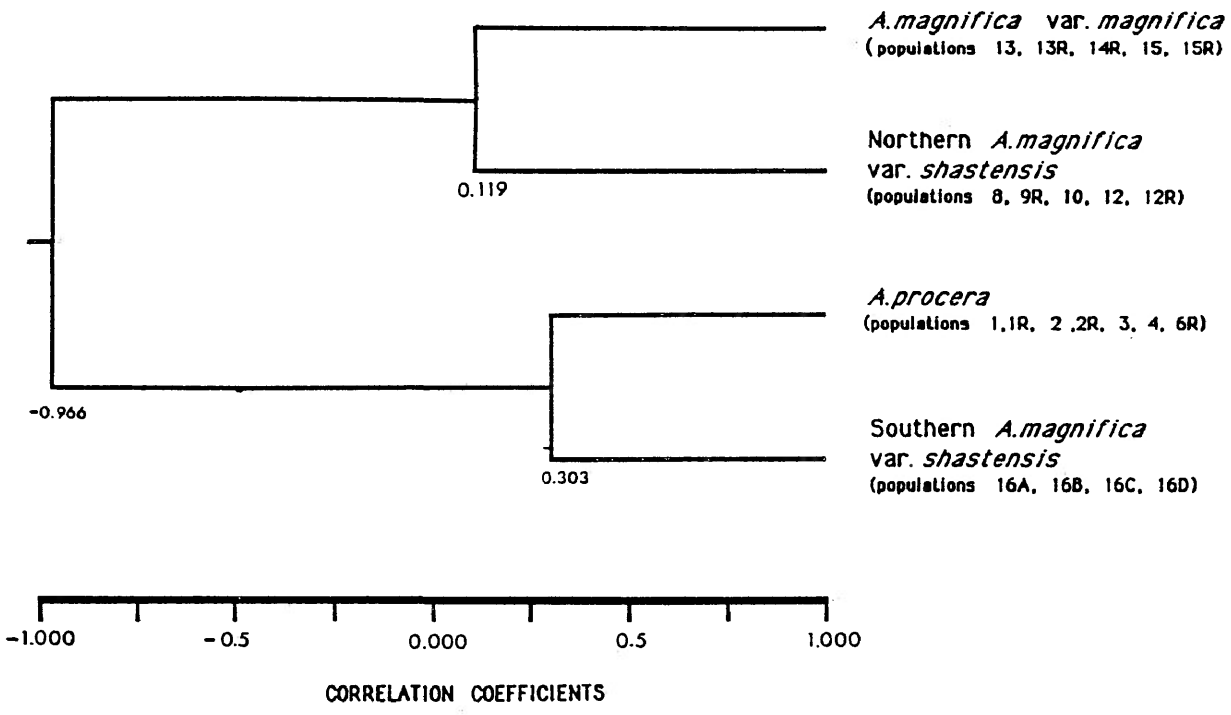


FIGURE 11. Dendrogram illustrating the results of the Weighted Pair-Group Method cluster analysis of flavonoid data for the four "operational" taxa of the *Abies* section *Nobilis*.

Discriminant Analysis

Discriminant analysis (DA) is an analytical tool that is used to find linear composites of predictor variables, which in this study are 24 flavonoid compounds; these linear composites maximize among-group versus within-group variability (Nei*et al.*,1975; Green,1978). As in PCA, each composite or axis is uncorrelated with previously-obtained axes, and each composite accounts for successively less variation. For a given analysis, the number of axes possible is equal to one less than the number of subgroups that the data set is partitioned into. These composites are

used to test whether or not the group centroids, the multivariate equivalents of sample mean, are significantly and for practical purposes different, and for how many of the discriminant axes.

In addition, DA can be used to assign new objects to the appropriate group, and can also determine which variables contribute most to discrimination among those groups. A measure of the discriminating power of the analysis and the reliability of its classification is the percentage of cases or individuals placed into the group they actually belong to. In this study, as an alternative to assigning new objects to appropriate groups, all of the individuals were classified according to the discriminant axes derived from the flavonoid data into the population or group each resembled most, and the pattern of correct/incorrect classification was used to look at the relationships between populations or groups.

The statistical theory behind DA assumes that the discriminating variables have a multivariate normal distribution and that the variance-covariance matrices between each group are equal. If the variance-covariance matrices are unequal, a bias occurs in the test for equality of group centroids, so that classification based on the discriminant axes tends to assign too many cases to the groups with larger variance-covariance matrices because they contribute more to the pooled within-groups covariance matrix (Green,1978,p.170). Nei*et al.* (1975,p.435) feel that the technique of DA presented in the SPSS subprogram DISCRIMINANT is very robust, so that the assumptions of normality and equal variance are not essential, and they have not been tested rigorously in this study. In addition, scatterplots of the cases by group for the first two discriminant functions all showed basically the random pattern indicative of normal distribution.

Two different kinds of DA were performed using the SPSS DISCRIMINANT subprogram, stepwise and direct. In the stepwise procedure, the independent or predictor variables are not all entered into the analysis together as they are in the direct procedure. The variable which is the most useful in discriminating between the partitioned groups is chosen for entry into the analysis first. Each of the remaining variables is sequentially judged in the same way, until an optimal set of variables is obtained. The criterion used in this study to determine whether or not a variable would be added to the variable set was the maximization of the multivariate F-ratio for a test of differences among group centroids. Maximization of the multivariate F-ratio minimizes Wilks' lambda, which is a measure of group discrimination. A stepwise procedure should be performed prior to the actual DA when correlation between variables is present, as is the case in this study.

Of the 24 variables in the data set, only 3 were consistently left out of the stepwise analysis - the lower aglycone 8A, the upper aglycone 16 and the acetylated laricytrin, spot 31 (Appendix IV.C). The number of groups or populations that the data set was partitioned into affected the order of entry of the variables; however the acetylated syringetin (spot 36) and the lower aglycone 5A were consistently chosen first and second respectively. Other variables with high discriminating power were lower aglycone 6, upper aglycone 13, the acetylated myricetin (29), the acetylated quercetin (34), the unidentified dihydroflavonol 45B and the dihydrokaempferol glycoside (48). These results are slightly different from the PCA results, in which the laricytrin acetyl (31) and the taxifolin glycoside (46) were important contributors to the variation. This is probably because spot 31 is fairly highly correlated with 36, 16 with 13, 8A with 5A and 46 with 48, so they were left to the end of the stepwise

procedure, or left out of the stepwise DA entirely.

The DA results of both approaches - entering all of the variables at the same time versus entering them sequentially with the order of entry dependent upon discriminating power - were essentially the same. Nine discriminant axes were obtained that were significant to *alpha* less than or equal to 0.05; they accounted for 93.53 percent of the variation in the data. (Appendix IV contains DA results.)

The eigenvalues from the stepwise procedure were actually slightly smaller than those produced by the direct procedure, and the values of Wilks' lambda were slightly larger. All of the variables were therefore retained for the classification analysis, a decision supported by visual assessment of the correlation coefficients discussed at the beginning of the Results and Analysis section.

The flavonoid data was partitioned several different ways, and each set was analyzed using the direct procedure in the SPSS DISCRIMINANT subprogram. A series of DA's were produced that showed increasing reliability for the purposes of classification, as presented in Table 7. Initially, the data set was partitioned into 21 subsets corresponding to the 21 unconsolidated mature and immature populations listed in Table 3. The use of all 21 populations achieved the lowest percentage of cases classified correctly, 75.65 percent, with the exception of when the 11 consolidated populations were used without allowing for disparate sample sizes (Table 7). When the DA of the populations consolidated by location were adjusted for sample size, 76.17 percent of the cases were correctly classified. Considering that only two species are traditionally recognized in the sample populations, and in light of the low PCA axes scores mentioned previously, this is quite a high level of reliability. It underlines that there are many flavonoid characters all contributing small, significant

TABLE 7. Summary of results of discriminant analysis (DA) classification based on flavonoid data partitioned into different populations (1) or groups. Population codes are in Table 3. Parentheses indicate that large differences in sample size are not accounted for.

GROUP MEMBERSHIP	% CASES CORRECTLY CLASSIFIED
21 populations, divided by location and tree age	75.65
11 populations, consolidated by location	(73.06)
11 populations as above, but DA adjusted for sample size	76.17
5 groups : consol. pop. 1,2,3,4-*A.procera* 5 & 6 - N *A.magnifica* var.*shastensis* 7 - Calif.Coast Range N *Amag.* var.*shast.* 8 & 9 - *A.mag.* var.*mag.* 10 & 11 - Smost *A.mag.* var.*mag.* & S *A.mag.* var.*shast.*	79.27
2 groups : consol. pop. 1 to 7 8 to 11	79.27
3 groups : consol. pop. 1 to 4 5 and 6 7 to 11	79.79
4 groups : consol. pop. 1 to 4 5 to 7 8 to 10 11	83.42
3 groups : consol. pop. 1 to 4 - *A.procera* 8 to 10 - *A.mag.* v.*mag.* 5 to 7 & 11 - *A.mag.* v.*shast.*, both N and S	87.56
3 groups : consol. pop. 1 to 4 and 11 5 to 7 8 to 10	90.16

amounts of variability to a fairly well-differentiated, and very variable gene pool.

Of particular interest are the summaries of group membership predicted from the various DA, as reproduced in Tables 8 and 9. For 21groups (Table 8), the 3 *A.magnifica* var.*magnifica* immature populations had all members classified correctly - Grizzly Ridge regeneration (9R), Alpine County (10R) and Mt. Tom regeneration (11R). Ten populations had 80 percent or more of their individuals correctly classified; only two of those ten populations were *A.procera*. Three populations had 50 percent or less of their individuals correctly classified - the *A.procera* population at Sleeping Beauty (3), and the southern *A.magnifica* var.*shastensis* populations at Jordan Peak (12A) and Sherman Peak (12B). The misclassified trees from Sleeping Beauty were all classed with other populations of *A.procera*, while the two populations from Tulare County were misclassified with populations of either *A.procera* or northern *A.magnifica* var.*shastensis*. No misclassified trees of these southern *A.magnifica* var.*shastensis* populations were placed with populations of *A.magnifica* var.*magnifica*.

It is interesting that, on the whole, a high percentage of trees from both immature and mature populations were classified to the correct population. While DA is designed to emphasize differences between groups, such a high percentage of correct assignments demonstrates that there are at least some differences between the occurrence of flavonoids in mature and those in immature trees at a given site. The very similar level of correct assignment for both consolidated and non-consolidated population data sets may indicate that the differences between immature and mature trees exist to the same extent as differences in flavonoids between populations from different locations.

It should be noted (Table 8) that a slight trend to greater variation in the flavonoid profiles of mature trees compared to immature trees exists. This trend is in accordance with the morphological results found by Maze and Parker(1983) in *A.procera*. The likelihood is that mature trees in a

TABLE 8. Results (1) of discriminant analysis classification of the flavonoid data partitioned into 21 populations (2) on the basis of location and maturity of sample trees.

	PREDICTED GROUP MEMBERSHIP (%)																				SAMPLE	(3)
POP.	1	1R	2	2R	3	4	5R	6	7	7R	8	8R	9	9R	10R	11	11R	12A	12B	12C	12D	SIZE	STATUS
1	80	10			10																	10	dist.
1R		60	20				20															5	dist.
2			71	29																		7	udist.,t
2R			20	60	20																	5	dist.
3		10		20	50	10	10															10	udist.,c
4						80	20															10	udist.
5R							60	20					10						10			10	?
6							12.5	75												12.5		8	udist.,t
7									90											10		10	udist.,sg
7R									10	90												10	?
8											80					10					10	10	udist.
8R												100										9	udist.
9										10		10	80									10	udist.,sg
9R							10							80			10					10	udist.
10R															100							10	?
11																80	20					10	udist.
11R																	100					9	udist.
12A	20	10			20					10								40				10	dist.
12B	10						30		10									10	40			10	dist.
12C						10	10													80		10	mdist.
12D											10									10	80	10	udist.

(1) Overall, 75.65 % of the trees were classified correctly.

(2) See Table 2 for population identification; table above is segregated into four taxa : *A.procera*, northern *A.magnifica* var.*shastensis*, *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis*; top to bottom and left to right, as indicated by the horizontal spacing and vertical lines.

(3) See Table 2 for more details on site status; c=a lot of competition from other species is evident; dist.=disturbed site; m=moderate disturbance; sg=second growth forest; t=thinning or selection cut evident; udist.=undisturbed; ?=site history details not available.

given stand are established in different years under a range of different selective pressures, while understory, immature or regeneration trees are established at about the same time. Despite this trend, the greatest number of misclassified individuals occurred in populations of immature southern *A.magnifica* var.*shastensis* trees collected from Tulare County, California. The high proportion of misclassification in two of the four populations of this taxon reflects the difficulty that the DA had in distinguishing these misclassified trees from the two northernmost taxa. Several other points should be noted from Table 8 :

1. Of the 7 *A.procera* populations, only Douglas County (5R) had individuals classified outside of this group; this is consistent with the frequency histograms which showed breaks in occurrence of some flavonoid compounds south of the Sardine Mt. populations, about latitude 44 degrees N. The population in this group that had the greatest percentage of its trees misclassified to several other locations within the group was Sleeping Beauty, which consisted only of mature trees. Such a high proportion of misclassified trees may indicate that this population is actually less variable than other populations of *A.procera*, as the DA was not able to consistently distinguish between members of the Sleeping Beauty population and other populations of the same taxon.

2. The northern *A.magnifica* var.*shastensis* populations were individually very cohesive. Mt. Ashland (unconsolidated population 6), the northernmost of these populations, had one tree classed with the *A.procera* of Douglas County (5R) and one tree with the southern *A.magnifica* var.*shastensis* population of Bone Meadow (12C); the Mt. Ashland population may contain hybrid trees (W.H.Parker,pers.comm.). One mature tree from Mt. Shasta (7) also grouped with Bone Meadow

while all of Mt. Shasta's immature individuals (7R) were classified to the correct location. All of the immature individuals from the Coast Ranges Brushy Mt. site were classified with that population, except for one of the mature Brushy Mt. trees that grouped with *A.magnifica* var.*magnifica* and one that grouped with southern *A.magnifica* var.*shastensis*.

3. The populations of *A.magnifica* var.*magnifica* were even more cohesive, with only a few individuals of Grizzly Ridge (9,9R) showing up as members of northern *A.magnifica* var.*shastensis* populations or of the *A.procera* population from Douglas County (5R). In addition, a few individuals from immature *A.magnifica* var.*magnifica* populations showed up in immature populations from other locations within the species grouping; otherwise *A.magnifica* var.*magnifica* populations were distinct from each other.

4. The populations of southern *A.magnifica* var.*shastensis* from Tulare County were also quite distinct from each other. Two populations, Jordan Peak (12A) and Sherman Peak (12B), had 50 percent or less of their individuals classified either correctly or with other populations from the county. Most of both of these populations, as well as two individuals from one of the other Tulare County populations, grouped with *A.procera*. As mentioned above, no individuals from any of the four populations of southern *A.magnifica* var.*shastensis* were classified with *A.magnifica* var.*magnifica*. The remaining misclassified individuals grouped with either northern *A.magnifica* var.*shastensis* or with other populations of southern *A.magnifica* var.*shastensis*. This is contrary to other studies of chemical variation in the section (Ustin,1976; Zavarin*et al.*,1978) which found populations of *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* more similar to each other in terpenes than either were to the other two taxa.

When the data set was partitioned into the 11 populations consolidated by location, and the DA was adjusted for different sample sizes, only slightly better overall classification results were achieved (Table 7), and the pattern of incorrect/correct classification by groups (Table 9.A) was essentially the same as for the 21 populations. There is some indication that populations of *A.magnifica* var.*shastensis* both north and south are more variable than populations of either *A.procera* or *A.magnifica* var.*magnifica* ; a larger proportion of individuals from both sets of populations were misclassified. Again, southern *A.magnifica* var.*shastensis* tends to be more like populations of northern *A.magnifica* var.*shastensis* and *A.procera* than like populations of *A.magnifica* var.*magnifica*, although a very small proportion of trees (2.5 percent) was classified with *A.magnifica* var.*magnifica* in this analysis. Both *A.magnifica* var.*magnifica* and *A.procera* seem to be very cohesive groups. Populations of northern *A.magnifica* var.*shastensis* likewise are essentially distinct from each other, which may argue for a pattern of clinal variation across that portion of the *Abies* section *Nobilis* complex.

The best classification result, 90.16 percent correctly classified (Table 7), was achieved by partitioning the data set into 3 groups :

1. *A.procera* /southern *A.magnifica* var.*shastensis* (consolidated populations 1, 2, 3 and 11);
2. northern *A.magnifica* var.*shastensis* (consolidated populations 5, 6 and 7); and
3. *A.magnifica* var.*magnifica* (consolidated populations 8, 9 and 10).

Groups one and three were mutually exclusive (Table 9.E),

TABLE 9. Results (1) of discriminant analysis classification of flavonoid data partitioned into different groups on the basis of location and taxonomic similarities. Some of the figures have been rounded to the closest integer.

A. 11 populations consolidated by location (2).

	PREDICTED GROUP MEMBERSHIP (%)											
POP.	1	2	3	4	5	6	7	8	9	10	11	SAMPLE SIZE
1	85	11		4								27
2	20	60	10	10								10
3	10		80	10								10
4	10			70	20							10
5				12.5	75						12.5	8
6		5				85					10	20
7							84	5		5	5	19
8						5	10	70	10	5		20
9									100			10
10								16		84		19
11	5	7.5		10	2.5	5	7.5			2.5	60	40

(1) Overall, 76.17% of the trees were correctly classified; the results were adjusted for the large differences in sample size.

(2) See Table 2 for population identification. The table above is segregated into three taxa: *A.procera*, northern *A.magnifica* var.*shastensis*, *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* ; top to bottom and left to right, as indicated by the horizontal spaces and vertical lines.

B. 3 populations, isolating the Cascade Mt. *A.magnifica* var.*shastensis*.

PRED.GROUP MEMBERSHIP (%)				
POP.	1	2	3	SAMPLE SIZE AND GROUP IDENTIFICATION
1	88	9	4	57 : pop.1 to 4 above, *A.procera*
2	17	75	14	28 : pop.5 & 6,*A.magnifica* var.*shastensis*
3	10	13	77	108 : pop.7 to 11, *A.magnifica*, both varieties

(1) Overall, 79.79% of the trees were classified correctly.

TABLE 9. (Continued).

C. 4 populations, consolidated by taxon.

PRED.GROUP MEMBERSHIP (%) POP.	1	2	3	4	SAMPLE SIZE AND GROUP IDENTIFICATION
1	89	2		9	57 : pop.1 to 4 above, *A.procera*
2	9	74	2	15	47 : pop.5 to 7, N *A.magnifica* var.*shastensis*
3		6	94		49 : pop.8 to 10, *A.magnifica* var.*magnifica*
4	17	10	73		40 : pop.11, S *A.magnifica* var.*shastensis*

(1) Overall, 83.42% of the trees were correctly classified.

D. 3 populations, consolidated by species and varieties.

PRED.GROUP MEMBERSHIP (%) POP.	1	2	3	SAMPLE SIZE AND GROUP IDENTIFICATION
1	95		5	57 : pop.1 to 4, *A.procera*
2		94	6	49 : pop.8 to 10, *A.magnifica* var.*magnifica*
3	18	2	80	87 : pop.5 to 7 &11, *A.magnifica* var.*shastensis*

(1) Overall, 87.56 % of the trees were correctly identified.

E. 3 populations consolidated by similarity of flavonoid profiles between populations.

PRED.GROUP MEMBERSHIP (%) POP.	1	2	3	SAMPLE SIZE AND GROUP IDENTIFICATION
1	85	2	13	47 : pop.5,6 &7; N *A.magnifica* var.*shastensis*
2	6	94		49 : pop.8,9 &10; *A.magnifica* var.*magnifica*
3	9		91	97 :pop.1 to 4 &11; *A.procera* & S *A.magnifica* v.*shastensis*

(1) Overall, 90.16% of the trees were correctly classified; adjusting for disparate sample sizes did not change the results.

although a few individuals from each were classified with northern *A.magnifica* var.*shastensis*. A few individuals from the northern *A.magnifica* var.*shastensis* group likewise were classified with each of the other two groups, which supports the idea of gene flow between northern *A.magnifica* var.*shastensis* and populations to the north and south of it.

The results of the DA are fairly clear. Almost all of the flavonoid compounds contribute to discrimination between groups, with only three correlated highly enough to other compounds to eliminate them from an "optimal" set of variable characters.

Populations of southern *A.magnifica* var.*shastensis* from Tulare County, California on average are most similar to populations of *A.procera*. There is considerable variability both within and between populations in this area, although very little of this variablilty is expressed as misclassification to *A.magnifica* var.*magnifica*.

The three consolidated populations of *A.magnifica* var.*magnifica* resemble each other fairly closely, and they are completely distinct by discriminant analysis from both the populations of *A.procera* north of Mt. Ashland, and the populations of southern *A.magnifica* var.*shastensis*. Only a few individuals from the northernmost *A.magnifica* var.*magnifica* population sampled grouped with northern *A.magnifica* var.*shastensis*.

The populations of northern *A.magnifica* var.*shastensis* appear to be more variable as a group than either the *A.procera* or *A.magnifica* var.*magnifica* populations, although not as much variability is evident in them as there is in the southern *A.magnifica* var.*shastensis*.

The populations of *A.procera* north of Mt. Ashland in turn form a cohesive group, with only Douglas County showing some similarities to

cohesive group, with only Douglas County showing some similarities to northern *A.magnifica* var.*shastensis*.

Cladistic Analysis

Cladistic analysis is a taxonomic tool similar to cluster analysis in that both procedures evaluate the resemblance between taxa and place them in a hierarchically-structured diagram from which deductions about their taxonomic status can be made. There are two major differences between cladistics, which sometimes is referred to as phylogenetic systematics, and cluster analysis, which is a tool of numerical taxonomy. Firstly, cladistic analysis looks for nested sets of unique characteristics or "evolutionary novelties", placing pairs and/or groups of taxa that share one or more evolutionary novelties together in the final cladogram. Cluster analysis instead uses overall resemblance based on as many characters as possible to organize clusters of taxa into dendrograms, picking out the pair with the highest correlation or degree of similarity first and adding the others sequentially in the same manner. Secondly, cladistic analysis usually goes a step further and attempts to hypothesize an evolutionary "history" using the cladogram, assigning ancestor and dependent status to the taxa involved. It is primarily because of this second step that the field of cladistics is currently rather controversial. Eldredge and Cracraft(1980) present a comprehensive and lucid explanation and defense of the technique and its place in modern taxonomy.

In this study, the flavonoid data was run through a computer algorithm designed by Farris and Mickevitch(1983) to compute and draw cladograms. Both the 21 mature and immature populations and the 11 populations consolidated by location were run through the program

separately. No attempt was made to use the resulting cladograms to rigorously interpret the evolutionary history of the populations assessed, partly because not all of the compounds have been identified fully. In addition, only 8 of the 24 compounds used as characters appeared in a single stem of the cladogram. While unique characters may disappear and reappear during the processes of speciation, Eldredge and Cracraft(1980) recommend that a novelty that defines a group at one hierarchical level should not be used to define subgroups at lower levels in the cladogram. Because of these two limitations, trends suggested by these cladograms are not strong (J.Maze,pers.comm.). Despite these major limitations, a cladistic analysis has been included in this thesis for comparative purposes because it is currently a widely used taxonomic technique.

In cladistic analyses, if adequate information is not available about the significance of character variation within the group being analyzed, then comparison is made to organisms outside the group (Eldredge and Cracraft,1980). In the absence of appropriate real outgroups, theoretical outgroups are used. For the cladistic analysis in this study, one theoretical ancestor with all of the variable compounds present and one theoretical ancestor with all of the variable compounds absent were both used as outgroups. No "legitimate" outgroups were used in the cladistic comparisons, so any implications of paraphylly may be suspect. Recoding on the basis of population averages for three character states should probably have been accompanied by recoding on the basis of absolute presence and absence to allow comparison between the two methods of coding. Eldredge and Cracraft(1980) also specify that the lowest-ranking taxon used in a cladistic analysis should be a species, since species are usually defined as the basic units of evolution. There are perhaps 4

species possible in the *Abies* section *Nobilis*, so the use of 21 and 11 populations contravenes this specification. The results of the cladistic analysis should, because of these limitations, be treated with caution.

Two cladograms were produced - one for the 21 mature and immature populations (Figure 12), and one for the 11 populations consolidated by location (Figure 13). Outgroup comparison at the same time to the two hypothetical ancestors was used in the calculation and construction of each cladogram. For both 21 and 11 populations, *A.procera* and *A.magnifica* var.*magnifica* were quite distinct from each other, and diverged at about the same point on the cladogram as the hypothetical ancestor with all compounds present. A major exception to this pattern was the Douglas County population from Oregon. This population grouped with *A.procera* for the analysis of 21 populations, where it was expected on the basis of taxon occurrence and morphology. It grouped between *A.magnifica* var.*magnifica* and the southernmost populations of northern *A.magnifica* var.*shastensis* in the analysis using the 11 populations consolidated by location, which might be expected if gene flow between northern *A.magnifica* var.*shastensis* and *A.procera* was occurring.

No compounds unique to a single stem were found in the analysis of 21 populations. This is not surprising, since 6 populations from different locations were each split into 2 separate samples, one immature and one mature, accounting for over half of the 21 populations. A number of immature populations did not group with their companion mature populations. This result supports the presence of some significant differences between the flavonoid complements of mature and immature trees. As mentioned in the analysis of DA results, this inconsistency may reflect generational rather than ontogenetic differences between

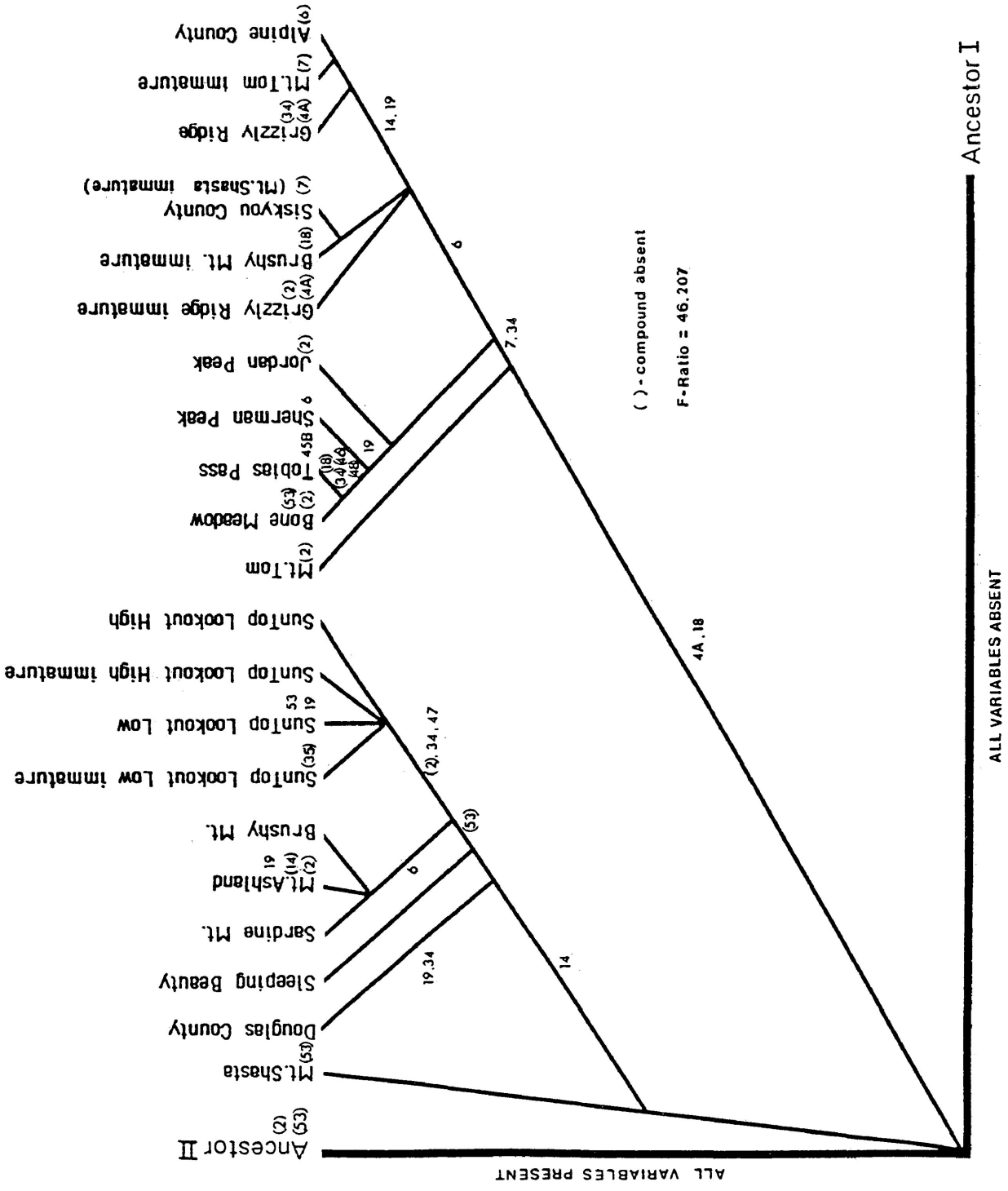


FIGURE 12. Cladogram illustrating the most parsimonious result of the cladistic analysis of flavonoid data for 21 immature and mature populations from 11 locations.

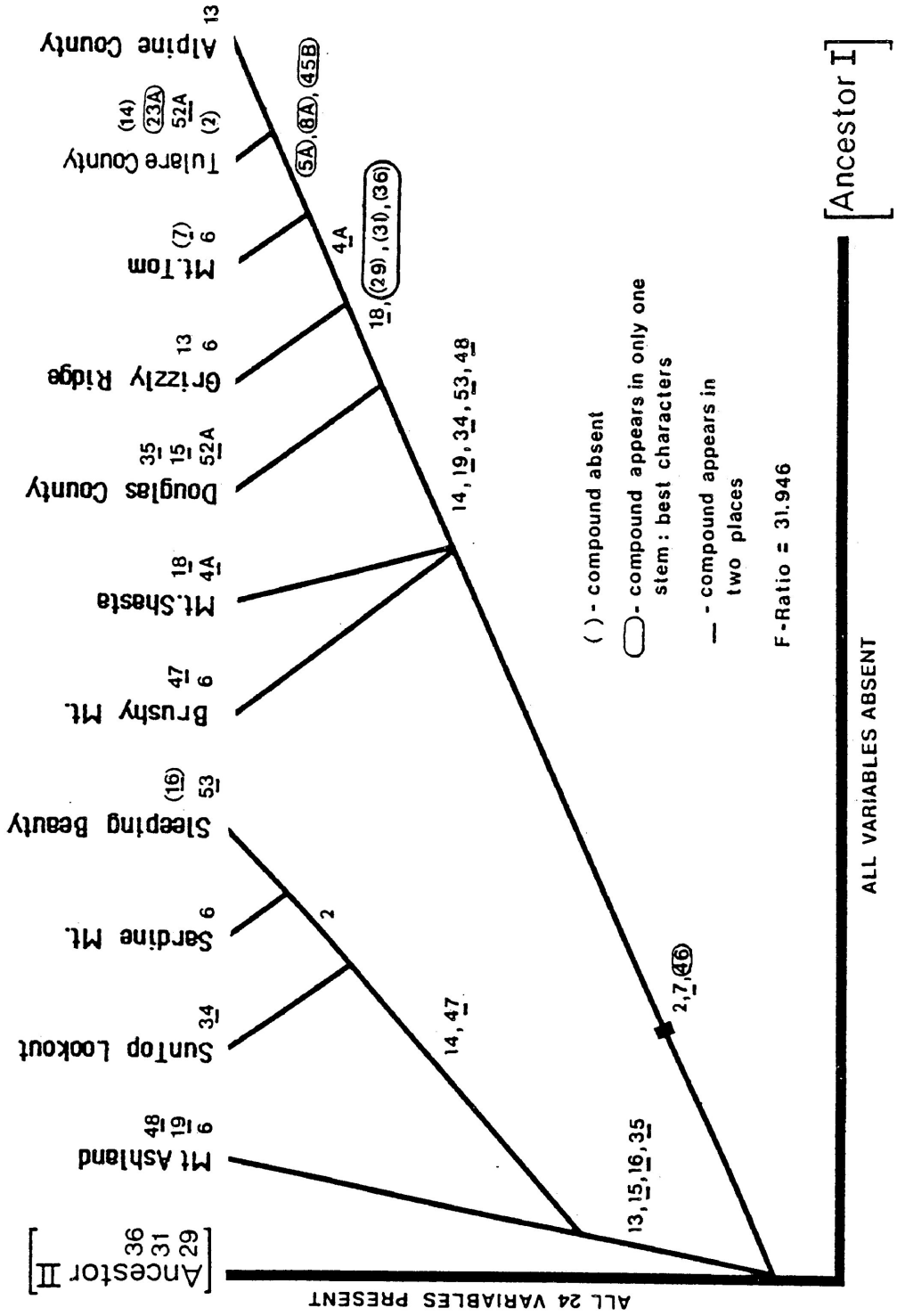


FIGURE 13. Cladogram illustrating the most parsimonious result of the cladistic analysis of flavonoid data for 11 populations consolidated by location.

immature and mature trees from the same location as reported by Maze and Parker(1983) for morphological characters in *A.procera.* The differences may also be statistical artifacts due to small sample size and the averaging of character states to accomodate the data set to the computer algorithm.

Because of these inconsistencies, the rest of this section will only present the results of the cladistic analysis that used the 11 consolidated populations (Figure 13).

One population of northern *A.magnifica* var.*shastensis* - Mt. Ashland - was the stem from which all of the *A.procera* populations except Douglas County arose. This is the site where hybridization and introgression are strongly suggested by the presence of a range of intermediate morphological characters. The other two populations of northern *A.magnifica* var.*shastensis* branched off of the stem that contained all of the rest of the populations, which included Douglas County, *A.magnifica* var.*magnifica* and the southern *A.magnifica* var.*shastensis.*

The absence of the acetylated myricetin, laricytrin and syringetin (spots 29, 31 and 36) distinguished *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* from all of the other populations, including those from Douglas County and the northern *A.magnifica* var.*shastensis* locations. This isolation of *A.magnifica* var.*magnifica* with southern *A.magnifica* var.*shastensis* is inconsistent with the DA results reported above.

In the cladistic analysis, the Tulare County southern *A.magnifica* var.*shastensis* and the *A.magnifica* var.*magnifica* population of Alpine County were split off as a terminal node on the basis of the presence of three compounds that appear only at the stem of this node - the lower

aglycones 5A and 8A, and the unidentified dihydroflavonol 45B. These two populations, according to cladistical theory, are the most derived populations. Compound 23A is a novel character that distinguishes between them. The population of southern *A.magnifica* var.*shastensis* where it occurs is therefore even more derived than the *A.magnifica* var.*magnifica* population from Alpine County.

Dr. J. Maze (pers.comm.) has pointed out that the northern and the southern *A.magnifica* var.*shastensis* do not come close to forming a node sharing an evolutionary novelty, which would be expected if they were as closely related as their current taxonomic status suggests. He also mentions that the populations of *A.procera* are the least derived or the most plesiomorphous; in effect, they are the most "primitive" of all of the populations, since they contain no evolutionary novelties. This conclusion contradicts accepted interpretations of the available fossil record, because *A.procera* fossils do not appear before fossils of other taxa in the section. However, Liu(1971,p.103) does suggest that exserted bracts like those typical of *A.procera* are a primitive character state.

These results of the cladistic analysis using flavonoid data imply that the *Abies* section *Nobilis* complex is probably not a monophyletic group, which is defined by Eldredge and Cracraft(1980) as a stem or ancestral species and all of the kinds of organisms hypothesized to have descended from it. Eldredge and Cracraft(1980) further state that an

> "ancestral species within any monophyletic group, if present in a sample and correctly analyzed, will be diagrammed as a monotypic sister-group of the remainder of the species contained in that group."(p.42)

Any of the northern *A.magnifica* var.*shastensis* could be perceived as possible ancestral groups, Mt.Ashland to *A.procera* and both Brushy Mt. and Mt.Shasta to *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis*. Given the similarities in flavonoid profiles between all of these groups, given the range of population variability not allowed for by the computer algorithm, and also given the fact that hypothetical ancestors were used for outgroup comparison, these conclusions are suspect.

A problem with cladistic analysis is its inability to distinguish hybrid or introgressive populations. Eldredge and Cracraft(1980, pp.100-101) suggest that fully interbreeding taxa are in effect one species, and should not be distinguished. In addition, if a new species has developed by hybridization between two pre-existing species, as evidenced by morphological intermediates, assessment in the absence of breeding data has to be based on an evaluation of closeness of evolutionary descent, which is difficult without a complete fossil record.

Nevertheless, some of the results of the cladistic analysis confirm the results of both the cluster and the discriminant analyses already presented. These results can be summarized as follows :

1. *A.procera* and *A.magnifica* var.*magnifica* are distinctly different, if closely related species.
2. Northern *A.magnifica* var.*shastensis* and southern *A.magnifica* var.*shastensis* are not a natural taxon.
3. Some populations of northern *A.magnifica* var.*shastensis* are more closely related to or more similar to *A.procera* while some appear to be more closely related to *A.magnifica* var.*magnifica*.
4. There appear to be differences in the flavonoid

complement of mature and immature trees from the same location, although it is not clear whether these are generational differences, ontological differences, or differences due to crown position.

Both the cladistic analysis and the cluster analysis of the flavonoid data produced two groups of populations. However, the component populations for each group in the two analyses were quite different. One major conflict exists among the results of the cladistic analysis, the cluster analysis and the discriminant analysis results. In the cladistic analysis, the southern *A.magnifica* var.*shastensis* is most similar to *A.magnifica* var.*magnifica*, where in the other analyses, it appeared on average to be most closely related to *A.procera.* In addition, the distinction between the four populations from Tulare County in the cladistic analysis was not as marked as it was in the other analyses.

DISCUSSION

It should be emphasized that the chemical characters used in this study do not reflect all of the variability present in the *Abies* section *Nobilis*. Nevertheless, flavonoid occurrence interpreted in light of other evidence in the literature suggests four major, complementary factors underlying the pattern of population variation in the section. The first factor is the Recent epoch migration of members of the section out of areas to which they were restricted during the climatic upheavals of the Pliocene and Pleistocene epochs. The second factor is hybridization between adjacent, but morphologically, chemically and/or ecologically differentiated populations of trees within the section. The last two factors are genetic variation first as a reflection of great genetic heterozygosity within the gene pool of the section, and secondly, the expression of a portion of that variability as survival and reproduction in different stages of forest succession.

ASSESSMENT OF FLAVONOID VARIATION IN THE *ABIES* SECTION *NOBILIS*

Comparison of flavonoid variation to other species groups

Of the five North American *Abies* species that have been analyzed for flavonoids, the flavonoid profile typical of only one species - *A.amabilis* - is distinct from the others. The flavonoid profiles characteristic of the noble fir species complex contain essentially the same compounds as the *A.balsamea /A.lasiocarpa* species complex. The biggest distinction between the flavonoid profiles of these two true fir groups is in the pattern of occurrence by taxon of rutinosides, acetylated monoglycosides and two dihydroflavonol glycosides, taxifolin and dyhydrokaempferol.

All of the trees sampled over the ranges of *A.procera* and *A.magnifica* in this study had very similar flavonoid profiles, with none of the variation in the occurrence of the rutinosides that is present in the section *Balsamea* (Parker and Maze,1984; Parker*et al.*,1984). Acetylated monoglycosides occur in *A.procera* and *A.magnifica* var.*shastensis* from the area of range sympatry between *A.procera* and *A.magnifica*, and for the most part are absent in *A.magnifica* var.*magnifica* collected south of this area. The dihydroflavonols occur in both varieties of *A.magnifica* and are usually absent in *A.procera* north of the area of range sympatry. The pattern of occurrence of these two sets of compounds is more clear-cut in the section *Balsamea*, where acetylated monoglycosides occur in populations of *A.lasiocarpa*, and the dihydroflavonol glycosides occur mostly in *A.balsamea*.

In the principal components analysis of individual tree flavonoid profiles in this study, each principal component accounted for a very small

proportion of the total variation in the data set. This finding is in contrast to two similar surveys of the *Abies* section *Balsamea* flavonoids, where a relatively large proportion of the total variability present in the data set was accounted for by the first two or three principal components (Parker and Maze,1984; Parker*et al.*,1984). Even the survey of the flavonoids of sixteen populations of the single species *A.lasiocarpa* had twice as much variation accounted for by the first two principal components as was accounted for in the first two principal components of the *Abies* section *Nobilis* analysis reported in this thesis (Parker and Maze,1984).

Two explanations for this major difference in principal components analysis of flavonoid data for the two *Abies* species complexes are suggested. Firstly, the *Abies* section *Nobilis* may simply contain less discrete flavonoid profiles than the section *Balsamea*, perhaps because of a more complicated evolutionary history. This may give the flavonoid profiles in the section *Nobilis* the appearance of greater variability within species and less between species. Two species are recognized in the *Abies* section *Nobilis* where three or four are recognized in the section *Balsamea*, which has a much larger range and potentially more opportunity for disjunction between component taxa (Liu,1971). Factor analysis results similar to those for the principal components analysis of the flavonoids of the *Abies* section *Nobilis* have been reported for the herbaceous genus *Limnanthes* (Parker and Bohm,1979). This genus contains about 9 species and an additional 9 varieties, and is distributed primarily in the same area as the *Abies* section *Nobilis*, an area with a complex paleogeographical history.

Secondly, similar flavonoids accumulated by the two *Abies* species complexes may not be affected by selective pressures or by the same selective pressures in each section. An assessment of the differences in

selective pressures acting on the *Abies* section *Balsamea* compared to the *Abies* section *Nobilis* is beyond the scope of this thesis.

Significance of flavonoid variation

The significance of the flavonoid differences among the taxa of the section *Nobilis* cannot be completely assessed since the function of each of the variable compounds is not known. However, differences in the flavonoids accumulated between closely related pioneer and climax species could be expected simply due to different selective pressures. As a simplified example, closed climax stands and open habitats that are in the process of being colonized have very different light regimes, so that plants adapted to one successional role have different light requirements (Spurr and Barnes,1973). Zavarin and Snajberk(1975) reported a difference in shade tolerance between chemical races of *Pseudotsuga menziesii* that were delineated using terpene variation. Populations of southern *A.magnifica* var.*shastensis* sampled showed considerable affinity to *A.procera*, which may be a reflection of parallel evolution resulting from similar selective pressures as well as or rather than phylogeny (Davis and Heywood,1963,p.449).

Photocontrol of flavonoid synthesis is evident at many points in the biosynthetic pathway (McLure,1975), with light increasing the activity of most enzymes involved in phenolic biosynthesis which can lead to increased accumulation of various phenols within the plant (McLure,1979). Although phenotypic plasticity as defined by Grant(1963) is not anticipated for leaf flavonoids (Harborne,1975), it is a possible source of variation. Some variation due to ontogeny is also possible (Alston and Turner,1963), although Maze*et al.*(1981) found no significant correlation between age

and phenotypic plasticity in morphological characters in populations of *A.lasiocarpa* and *A.amabilis* collected from north coastal British Columbia. Species in the genus *Abies* are noted for morphological plasticity (Liu,1971). While flavonoid and morphological variation are not always correlated (Crawford and Mabry,1978; Grant,1971; Davis and Heywood,1963), flavonoid variation due to variable environments affecting tree species that exhibit morphological plasticity needs to be examined. In the absence of common garden experiments or provenance tests for flavonoid variation, modification of the flavonoids of maturing *Abies* trees in response to environmental differences cannot be ruled out, especially as a source of differentiation between mature and immature populations from the same locality.

IMPLICATIONS OF FLAVONOID VARIATION TO EVOLUTION IN THE *ABIES* SECTION *NOBILIS*

Five possibly complementary hypotheses were presented in the Introduction of this thesis to explain the pattern of taxon occurrence in the *Abies* section *Nobilis*. These five hypotheses are summarized below, and then discussed in the following four sections :

1. *A.magnifica* var.*shastensis* is a discrete species that has a set of characteristics that are intermediate between *A.magnifica* var.*magnifica* and *A.procera* ;

2. *A.magnifica* var.*shastensis* is a hybrid taxon, consisting of individuals that exhibit different sets of characters that range

between those typical of *A.magnifica* var.*magnifica* and those typical of *A.procera* ;

3. of the three recognized taxa in the *Abies* section *Nobilis*, *A.magnifica* var.*shastensis* most closely resembles an "ancestral race" from which the modern taxa of the section have been derived;

4. *A.magnifica* var.*shastensis* is one, two or three relict subspecies that have survived climatic changes in isolated pockets while intermediate forms have been extinguished; or

5. the *Abies* section *Nobilis* is a single polytypic species complex that exhibits ecotypic or clinal variation.

Available paleobotanical evidence does not conclusively eliminate any of the above hypotheses. Fossils of the *Abies* section *Nobilis* have been recovered from very little of the present range of the section, with no reliable fossils assigned to the section recovered from Washington, the Klamath-Siskyou mountain complex, the Pacific coastal mountains or south-central California. Most of the fossil specimens identified as belonging to the section have been recovered from states to the interior - Idaho, Montana and Nevada. The oldest reported fossil occurrence of the *Abies* section *Nobilis* was recovered from a temperate, upland, Eocene flora in northwestern Nevada (Axelrod,1976; 1964), and has been identified as the fossil equivalent of *A.magnifica* var.*shastensis*. No reliable fossils of *A.procera* have been found, although a number that are considered to be the fossil equivalent of *A.magnifica* var.*magnifica* have been reported. While Liu(1971,p.103) suggests that elongated bracts represent a primitive character and included bracts represent an advanced character, this fossil evidence suggests that *A.procera* may be a relatively late divergence from the *Abies* section *Nobilis* stock (cf.

Grant,1963,p.570).

Each of the five hypotheses are addressed below in light of the four factors outlined at the beginning of the discussion, where appropriate.

A.magnifica var.shastensis - a discrete species

Shasta red fir has been recognized at the taxonomic level of noble fir and red fir as the species *A.shastensis* Lemm. To be considered a discrete species, *A.magnifica* var.*shastensis* should be either consistently distinguishable from all other species in the genus, or should be reproductively isolated from closely related sympatric species (Eldredge and Cracraft,1980; Dobzhansky*et al.*,1977; Sneath and Sokal,1973).

Conclusive argument in favour of the specific status of *A.magnifica* var.*shastensis* is not supported by either ecological, morphological or previously-reported chemical evidence. Ecologically, *A.magnifica* var.*shastensis* exhibits a range of attributes between those exhibited by *A.procera* and *A.magnifica* var.*magnifica*. Morphologically, *A.magnifica* var.*shastensis* exhibits either a range of cone and needle morphology, or is intermediate between *A.procera* and *A.magnifica* var.*magnifica* in characteristics like cotyledon number and seed weight. In terpene composition, southern occurrences of *A.magnifica* var.*shastensis* tend to be more like *A.magnifica* var.*magnifica* with only a few trees in sampled populations similar to *A.magnifica* var.*shastensis* from the area of range sympatry between *A.procera* and *A.magnifica* var.*magnifica*. A disjunction in terpene composition between populations of "pure" *A.procera* and those south of latitude 44 degrees N suggest the existence of "species-level" differences

between *A.procera* and *A.magnifica* var.*shastensis*, but these differences could be interpreted as evidence of clinal variation between the taxa.

No reports of reproductive isolation between *A.magnifica* var.*shastensis* and *A.magnifica* var.*magnifica* have been published. Hybrids between *A.magnifica* var.*magnifica* and *A.procera* and between *A.magnifica* var.*shastensis* and *A.procera* have been reported under both artificial and natural conditions (Liu,1971; Lofting,1967; Silen*et al.*,1965). Artificial reciprocal crossings of *A.procera* and *A.magnifica* have produced as high percentages of sound seed as intraspecies pollinations (Sorenson*et al.*,1976). Given these reports, and given the high degree of interfertility between sympatric as well as allopatric members of the genus *Abies* (Liu,1971; Klaehn and Winieski,1962), reproductive isolation capable of maintaining *A.magnifica* var.*shastensis* as a separate species (Dobzhansky*et al.*,1977; Grant,1963) seems unlikely. This argument is weakened by similar findings between other species of North American conifers (Fowells,1965), and by the probability of minimal gene exchange between disjunct stands (Ehrlich and Raven,1969). Maintenance of differentiated populations due to geographical isolation among mountain sites cannot, therefore, be ruled out.

Analysis of the flavonoid profiles of the *Abies* section *Nobilis* does not support the combining of all of the populations of *A.magnifica* var.*shastensis* into one taxon. In the discriminant analysis of flavonoid data, populations of northern *A.magnifica* var.*shastensis* were distinct from each other, with some trees misclassified to populations outside of this operational taxon, only half of them to southern *A.magnifica* var.*shastensis*. A similar level of distinctness between populations was

found in southern *A.magnifica* var.*shastensis*, but twice as many trees from these populations were misclassified to other taxa, most of them to *A.procera*, not to northern *A.magnifica* var.*shastensis*. *A.magnifica* var.*magnifica* populations showed the same level of distinctness between populations and greater cohesiveness as a taxon compared to northern *A.magnifica* var.*shastensis*. In *A.procera*, a substantial number of trees were classified to the wrong locality within the taxon, but very few of these were classified outside of the taxon.

Thus, while northern *A.magnifica* var.*shastensis* has only slightly less cohesiveness compared to *A.procera* and the typical variety of *A.magnifica*, southern *A.magnifica* var.*shastensis* is much less cohesive. The distinction between populations of *A.magnifica* var.*magnifica* is on par with the distinctness of the populations of both occurrences of *A.magnifica* var.*shastensis*, which suggests that *A.magnifica* var.*shastensis* could be considered part of a very variable *A.magnifica*, in which geographically distant populations are well differentiated from each other. Given the lack of differentiation evident between populations of *A.procera*, this conclusion is consistent with the contention that plant species ecologically adapted to stable, climax communities will differentiate in accordance with the conditions specific to that locality (Hamrick*et al.*,1979a; 1979b; Rehfeldt and Lester,1969).

The lack of overlap in flavonoid profile between northern *A.magnifica* var.*shastensis* and southern *A.magnifica* var.*shastensis* undermines any argument that these two occurrences of the variety *shastensis* should be considered the same taxon at the species level and certainly not at the level of variety. If particular flavonoid structures almost always persist in all populations of a taxon or populations of closely related taxa (Crawford and Mabry,1978;

Mabry,1973), the two varieties of *A.magnifica* should have very similar flavonoid profiles, especially compared to *A.procera*. This is not the case; in none of the analyses of flavonoid data were the northern and southern occurrences of *A.magnifica* var.*shastensis* grouped together separately from the other taxa. The occurrence of some flavonoid compounds in the southern *A.magnifica* var.*shastensis* populations that do not appear in any other populations is further evidence of the lack of correlation between the two occurrences of the variety *shastensis*.

On the basis of these arguments then, there is insufficient evidence to recognize *A.magnifica* var.*shastensis* as a species distinct from the other two species in the *Abies* section *Nobilis*. At this point, some mention of the varietal status of *A.magnifica* var.*shastensis* should be made.

Two of the three consolidated populations of northern *A.magnifica* var.*shastensis* were collected from the area of range overlap between *A.procera* and *A.magnifica* var.*magnifica* ; these are the populations at Mt.Shasta and at Mt.Ashland. The third population of northern *A.magnifica* var.*shastensis*, Brushy Mt., is located near the southernmost occurrence of *A.magnifica* var.*magnifica* in the northern California Coast Ranges (Little,1971). Morphological and preliminary flavonoid data supported the identification of *Abies* section *Nobilis* trees in this area as *A.magnifica* var.*shastensis* (Heckard and Hickman,1984; Griffin and Critchfield,1972; Liu,1971), and the Brushy Mt. population has been treated as such in this study rather than as *A.magnifica* var.*magnifica* (Munz and Keck,1959). Despite this assumption, Brushy Mt. is the only population of northern *A.magnifica* var.*shastensis* that appears to be more similar to *A.magnifica*

var.*magnifica* than to *A.procera* in all three numerical analyses of the flavonoid data. In addition, none of the southern *A.magnifica* var.*shastensis* populations showed affinity to populations of the typical variety, a result that is in keeping with Ustin's(1976) morphological findings. These results contradict the assignment of a single variety *shastensis* simply to the species *magnifica.*

A.magnifica var. *shastensis* - a hybrid between *A.procera* and *A.magnifica* var.*magnifica*

Liu(1971,p.320), in his monograph of the genus *Abies*, identifies Shasta red fir as *Abies* x *shastensis* Lemm.,emend Liu. He feels that "typical" Shasta red fir from southern Oregon and northern California exhibits morphological characters that are "strikingly" intermediate between *A.procera* and *A.magnifica* var.*magnifica* and should therefore be considered a hybrid between them. Liu(1971,p.249) notes that it is not possible to determine the taxonomic status of other intermediate forms until more genetic or paleobotanical evidence is available. The identification of Shasta red fir as a hybrid between *A.magnifica* var.*magnifica* and *A.procera* does not account for the occurrences of the intermediate variety at the southern extremes of the range of *A.magnifica* var.*magnifica* in the California Coast Ranges and the Sierra Nevada Ranges. Both of these regions are well outside the area of purported sympatry between the ranges of the typical species. While hybridization between *A.procera* and *A.magnifica* var.*magnifica* prior to the Pleistocene is possible, the restriction of such hybrid products to their current south-central Californian distribution is unlikely given the pattern of subsequent migration, with *A.procera*

restricted to above 40 degrees N latitude.

Adams(1977) in a discussion of population differentiation and variation in *Juniperus asheii* Bucholz, found that ancestral populations were more distinct from each other than were recently established populations. Populations of *A.procera* were much more similar to each other in the discriminant analysis of flavonoids than were populations of *A.magnifica*. This difference suggests that *A.procera* is perhaps a more recently-derived taxon than *A.magnifica* var.*magnifica* or var.*shastensis*. If this is the case, ancient hybridization between *A.procera* and *A.magnifica* var.*magnifica* could not have occurred.

While *A.procera* and *A.magnifica* var.*magnifica* are distinct species ecologically, with different forest successional roles, natural hybrids are very likely if mature trees of both species are growing together. According to Grant(1963), barriers to reproduction between closely related allopatric species are weak, so that if their ranges overlap, hybrids with a wide range of ecological characeristics are to be expected. *Abies* species are what Grant(1971) calls "promiscuous" plants, with some gene flow possible over fairly long distances due to wind pollination. However, the extent of gene flow in closely related tree taxa that are ecologically distinct is open to some debate (Wright,1976; Ehrlich and Raven,1969; Grant,1963). The extent of distinctness between populations in the discriminant analysis suggests that gene exchange is not occurring between populations of *A.magnifica*, but may be occurring in *A.procera* (Grant,1963,p.432). On the other hand, successional status may be the determining factor in the relative homogeneity of populations of *A.procera* (Hamrick*et al.*,1979a; 1979b).

Nevertheless, if *A.procera* and *A.magnifica* var.*magnifica* are genetically distinct species, then hybridization between them could be

expected to produce a wide range of variability in second-generation hybrid offspring (Grant,1971; etc.). No populations in the area of range sympatry between the two typical species with a range of variation expected of hybrid swarms have been reported in the literature or pinpointed by the flavonoid survey in this report. Similarly, no such higher levels of variation have been reported for hybrids of *Picea glauca* (Moench.)Voss and *P.englemannii* Parry occurring along elevational transects (Daubenmire,1974). In this case, however, hybridization is occurring between a high elevation and a low elevation species, so that surviving hybrids sort themselves out along an elevational transect rather than as a hybrid swarm.

Four explanations can be offered to explain a lack of obvious hybridization between *A.procera* and *A.magnifica* where it is expected. Firstly, hybridization and therefore gene flow may not be occurring because of ecological barriers to reproduction. It is extremely likely that some interbreeding between "typical" *A.procera* and "typical" *A.magnifica* has taken and is taking place between 44 degrees N latitude and about 40 degrees N latitude, given the evidence of previously published systematic papers in the section. *A.procera* and *A.magnifica* do not appear to be differentiated ecologically to the point where they are restricted into separate areas and niches where their ranges overlap. This lack of habitat distinction lends support to reports that hybridization is occurring between the two taxa (Clarke,1979; Franklin and Dyrness,1973; Grant,1963).

Secondly, perhaps no second-generation hybrids are produced, even though intermediate first-generation hybrid trees that are very similar to one of the parents survive to bear cones (Dobzansky*et al.*,1977; Raven,1976; Grant,1971,p.153). Alternatively, *A.procera* and

A.magnifica var.*magnifica* may have genotypes that are similar enough to allow intergradation in the form of gene flow that cannot be readily detected as hybrids between the two species. These two alternatives cannot be proved or disproved using the results of this study. The limited crossing information that has been published for the section *Nobilis* suggests that hybrids would be intermediate in at least some morphological characters, as is apparent at the Mt.Ashland site in the area of range sympatry (W.H.Parker, pers. comm.). Intermediacy in physiological attributes like shade tolerance might allow survival to reproductive age of first-generation hybrid individuals. At the same time, survival of second-generation hybrid individuals not adapted to the stage of forest succession they germinated under would be unlikely (Maze*et al.*,1981; Lewis,1969). If there is strong selection pressure occurring in the area of range sympatry, then most hybrid intermediates would not survive (Rehfeldt and Lester,1969; Grant,1963,p.455).

Finally, the characteristics that have been assessed in this survey of flavonoids in the *Abies* section *Nobilis* may not be sensitive enough, or sampling may not have been intensive enough, to uncover evidence of hybridization. There were no large negative correlations between compounds, which suggests that "replacement" characters typical of dominant/recessive pattern of inheritance in flavonoids (Harbourne,1975; Hegnauer,1969) are not evident in the *Abies* section *Nobilis*. However, very large negative correlations did occur between some of the operational taxa in the cluster analyses. It is therefore not clear whether flavonoid inheritance in the leaves of the section is under dominant/recessive gene control or additive gene control. Most hybrids that have had their flavonoids assessed exhibit additive inheritance of flavonoids (Harbourne,1975; Jones and Siegler,1975).

Jones and Seigler(1975) present an example of the utility of flavonoids in uncovering hybridization where morphological and habitat intermediacy is present in *Populus* x *accuminata* Rydb. The two putative parents of this hybrid share very few flavonoids, whereas *P.* x *accuminata* has almost all of the flavonoids of both parents and one extra flavonoid not found in either parent. In this case, it was very easy to pinpoint which species were most likely hybridizing to produce the intermediate. Given simple additive inheritance of flavonoids, it should be easy to isolate first generation hybrids between *A.magnifica* var.*magnifica* and *A.procera* because the hybrids would most likely have flavonoid profiles like *A.procera* with acetylated monoglycosides, with the addition of the taxifolin and dihydrokaempferol glycosides. This combination is evident in at least half of the individuals in each consolidated population between and including Douglas County, Washington and Brushy Mt., California except for Mt.Ashland, California. In the population at Mt.Ashland, the taxifolin glycoside did not occur at all, and the dihydrokaempferol glycoside was present in only one-quarter of the population. The sample size at Mt.Ashland was the smallest of all of the consolidated populations, so this exception may be due to experimental bias. Given the fact that the members of the section *Nobilis* appear to interbreed freely, these "combination" profiles are fairly strong evidence of close genetic similarity and/or gene flow among these "intermediate" trees.

However, given the current taxonomic status of the three taxa in the *Abies* section *Nobilis*, interpretation of flavonoid variation by population is difficult because of the similarity in overall flavonoid profile and overlap in occurrence of specific profiles between *A.procera* and *A.magnifica.* Intermediacy in flavonoid profiles can be very difficult to pinpoint in closely

related plant taxa (Mabry,1973). Presence of a compound is a more reliable indication of the ability of a plant taxon to produce that compound than absence is an indication of inability to produce that compound. The abililty to accumulate a given secondary plant product, not simply the ability to produce that product, is therefore the characteristic used in most systematic studies using flavonoids (Hegnauer,1969). Because of the low level of taxonomic distinction being examined, compound occurrence was coded in this study to include the presence of a variable compound in trace amounts, which can be difficult to determine. Seven of the twenty-four flavonoids used as characters in this study typically occurred as trace components in the flavonoid profile of an individual tree. Inclusion of these compounds as well as others that were typically accumulated, when they occurred in trace amounts, may have obscured evidence of hybridization in populations sampled.

While some hybridization is likely between *A.procera* and *A.magnifica* in the area of range sympatry, it appears to be obscured because of genetic similarity and minimal gene exchange between stands. *A.magnifica* var.*shastensis* is thus not a taxon that can be unequivocably assigned hybrid status, even in areas where the ranges of *A.procera* and *A.magnifica* var.*magnifica* overlap.

A.magnifica var.*shastensis* - ancestral race or relict subspecies

Migration and adaptation of plant species to changing and diversified environments is very evident in the modern and past floras of the west-coastal United States (eg. Barbour and Major,1977; Franklin and Dyrness,1973; Munz and Keck,1959). Harborne(1975) and Mabry(1973) state that when a new ecosystem that requires physiological adaptation is

colonized by a given taxon, one might expect that the plants that survive would show modification of their natural products chemistry. Paleobotanical evidence suggests that the subalpine and montane forests which included *A.magnifica* var.*magnifica* and var.*shastensis* that were growing east of the Cascade-Sierran crest as late as the Pliocene epoch, were displaced to the coastal mountains because of increasing interior aridity prior to the glacial age. Cordilleran glaciation occurred in western North America south of the continental ice sheets during the Pleistocene epoch throughout most of the Cascade mountains of Washington and Oregon and throughout all except the southernmost portion of the Sierra Nevada Range (see Figure 1). Although conclusive fossil evidence is not available, current authorities maintain that these displaced interior forests and the northern coastal forests were preserved over the course of glaciation in the Klamath-Siskyou mountain complex of what is now northwestern California and southwestern Oregon (eg.Waring,1969).

A.magnifica var.*magnifica* and *A.procera* are probably both recent immigrants from the Klamath Mts. into most of their current distributions, which have been achieved since the last glacial retreat (Heckard and Hickman,1984; Wells,1983; Axelrod,1981; 1976). Unglaciated areas of Washington and Oregon are far enough north to have been too cold to support trees during periods of greatest glacial advance (Wells,1983; Zavarin and Snajberk,1973). This conclusion is consistent with the vigorous growth and relatively uniform distribution found in northernmost *A.procera* (Gessel and Oliver,1982,p.182); an unbroken marginal distribution is typical of a species that is extending its range (Detling,1953). Corresponding growth characteristics have not been reported for the southernmost occurrences of *A.magnifica*

var.*magnifica*, although Heckard and Hickman(1984) report vigorous stands of *A.magnifica* var.*shastensis* at the range limit of that species in the North California Coast Mts. The distribution of the *Abies* section *Nobilis* in that area is patchy, which is sometimes indicative of retreating populations (Detling,1953). Because of this contradiction, no firm conclusions can be drawn about the current migrational status of that species.

The lack of correlation in morphological characters between *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* reported by Ustin(1976) and in the flavonoid profiles reported by this study support the acceptance of the southern portion of the variety *shastensis* as a relict subspecies that has been separated from the rest of the section since the ecological changes of the Pliocene epoch. The possibility exists that the southern Sierra Nevada and/or the southern mountains of California acted as a refuge area for elements of the displaced interior forest (Stebbins,1982; Axelrod,1966; 1959). As small, isolated populations, the trees in this area would be subjected to random changes in genotype which may have been expressed as the accumulation of several different flavonoids which are typically not found in the rest of the section (Harborne,1975; Mabry,1973). Alternatively, these flavonoids may have been present in the flavonoid profiles of these populations prior to their isolation; simplification of flavonoid profiles is sometimes judged to be evidence of a derived condition (Levy,1983; Crawford and Mabry,1978; Harborne,1975).

Grant(1971,p.116) points out that races and species with "deviant" characters occur in geographically isolated positions on or off of the periphery of an ancestral species area. Given the lack of reliable fossil

remains of *A.procera* and the similarities in cone morphology of extant *A.magnifica* var.*magnifica* and var.*shastensis*, and given the pattern of species migration in the western United States, it is tempting to suggest that northern *A.magnifica* var.*shastensis* is an ancestral taxon from which *A.procera* has differentiated to the north of the Klamath-Siskyou mountain complex and *A.magnifica* var.*magnifica* to the south. *A.magnifica* var.*shastensis* as it occurs today is a taxon that exhibits more ecological and phenological amplitude compared to the other two taxa in the section *Nobilis* (Franklin and Dyrness,1973). Adams(1977), in his discussion of variation in *Juniperus ashei*, found that ancestral populations showed more variability in both terpene and morphological data than "recent" populations. This assertion was qualified with the note that small pockets of relict populations showed less variability than larger pockets (cf. Zavarin and Snajberk,1973). While ecological and phenological amplitude are not strictly equivalent to variability, the point can be made that a variable gene pool, sometimes identified by observable variability, would be more apt to be the source of different races than a more uniform gene pool.

Grant(1963) discusses ecological competition between large organisms like trees as a stimulus for speciation. While *A.procera* and *A.magnifica* var.*magnifica* have mostly allopatric distributions, they probably would not compete for space even in a sympatric distribution because they occur in different stages of forest succession. However, *A.magnifica* var.*shastensis* as described in the literature (see esp. Franklin and Dyrness,1973) would compete with both of the other taxa. This difference in successional role suggests that given the opportunity for migration into new localities as apparently occurred after Wisconsin glaciation, ecological races present in a very variable, ancestral

A.magnifica var.*shastensis* population could have increased their distribution. This contention assumes that the flavonoid profile reflects ecotypic variation, an assumption that is addressed at the beginning of this Discussion.

Stebbins(1975), citing Grant(1971), asserts that species can differentiate in as little as two to one hundred generations, so that adequate time has been available since the last glacial retreat for the dispersal of *A.procera* and *A.magnifica* var.*magnifica* into ranges that suggest that they are species discrete from *A.magnifica* var.*shastensis*. As mentioned above, *A.procera* growth characteristics do not decline from the centre to the northern limit of the range of that species, which suggests that northward migration of *A.procera* may still be taking place (Gessel and Oliver,1982).

Templeton(1981) cautions that habitat divergence as a mode of speciation occurs relatively rarely in the absence of isolating barriers, because it requires unoccupied niches. However, Raven(1976,p.300) points out that "patterns of differentiation involving saltational speciation appear to be characteristic of Mediterranean and arid areas in general". While western Washington, Oregon, and northern California have "humid" to "super-humid" climates (Little,1971), the pattern of precipitation is "mediterranean", with lots of winter precipitation and extended dry periods in the summer (Franklin and Dyrness,1973; Sprague,1941). Thus the area to which most of the *Abies* section *Nobilis* has been restricted to since the Pleistocene epoch is the kind of environment in which rapid differentiation of polytypic species would be expected. In addition, Grant(1971) points out that wind-pollinated woody plant taxa are commonly isolated as species by external factors like geography and so display a gradual divergence of ecological preference.

The assertion that ecological differentiation has guided species differentiation in the *Abies* section *Nobilis* assumes that gene flow between populations is not sufficient to obscure discrete ecological distribution (Templeton,1981; Davis and Heywood,1963; Stebbins,1950). The distinction between populations of the same operational taxa in this study except for southern *A.magnifica* var.*shastensis* that is indicated by the discriminant analysis supports this assumption. Similarily, as mentioned above, ancestral populations of *Juniperus ashei* have been found to be more distinct from each other than derived populations (Adams,1977).

Mattfeld(1930), in a discussion of the phylogeny of a European *Abies* complex, rejects the possibility of the intermediate race /species as an ancestral "gene centre" because it does not contain any unique genes or genotypes that are not present in the other two species. Analysis of more populations of northern *A.magnifica* var.*shastensis* from the Klamath-Siskyou mountain complex might be in order to acertain if flavonoid profiles are present in this region that are not present in either *A.magnifica* var.*magnifica* or *A.procera*. This lack of unique flavonoids is the strongest evidence against *A.magnifica* var.*shastensis* being considered as a taxon ancestral to the rest of the *Abies* section *Nobilis*, and it assumes that more complex flavonoid profiles are the primitive condition. Nevertheless, the conclusion can be made that at least southern *A.magnifica* var.*shastensis* is a relict subspecies or race.

Abies section *Nobilis* - a single polytypic species

A polytypic species is defined by Grant(1971) as "a plant group which contains numerous and diverse forms which intergrade with one another in their morphological characters and ecological preferences (p.295). Polytypic species appear to be the norm in the genus *Abies*. As a classic example, Stebbins(1950,p.280-282) discusses the origin of a new race of *Abies* through ancient hybridization that has taken place in Europe as reported by Mattfeld(1930). While three distinct species are named taxonomically in this European true fir complex, Stebbins suggests that these three species "should be treated as races of a single polytypic species".

Jacobs*et al.*(1984) have assessed populations of a portion of the *Abies* section *Balsamea*. In the eastern United States a systematic problem similar to that between *A.procera* and *A.magnifica* exists between *A.balsamea* and *A.fraseri* (Pursh)Poir. *A.balsamea* var.*phanerolepis* Fern. is a taxon with partly extruded cone scale bracts that is intermediate in some other respects between *A.balsamea* var.*balsamea* and *A.fraseri*. Electrophoretically, populations of *Abies* in the eastern United States appear to be one species, with the variety *phanerolepis* an intermediate that is likely not of hybrid origin. Jacobs*et al.*(1984) suggest that *A.fraseri* was derived during the Pleistocene epoch from an "adaptive line" characterized by *A.balsamea* var.*phanerolepis*. They found some correlation between bract exsertion and cool, moist habitats, and suggest that genetic drift and differential selection within isolated southeastern populations has accentuated differences that were initially clinal. Similarily, as discussed above, *A.procera* may have been derived from an "adaptive line" characterized

by *A.magnifica* var.*shastensis*.

Hamrick and Libby(1972), using common garden experiments and field data for *A.concolor*, uncovered four major morphological divisions in that species, including one in central Oregon and northwestern California and another in south-central Oregon and central and northeastern California. Some of the characteristics varied clinally and some ecotypically with latitude and/or elevation.

These three sets of results all support the contention that the *Abies* section *Nobilis* could be considered a single, very variable species. In these other *Abies* species, some ecotypic variation has shown a clinal pattern. Ecotypic and especially clinal variation can lead to geographic speciation (Templeton,1981; Grant,1971; Stebbins,1950). This is a conservative type of speciation (Grant,1971,p.114) and often results from an accumulation of differences between populations that arise as a result of a graded environmental factor, graded natural selection, or long-range hybridization and introgression (Heslop-Harrison,1963).

Visual assessment of histograms of variable flavonoid compounds (Results section; Appendix I) suggests a stepped cline in percent occurrence from northern populations through southern populations and vice versus in some of the compounds present in the *Abies* section *Nobilis* complex. However, the pattern of decreasing or increasing percent occurrence from one population to the next is not consistent across all of the populations sampled. Developmental changes from mature to immature trees and differential selection at different locations could account for this inconsistency (M.Knowles,pers.comm.).

While some ecotypic differentiation is expected in plant species (Antonovics,1971; Bradshaw,1972; Jain and Bradshaw,1966), a pattern of clinal variation in the flavonoids of the *Abies* section *Nobilis* is not

supported by the numerical analyses in this study, except in that most populations were fairly distinct from each other in the discriminant analyses. Numerical analysis of three different sets of morphological data for some of the same populations of *A.procera* and *A.magnifica* that were assessed in this study of flavonoid variation likewise did not show a simple clinal pattern of variation (W.H.Parker, pers. comm.). Using morphological attributes, these populations did not segregate out according to latitude, with the most "typical" form of *A.magnifica* var.*magnifica* not occurring where expected, and with the population of *A.procera* most similar to *A.magnifica* the one with the most northerly location in Washington.

Ustin(1976) reported a steep cline in morphological characteristics between *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis* in Tulare County, California. It is difficult to distinguish clinal variation in a species from the re-uniting of previously geographically isolated and morphologically divergent populations through hybridization (Heslop-Harrison,1963; Templeton,1981). This is especially true where ecological boundaries have recently been destroyed, for example after glacial retreat. Millar(1983) reports a steep cline in *Pinus muricata* D.Don from California similar to that reported by Ustin(1976) between *A.magnifica* var.*magnifica* and southern *A.magnifica* var.*shastensis*. Millar deduces that this cline is a result of secondary contact of formerly allopatric populations of *P.muricata* that has occurred since the climate has cooled and become wetter over the last 4 000 years. The pattern of climatic change and consequent plant migration in western North America make this a likely explanation for the current pattern of morphological variation in the *Abies* section *Nobilis* in south-central California. However, it does not fully explain the results of

the flavonoid analysis reported in this thesis. Many of the southern *A.magnifica* var.*shastensis* individuals that were assessed were most similar to *A.procera* in their flavonoid profiles, even more similar than individuals of northern *A.magnifica* var.*shastensis* which are geographically much closer to *A.procera*.

The amount of polymorphism that is present in a population or species is a function of the number of suitable habitats available (Dobzhansky,1950), as well as certain life forms and silvical characteristics (Hamrick*et al.*,1979b). Adaptive divergence occurs rapidly in plants in the presence of a changing environment (Stebbins,1947). Mountainous habitats are noted for drastic environmental changes over short distances (Hamrick,1979,p.96; and eg.Munz and Keck,1959). Extensive geologic activity in the western United States since the Eocene epoch has led to great habitat and ecological diversification in western North America (eg.Edmonds,1982). This kind of environmental diversity presumably would result in an accompanying diversification in the species that survived there. In the face of repeated migration and partial extinction, only those populations with a lot of variability in the potential characteristics of their offspring - ie. a large gene pool - could sustain themselves. Stebbins(1950, p.47) states that the presence of diverse and discontinuous habitats will result in fairly distinct ecotypes within species evolving under those conditions.

Grant(1963) maintains that polymorphic populations have an adaptive advantage, and he refers to "allopatric speciation". This occurs if an ancestral species has a large, variable gene pool which may allow a species to extend its range into new habitats/niches as they become available. Grant(1963) suggests that permanent heterozygosity is most

easily borne by plants which form regular dominant or subdominant elements in stable or closed communities, like climax tree species, for example *A.magnifica* var.*magnifica*. While local differentiation of successionally advanced species is expected (Hamrick*et al.*,1979b; Rehfeldt and Lester,1969), colonizing tree species like *A.procera* tend to be relatively uniform in genetic variability. These assumptions are confirmed by the flavonoid analyses in this study. Levin(1971) suggests that populations under strong selective pressure for a particular phenolic will have the fewest phenolic variants, as they will be under "strong stabilizing selection". *A.procera* appears to be less variable in many characteristics, including flavonoid profile, compared to other taxa in the section. Diversity of flavonoid profile in the section does seem to be correlated to successional role, and perhaps even to ecological factors like insect and disease resistance. No evidence is available concerning the biosynthesis of, the significance to survival of a tree or the mode of inheritance of the variable flavonoids in the *Abies* section *Nobilis*. However, *A.procera* is reported to have virtually no significant insect or disease pests (Franklin,1982). The significance of chance as a factor in assessing this correlation cannot be addressed with the information at hand. The homogeneity between populations of *A.procera* contrasted to the heterogeneity of populations of *A.magnifica* may have led to an overestimation of the taxonomic and genetic differences between the two species.

SUMMARY

The *Abies* section *Nobilis* appears to have a large, polymorphic gene pool. This gene pool contains at least two specialized phenetic "lines" that are well-differentiated ecologically, morphologically and chemically, as well as a more amorphous third entity. One line is represented by a fairly uniform pioneering taxon that may have the potential to expand its range northward - *A.procera*. The other line, represented by the taxon *A.magnifica* var.*magnifica*, is a climax tree with locally distinct populations that is probably at the limits of its distribution, especially to the south.

The section has survived in diverse and changing habitats since the Pliocene epoch. Survival and reproduction under such long-term stressful conditions have resulted in a lot of variability in most western North American fir species, particularly compared to eastern species (Stebbins,1950). This variability is evident in the needle flavonoid profiles of the *Abies* section *Nobilis* as well as in morphological and physiological characters. Environmental conditions since the last glacial retreat have apparently enabled populations exhibiting specific segments of this variability to migrate into new and relatively stable ecosystems. The two segments of this variability that are recognized taxonomically as species are distinct from each other throughout most of their allopatric ranges.

The "amorphous third entity" referred to above is represented by the taxon *A.magnifica* var.*shastensis* which cannot be consistently distinguished from *A.magnifica* var.*magnifica* or from *A.procera* on the basis of ecological preference, morphology or chemical characteristics. It is thus difficult to assign specific status to this taxon, even though the *Abies* section *Nobilis* appears to have more diverse elements than are adequately represented by the two recognized species designations.

One extant element may closely resemble a taxon postulated to be ancestral to the *Abies* section *Nobilis* that is most conveniently designated as *"A.shastensis"*. Populations of *"A.shastensis"* may have survived the ecological upheavals of the Pliocene and Pleistocene epochs as the currently recognized taxon *A.magnifica* var.*shastensis* in two widely separated locations. Subsequently, populations from the Klamath Mts. that straddle the border between northwestern California and southwestern Oregon may have extended their ranges east into the Cascade Mts. and south into the North Coast Range of California, while populations in south-central California at the southern extreme of the Sierra Nevada Mts. remained restricted in distribution. These three modern occurences of *A.magnifica* var.*shastensis* - north, west and south of the typical variety - are somewhat differentiated chemically, as expected for taxa that have been separated into different environments for an extended period of time.

Some gene flow or hybridization is likely occurring between the species *A.procera* and *A.magnifica* between 44 degrees N latitude and 40 degrees N latitude in the Cascade Mts. of Oregon and northern California. These hybrid products, designated *Abies* x *shastensis* by Liu(1971), are difficult to distinguish from those elements of the gene pool

that most resemble the ancestral taxon, *"A.shastensis "*.

While the *Abies* section *Nobilis* might be considered functionally a single, polytypic species, ecological, morphological and chemical, especially flavonoid, characteristics are diverse enough to warrant the recognition of at least three taxa :

1. *A.procera* Rehd., Rhodora 42:522,1940 - a species that grows principally in Washington and Oregon between about 48 degrees 30 minutes N latitude and 44 degrees N latitude;
2. *A.magnifica* A.Murray var.*magnifica*,. Proc.Roy.Hort.Soc. 3:318,f.25-33,1863 - a species that grows principally in the Sierra Nevada Mts. of California; and
3. *A.magnifica* A.Murray var.*shastensis* Lemm., Rep.Calif.State Bd.For. III:145, 1890 - all trees that appear intermediate in morphological characteristics between *A.procera* and *A.magnifica* var.*magnifica*.

A.magnifica var.*shastensis* might more correctly be identified as three or four separate taxa :

1. the hybrid product *A.procera* x *A.magnifica* (*Abies* x *shastensis sensu* Liu,1971);
2. the relictual populations of *A.magnifica* var.*shastensis* in Tulare County, California and the Klamath Mts., which are differentiated populations; and
3. *A.magnifica* var.*shastensis* in the North Coast Ranges of California, which probably consists of relatively recent immigrants from

the relictual *A.magnifica* var.*shastensis* in the Klamath Mts.

The assignment of new varietal status to *A.magnifica* var.*shastensis* from Tulare County, California should be considered after a thorough morphological analysis of populations from that locale is made. Resolution of inconsistencies in the flavonoid results would require (1) a study comparing the flavonoids of immature to the flavonoids of mature trees in the *Abies* section *Nobilis* to determine the importance of flavonoid variation due to age of the tree, (2) a study designed to assess phenotypic plasticity of needle flavonoids in the *Abies* section *Nobilis* and (3) increased, uniform sample sizes to allow better distinction between flavonoid profiles that overlap in variable compound occurrence. As well, more populations in the Coast Ranges and the Klamath-Siskyou Mts. complex should be sampled to fully complete the assessment of flavonoid variation in the *Abies* section *Nobilis*.

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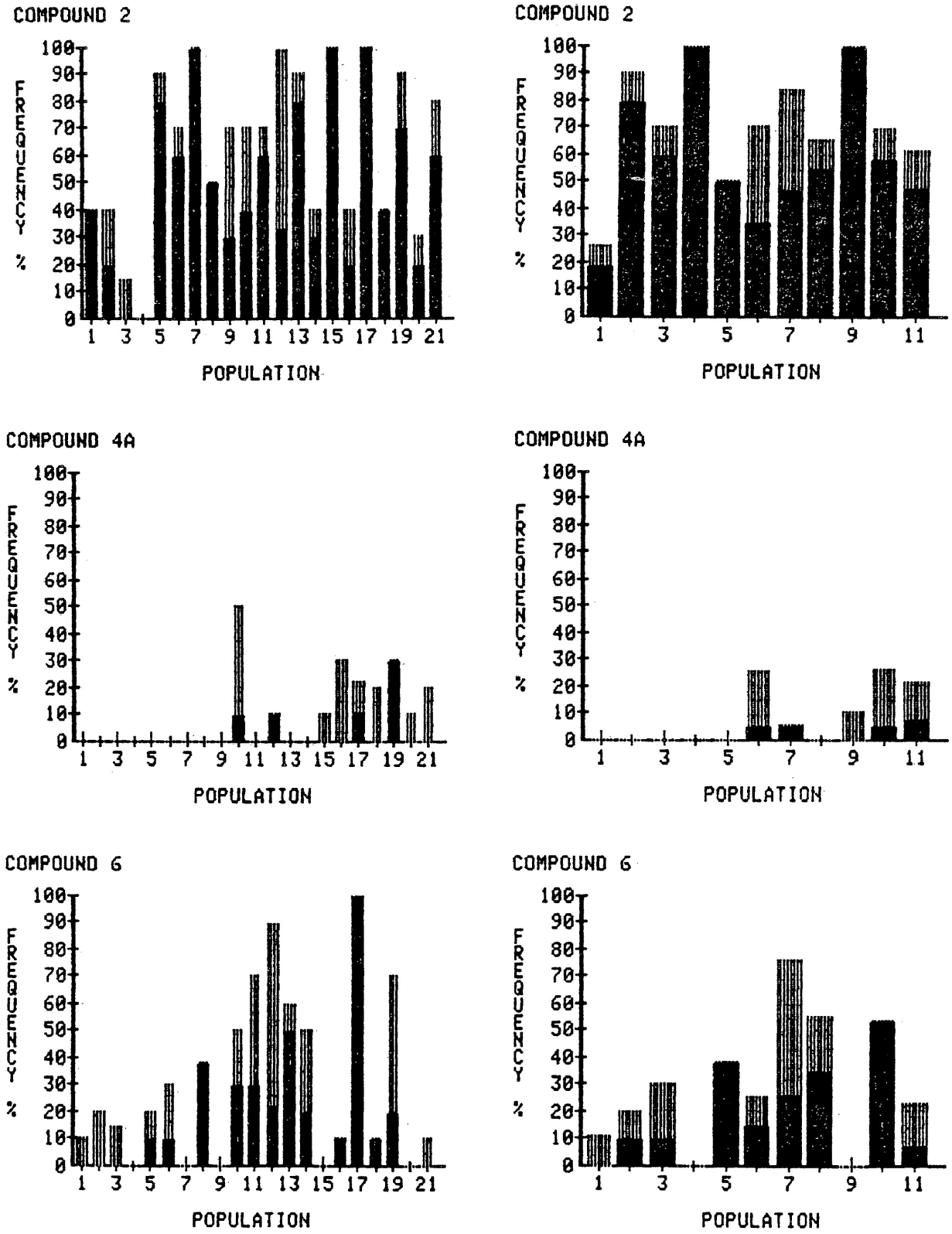
APPENDICES

APPENDIX I

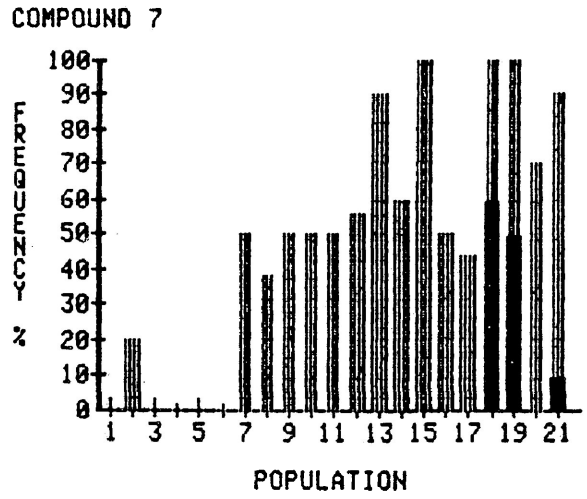
FREQUENCY HISTOGRAMS OF VARIABLE COMPOUNDS

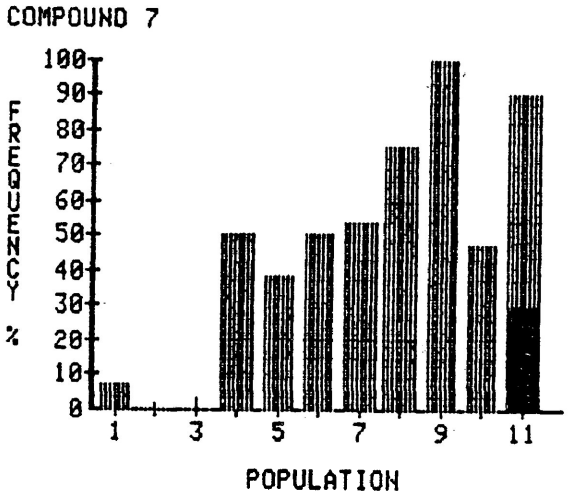
I.A. Table of population codes for frequency histograms of variable compounds.

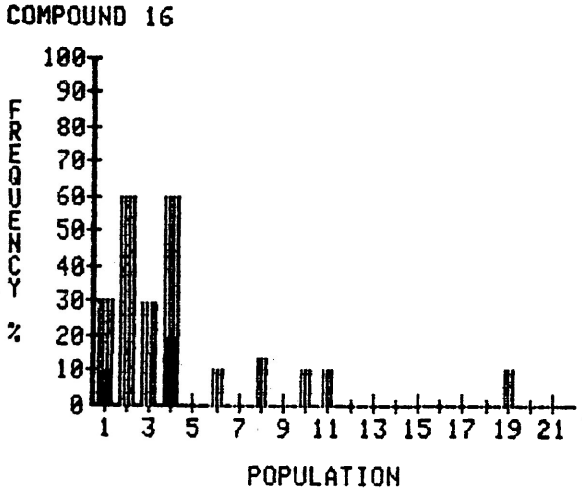
UNCONSOL. POP.CODE	CONSOL. POP.CODE	POPULATION IDENTIFICATION
A.procera		
1	1	Suntop Lookout High - mature
2	1	Suntop Lookout High - immature
3	1	Suntop Lookout Low -mature
4	1	Suntop Lookout Low - immature
5	2	Sleeping Beauty - mature
6	3	Sardine Mt. - mature
7	4	Douglas Co. - immature
Northern *A.magnifica* var.*shastensis*		
8	5	Mt. Ashland - mature
9	6	Mt. Shasta - mature
10	6	Mt. Shasta - immature (Siskyou Co.)
11	7	Brushy Mt. - mature
12	7	Brushy Mt. - immature
A.magnifica var.*magnifica*		
13	8	Grizzly Ridge - mature
14	8	Grizzly Ridge - immature
15	9	Alpine Co. - immature
16	10	Mt. Tom - mature
17	10	Mt. Tom - immature
Southern *A.magnifica* var.*shastensis*		
18	11	Tulare Co., Jordan Peak - immature
19	11	Tulare Co., Sherman Peak - immature
20	11	Tulare Co., Bone Meadow - immature
21	11	Tulare Co., Tobias Pass - immature

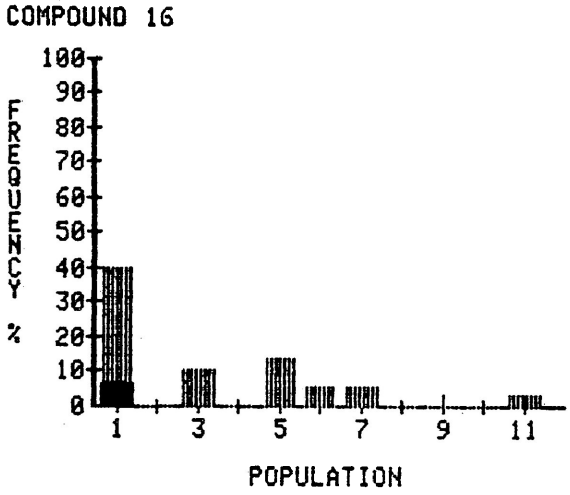


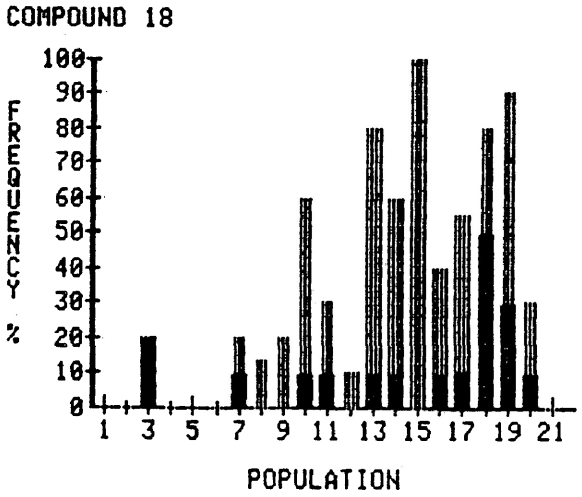
I.B. Frequency histograms of occurrence of variable compounds 2, 4A and 6. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are listed in I.A. Light shading = present in trace amounts; dark shading = present in more than trace amounts. For compound identification, see text.

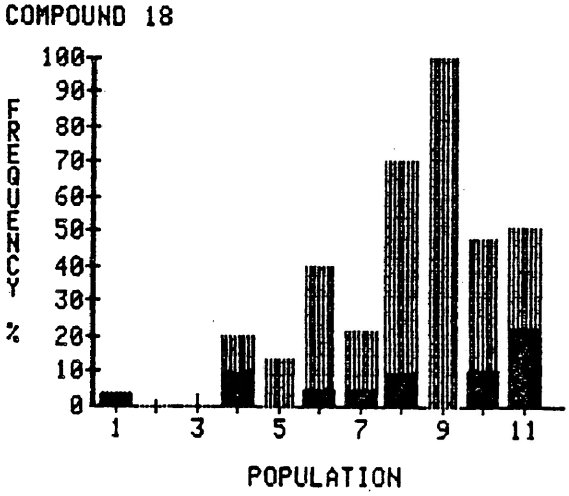




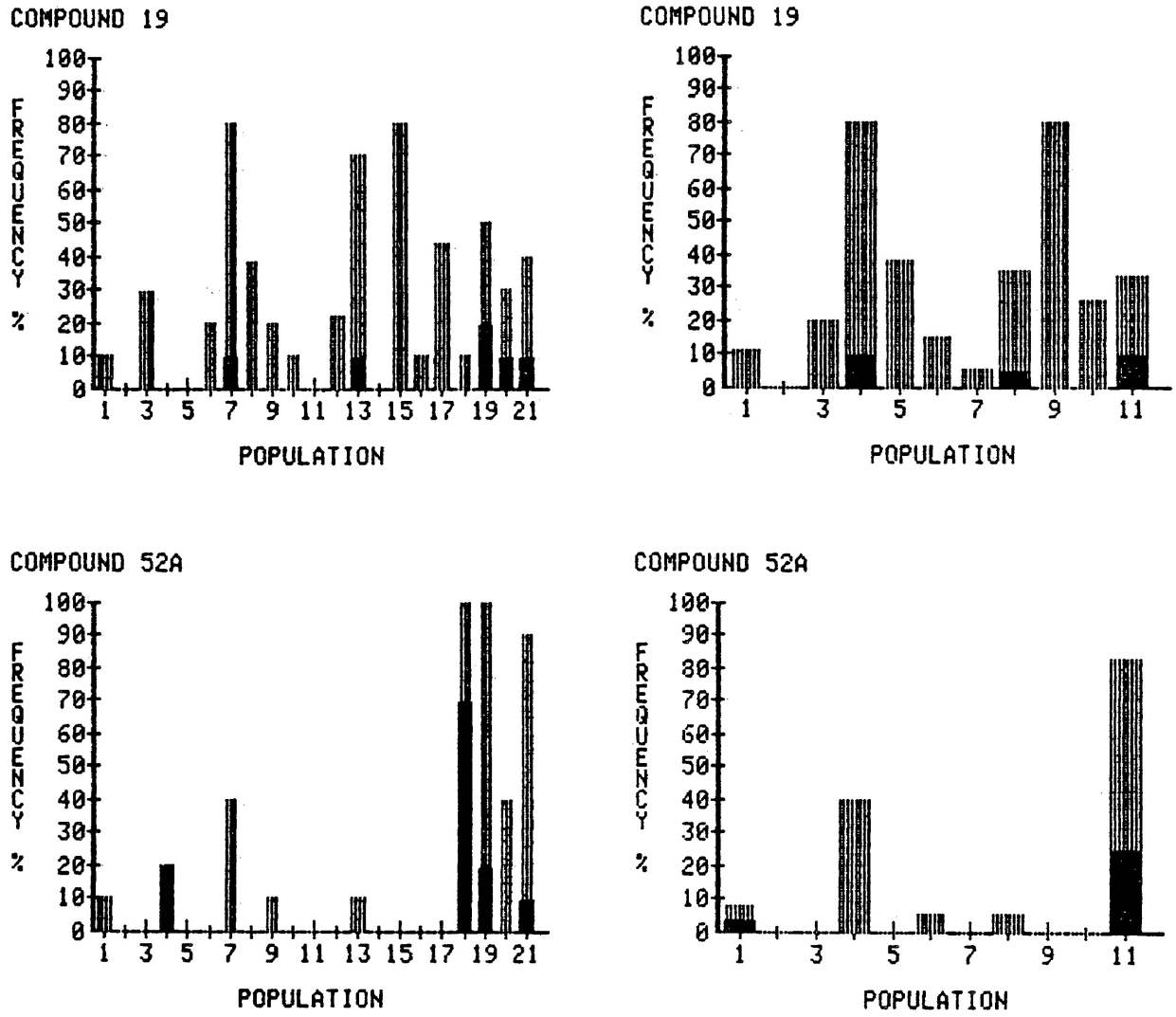








I.C. Frequency histograms of occurrence of variable compounds 7, 16 and 18. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are listed in I.A. Light shading = present in trace amounts; dark shading = present in more than trace amounts. For compound identification, see text.



I.D. Frequency histograms of variable compounds 19 and 52A. Unconsolidated populations are on the left, consolidated populations are on the right. Population codes are listed in I.A. Light shading = present in trace amounts; dark shading = present in more than trace amounts. For compound identification, see text.

APPENDIX II

BASIC DATA MATRIX OF THE OCCURRENCE EACH VARIABLE
COMPOUND IN EACH TREE,
BY POPULATION

II. Basic data matrix for numerical analyses of Abies section Nobilis.

	(1)	VARIABLE COMPOUNDS (2)																								
POPULATION		2	4A	5A	52A	6	7	8A	23A	13	14	15	18	19	29	31	36	34	35	47	53	16	48	46	45B	
TREE																										
1	AOO	2	0	0	0	0	0	0	1	2	2	2	0	0	1	2	2	0	0	1	0	1	0	0	0	
	AOP	0	0	0	0	0	0	0	0	2	2	1	0	0	2	2	2	0	0	0	0	1	0	0	0	
	AOQ	0	0	0	0	0	0	0	0	2	2	2	0	0	1	2	2	1	0	0	0	0	0	0	0	
	AOR	0	0	0	0	0	0	0	0	2	1	1	0	0	2	2	2	0	0	0	0	0	0	0	0	
	AOS	2	0	0	0	0	0	0	0	2	2	2	0	1	2	2	2	0	0	0	0	0	0	0	0	
	AOT	2	0	0	0	1	0	0	0	2	2	2	0	0	2	2	2	0	0	0	1	2	1	0	0	
	AOU	0	0	0	1	0	0	0	0	1	1	0	0	0	1	2	2	0	1	0	0	0	0	0	0	
	AOW	2	0	0	0	0	0	0	0	2	2	1	0	0	1	2	2	0	0	1	0	0	0	0	0	
	AOV	0	0	0	0	0	0	0	0	1	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0	
	AOX	0	0	0	0	0	0	0	0	2	1	1	0	0	2	2	1	0	0	0	0	0	0	0	0	
1R	AO1	1	0	0	0	0	0	0	0	1	1	0	0	0	1	2	2	1	0	2	0	0	0	0	0	
	AO2	2	0	0	0	1	1	0	0	1	2	0	0	0	2	2	2	1	0	2	0	1	0	0	2	
	AO7	0	0	0	0	0	0	0	0	2	2	2	0	0	2	2	2	0	1	2	0	1	0	0	0	
	AO8	0	0	0	0	0	0	0	0	1	2	2	0	0	2	2	2	0	0	2	0	0	0	0	0	
	AO9	0	0	0	0	0	0	0	0	2	2	2	0	0	2	2	2	1	0	2	0	1	0	0	0	
2	API	1	0	0	0	0	0	0	0	2	2	1	0	1	2	2	2	1	1	0	1	0	0	0	0	
	APJ	0	0	0	0	0	0	0	0	2	2	2	0	0	1	2	2	1	1	2	1	0	0	0	0	
	APK	0	0	0	0	0	0	0	0	2	2	1	0	0	1	2	2	1	0	1	1	1	0	0	0	
	APL	0	0	0	0	1	0	0	0	2	2	2	0	0	1	2	2	0	0	0	1	1	1	0	0	
	APM	0	0	0	0	0	0	0	0	1	2	1	0	0	2	2	2	2	0	2	0	0	2	1	0	
	APN	0	0	0	0	0	0	0	0	2	2	1	0	0	2	2	2	1	0	1	0	0	0	0	0	
	APO	0	0	0	0	0	0	0	0	2	2	2	0	0	2	2	2	1	1	0	1	0	0	0	0	
2R	AO11	0	0	0	0	0	0	0	0	1	2	1	0	0	2	2	2	1	0	2	1	0	0	0	0	
	AO12	0	0	0	0	0	0	0	0	1	2	0	0	0	2	2	2	2	0	2	1	0	0	0	0	
	AO14	0	0	0	2	0	0	0	0	2	2	2	0	0	2	2	2	2	0	2	0	1	0	0	0	
	AO14	0	0	0	0	0	0	0	0	2	2	2	0	0	2	2	2	1	0	2	1	2	0	0	0	
	AO15	0	0	0	0	0	0	0	0	2	1	1	0	0	2	2	2	1	0	2	1	0	0	0	0	
3	ASH	2	0	0	0	0	0	0	0	0	1	0	0	0	1	2	2	0	1	0	1	0	0	0	0	
	ASI	2	0	0	0	0	0	0	0	1	1	1	0	0	2	2	2	0	1	0	2	0	0	0	0	
	ASJ	2	0	0	0	0	0	0	0	1	1	1	0	0	1	2	2	0	0	1	0	0	0	0	0	
	ASK	2	0	0	0	0	0	0	0	2	1	2	0	0	1	2	2	0	0	1	1	0	0	0	0	
	ASL	0	0	0	0	0	0	0	0	1	2	1	0	0	1	2	2	0	0	0	1	0	0	0	0	
	ASM	2	0	0	0	0	0	0	0	1	1	0	0	0	2	2	2	0	0	0	1	0	0	0	0	
	ASN	2	0	0	0	2	0	0	0	0	0	0	0	0	1	2	2	0	0	0	1	0	1	1	0	
	ASO	2	0	0	0	0	0	0	0	1	1	1	0	0	1	2	2	0	1	1	1	0	0	0	0	
	ASP	2	0	0	0	0	0	0	0	1	1	1	0	0	2	2	2	0	0	0	1	0	0	0	0	
	ASQ	1	0	0	0	0	0	0	0	1	2	1	0	0	2	2	2	0	1	0	1	0	0	0	0	

II. (Continued).

	(1)	VARIABLE COMPOUNDS (2)																								
POPULATION		2	4A	5A	52A	6	7	8A	23A	13	14	15	18	19	29	31	36	34	35	47	53	16	48	46	45B	
	TREE																									
4	ARX	1	0	0	0	1	0	0	0	1	1	1	0	0	1	2	2	1	1	0	0	0	0	0	0	
	ARY	2	0	0	0	0	0	0	0	2	2	1	0	0	1	2	2	0	0	0	0	0	0	1	0	
	ARZ	0	0	0	0	0	0	0	0	2	0	2	0	0	0	2	1	0	0	0	1	0	1	0	0	
	ASA	0	0	0	0	1	0	0	0	2	2	1	0	0	2	2	2	1	1	0	1	0	0	0	0	
	ASB	2	0	0	0	0	0	0	0	0	2	0	0	0	1	1	2	0	1	1	2	0	0	0	0	
	ASC	2	0	0	0	0	0	0	0	2	2	1	0	1	1	2	2	1	0	0	1	0	0	0	0	
	ASD	2	0	0	0	0	0	0	0	1	2	1	0	1	2	2	2	0	1	0	1	0	0	0	0	
	ASE	2	0	0	0	0	0	0	0	2	2	2	0	0	1	2	2	1	1	0	0	0	0	0	0	
	ASF	2	0	0	0	2	0	0	0	2	2	1	0	0	2	2	2	0	1	0	1	1	0	0	0	
	ASG	0	0	0	0	0	0	0	0	0	2	1	0	0	2	2	1	0	1	0	0	0	0	0	0	
5R	AP250	2	0	2	1	0	1	0	0	2	2	2	2	1	2	2	2	2	0	0	1	0	2	2	0	
	AP252	2	0	0	1	0	1	0	0	1	2	2	0	1	2	2	2	1	0	0	1	0	2	1	0	
	AP253	2	0	0	0	0	1	0	0	0	2	2	1	1	2	2	2	1	0	0	1	0	2	0	0	
	AP255	2	0	0	0	0	0	0	0	0	2	2	0	1	2	2	2	1	0	0	1	0	2	1	0	
	AP257	2	0	0	1	0	1	0	0	0	2	0	0	0	2	2	2	1	0	0	1	0	0	0	0	
	AP259	2	0	0	0	0	0	0	0	0	2	2	0	1	2	2	2	0	1	0	1	0	2	1	0	
	AP260	2	0	0	0	0	0	0	0	0	2	2	0	0	2	2	2	1	0	0	1	0	2	1	0	
	AP261	2	0	0	1	0	1	0	0	0	2	2	0	1	2	2	2	1	0	0	1	0	2	1	0	
	AP275	2	0	0	0	0	0	0	0	0	2	2	0	2	2	2	2	1	0	0	1	0	0	0	0	
	AP276	2	0	0	0	0	0	0	0	0	2	2	0	1	2	2	2	0	0	0	1	0	2	0	0	
6	ARO	2	0	0	0	2	0	0	0	2	2	2	0	0	2	2	2	1	0	0	0	0	0	0	0	
	ARP	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	0	0	0	0	0	2	0	0	
	ARQ	0	0	0	0	0	1	0	0	1	0	1	0	1	2	2	2	0	0	0	0	0	0	0	0	
	ARS	2	0	0	0	2	1	0	0	2	2	2	0	1	2	2	2	1	1	0	0	1	1	0	0	
	ART	0	0	0	0	2	0	0	0	1	1	1	0	0	1	2	2	0	0	0	0	0	1	0	0	
	ARU	0	0	0	0	0	0	0	0	1	0	0	0	0	1	2	2	0	0	0	0	0	1	0	0	
	ARV	2	0	0	0	0	1	0	0	2	1	1	0	0	1	2	2	0	0	0	0	0	0	0	0	
	ARW	2	0	0	0	0	0	0	0	2	2	2	0	0	2	2	2	0	0	0	1	0	1	0	0	
7	ARD	2	0	0	0	0	1	0	0	0	0	0	1	0	1	2	2	0	0	0	0	0	1	1	0	
	ARE	1	0	0	0	0	1	0	0	2	1	1	0	0	1	1	2	0	0	0	0	0	0	0	0	
	ARF	0	0	0	0	0	1	0	0	1	0	0	0	1	1	2	2	0	0	0	0	0	0	0	0	
	ARG	2	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	
	ARH	1	0	0	0	0	1	0	0	2	1	1	0	0	1	2	2	0	0	0	0	0	0	0	0	
	ARI	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	2	0	0	0	0	0	0	0	1	
	ARJ	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0	0	
	ARK	0	0	0	1	0	1	0	0	2	0	1	1	1	2	2	2	0	0	0	0	0	0	0	0	
	ARL	2	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	ARM	1	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	0	0	0	1	0	2	1	0	

II. (Continued).

	(1)	VARIABLE COMPOUNDS (2)																								
POPULATION		2	4A	5A	52A	6	7	8A	23A	13	14	15	18	19	29	31	36	34	35	47	53	16	48	46	45B
	TREE																									
7R	AP300	1	0	0	0	0	0	0	0	1	1	0	0	0	2	2	2	0	0	0	2	0	2	1	0
	AP301	1	2	0	0	0	0	0	0	0	0	0	1	0	2	2	2	1	0	0	1	0	2	2	0
	AP302	0	0	2	0	0	1	0	0	1	0	0	1	0	2	2	2	1	0	0	1	0	2	1	0
	AP303	2	0	0	0	1	1	0	0	2	2	0	1	0	2	2	2	0	0	0	2	1	2	1	0
	AP304	1	1	0	0	2	1	0	0	0	2	0	2	0	2	2	2	1	0	0	2	0	2	2	0
	AP305	2	1	0	0	2	0	0	0	0	2	0	2	1	2	2	2	1	0	0	1	0	2	2	0
	AP306	2	0	0	0	2	0	0	0	1	2	0	1	0	2	2	1	1	0	0	0	0	2	1	0
	AP307	2	0	0	0	1	1	0	0	1	1	0	0	0	2	2	2	1	0	0	2	0	0	0	0
	AP308	0	1	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	2	1	0
	AP309	0	1	0	0	0	1	0	0	0	0	0	0	0	2	2	2	1	0	0	0	0	1	1	0
8	APP	2	0	0	0	2	1	0	0	1	1	1	2	0	1	2	2	0	0	1	1	0	2	1	0
	APQ	0	0	0	0	2	1	0	0	1	0	0	0	0	1	1	1	0	0	0	2	0	0	0	0
	APR	0	0	0	0	2	1	0	0	1	0	0	0	0	1	2	2	1	0	0	0	0	0	0	0
	APS	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0
	APT	2	0	0	0	1	1	0	1	1	1	1	1	0	1	2	2	0	0	0	1	1	0	0	0
	APU	2	0	0	0	2	1	0	0	1	0	0	0	0	1	2	2	0	2	0	2	0	0	0	0
	APV	2	0	0	0	1	0	0	0	2	1	1	0	0	0	1	2	0	0	0	1	0	0	0	0
	APW	2	0	0	0	0	0	0	2	1	2	2	0	0	0	1	2	0	0	0	0	0	2	0	0
	APX	2	0	0	0	1	1	0	0	1	0	0	1	0	1	2	2	0	0	0	0	0	2	1	0
	APY	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	1	0	0	0	0
8R	AO21	1	0	0	0	1	0	0	0	0	0	0	0	0	2	2	2	1	0	0	2	0	2	2	0
	AO22	2	0	0	0	1	1	0	0	0	0	0	0	0	2	2	2	1	0	0	2	0	2	1	0
	AO23	2	2	0	0	1	0	0	0	0	0	0	0	0	2	2	2	0	0	0	2	0	0	0	0
	AO24	1	0	0	0	2	0	0	0	1	0	0	1	1	2	2	2	1	0	0	1	0	2	1	0
	AO25	2	0	0	0	2	1	0	0	0	0	0	0	0	2	2	1	1	0	1	0	0	2	1	0
	AO26	1	0	0	0	1	1	0	0	1	0	0	0	0	2	2	2	1	0	2	1	0	2	1	0
	AO27	1	0	0	0	1	1	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	0	0
	AO28	1	0	0	0	1	1	0	0	0	0	0	0	0	2	2	2	1	0	0	1	0	0	0	0
	AO29	1	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	1	0	0	1	0	2	1	0
9	AQT	1	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	2	1	0
	AQU	2	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0
	AQV	0	0	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2	0	0
	AQW	2	0	0	0	2	1	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	2	1	0
	AQX	2	0	0	0	2	1	0	0	2	1	0	1	1	1	2	2	0	0	0	1	0	2	0	0
	AQY	2	0	0	0	2	1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
	AQZ	2	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	2	0	0
	ARA	2	0	0	1	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	2	0	2	1	0
	ARB	2	0	0	0	2	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	1	0
	ARC	2	0	0	0	0	1	0	0	1	2	2	0	0	0	0	0	0	0	0	1	0	2	1	0

II. (Continued)

POPULATION	(1) TREE	VARIABLE COMPOUNDS (2) 2	4A	5A	52A	6	7	8A	23A	13	14	15	18	19	29	31	36	34	35	47	53	16	48	46	45B
9R	AO41	2	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2	0	2	1	0
	AO42	0	0	0	0	0	1	0	0	2	2	0	1	0	0	0	0	1	0	0	2	0	2	1	0
	AO43	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0
	AO44	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	0	1	0	0	2	0	2	2	0
	AO45	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	2	0	1	1	0
	AO46	2	0	0	0	0	1	0	0	2	2	0	0	0	0	0	1	1	0	0	2	0	2	2	0
	AO47	0	0	0	0	2	1	0	0	1	2	0	1	0	0	0	0	1	0	0	2	0	2	1	0
	AO48	0	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	2	0	0	2	0	1	0	0
	AO49	2	0	0	0	1	1	0	0	1	1	0	2	0	0	0	0	1	0	0	1	0	2	2	0
	AO50	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	2	0	2	1	0
10R	320	2	0	0	0	0	1	2	0	1	1	1	1	1	0	0	1	1	0	0	1	0	2	1	2
	321	2	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	2	0	0	1	0	2	1	0
	325	2	1	0	0	0	1	2	0	1	1	0	1	0	0	0	0	2	0	0	1	0	2	1	2
	328	2	0	2	0	0	1	0	0	0	1	0	1	1	0	0	0	2	0	0	1	0	2	2	0
	329	2	0	0	0	0	1	2	0	2	2	1	1	1	0	0	0	2	0	0	1	0	2	1	0
	330	2	0	0	0	0	1	0	0	1	2	0	1	1	0	0	0	1	0	0	1	0	1	0	0
	331	2	0	2	0	0	1	0	0	1	1	0	1	1	0	0	0	2	0	0	1	0	2	1	2
	349	2	0	0	0	0	1	0	0	1	2	0	1	0	0	0	0	0	0	0	1	0	2	0	2
	350	2	0	0	0	0	1	0	0	2	2	1	1	1	0	0	0	2	0	0	1	0	2	0	2
	352	2	0	0	0	0	1	0	0	1	1	0	1	1	0	0	1	2	0	0	1	0	1	1	2
11	AQJ	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	AQK	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
	AQL	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	AQM	2	1	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0
	AQN	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	AQO	2	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	2	0	2	1	0
	AQP	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	1	0	2	0	0
	AQQ	0	1	0	0	0	1	0	0	2	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
	AQR	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	AQS	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0
11R	AO31	2	0	0	0	2	1	0	1	2	2	0	0	0	0	0	0	0	0	0	1	0	2	0	0
	AO32	2	0	0	0	2	0	0	1	0	1	0	0	1	0	0	0	2	0	0	1	0	2	1	0
	AO33	2	0	0	0	2	0	0	0	2	2	0	1	0	0	0	0	2	0	0	1	0	2	1	0
	AO35	2	0	0	0	2	0	0	0	0	1	0	2	0	0	0	0	2	0	0	2	0	2	2	0
	AO36	2	0	0	0	2	1	0	0	2	1	1	1	0	1	0	1	1	0	0	1	0	2	2	0
	AO37	2	0	1	0	2	1	0	0	1	1	0	1	1	0	0	0	1	0	0	0	0	2	1	0
	AO38	2	0	2	0	2	1	0	0	1	1	0	1	0	0	0	0	1	0	0	1	0	2	1	0
	AO39	2	2	1	0	2	0	0	0	2	2	0	0	1	0	0	0	1	0	0	1	0	2	1	0
	AO40	2	1	0	0	2	0	0	0	1	1	0	0	1	0	0	0	1	0	0	0	0	2	1	0

II. (Continued)

(1) POPULATION	TREE	VARIABLE COMPOUNDS (2) 2	4A	5A	52A	6	7	8A	23A	13	14	15	18	19	29	31	36	34	35	47	53	16	48	46	45B
12A	81-1	0	0	2	2	0	1	0	0	0	0	0	2	0	1	1	0	2	0	0	1	0	2	1	2
	-2	2	0	2	2	0	1	0	1	2	1	0	1	0	0	0	0	1	0	0	2	0	2	1	2
	-3	0	1	2	2	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	2	0	1	1	2
	-4	0	0	2	1	2	2	0	0	1	1	0	2	0	0	0	0	0	0	0	1	0	2	2	2
	-5	0	0	2	2	0	1	2	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0
	-6	2	1	2	2	0	2	0	0	2	0	0	2	0	0	0	0	1	0	0	2	0	2	1	2
	-7	2	0	2	2	0	2	0	1	0	0	0	2	1	0	0	0	1	0	0	1	0	1	1	0
	-8	0	0	2	1	0	2	2	0	2	0	0	1	0	0	0	0	1	0	0	2	0	2	1	2
	-9	0	0	2	1	0	2	2	1	0	0	0	0	0	1	1	0	2	0	0	0	0	1	1	0
	-10	2	0	0	2	0	2	0	1	0	0	0	2	0	1	2	1	1	0	0	1	0	2	1	2
12B	81-11	2	0	2	2	1	2	1	1	0	0	0	1	0	0	0	0	1	0	0	1	0	2	1	2
	-12	0	2	2	1	0	1	1	1	1	0	0	1	1	0	0	0	1	0	0	1	0	1	1	2
	-13	2	0	2	2	0	2	0	1	2	0	0	2	0	0	0	0	1	0	0	1	0	2	2	2
	-14	2	0	2	1	0	2	0	1	1	0	1	1	0	0	0	0	1	0	0	1	1	2	1	2
	-15	2	0	2	1	1	1	0	0	0	0	0	2	0	1	1	1	0	0	0	1	0	2	1	2
	-16	2	0	2	1	2	2	0	0	0	1	0	0	2	0	0	0	1	0	0	1	0	2	1	2
	-17	2	2	2	1	2	1	0	0	0	0	0	1	0	2	2	2	0	0	0	1	0	1	0	0
	-18	1	0	2	1	1	1	0	1	1	1	0	2	2	0	0	0	1	0	0	1	0	2	0	2
	-19	1	2	2	1	1	2	0	1	0	0	0	1	1	1	1	1	1	0	0	1	0	2	0	2
	-20	2	0	2	1	1	1	0	0	1	0	0	1	1	1	1	1	0	0	0	1	0	2	1	1
12C	81-31	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	-32	0	0	2	0	0	0	0	0	2	0	2	0	1	0	0	0	1	0	0	0	0	0	0	0
	-33	0	1	2	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	-34	0	0	2	0	0	1	0	0	2	0	0	0	2	0	0	1	1	0	0	0	0	0	0	2
	-35	0	0	2	0	0	1	2	0	2	2	0	1	1	0	0	0	0	0	0	1	0	0	0	2
	-36	0	0	2	0	0	1	0	0	2	0	0	2	0	0	0	1	1	0	0	0	0	2	1	2
	-37	0	0	2	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
	-38	1	0	2	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	-39	2	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	-40	2	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	2	0	0
12D	81-41	2	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	-42	1	0	0	1	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	-43	2	0	2	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
	-44	2	0	2	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	-45	2	1	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0
	-46	2	0	2	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1
	-47	1	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	-48	2	0	2	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0
	-49	0	0	0	0	0	1	2	0	2	0	0	0	1	0	0	0	0	0	0	1	0	2	1	0
	-50	0	0	2	1	0	2	0	1	2	1	0	0	2	1	1	1	1	0	0	0	0	2	1	0

(1) Population codes are in Table 2.

(2) 0 = absent; 1 = present in trace amounts; 2 = present.

APPENDIX III

SUMMARY OF CORRELATION CO-EFFICIENTS BETWEEN VARIABLES

Summary of correlation coefficients between variables that are greater than |0.40|. Negative correlations are indicated by brackets. Group composition was dictated solely by ease of presentation.

GROUP 1

	52A	5A	7	23A	45B
52A	1				
5A	0.61363	1			
7	0.52181	0.49067	1		
23A	0.48454	0.41084	(0.39435)	1	
45B	0.44031	0.49730	0.48141	(0.34276)	1

GROUP 2

	5A	7	14	15	29	31	36
5A	1						
7	(0.52181)	1					
14	(0.37534)	(0.27483)	1				
15	(0.28377)	(0.35255)	0.57351	1			
29	(0.37029)	(0.34319)	(0.31616)	0.42257	1		
31	0.45534	0.42450	(0.27978)	0.43995	0.85389	1	
36	0.46466	0.43983	(0.33282)	0.47728	0.84097	0.92951	1

GROUP 3

	7	18	34	48	46	45B
7	1					
18	0.42152	1				
34	(0.18058)	(0.28924)	1			
48	(0.35176)	0.52742	(0.33774)	1		
46	(0.31365)	0.51367	0.43929	0.72484	1	
45B	(0.48141)	0.44972	(0.27621)	(0.26639)	(0.19807)	1

GROUP 4

8A - slight correlation with 7 (less than 0.31)
13 - slight correlation with 14 and 15 (less than 0.36)
53 - slight correlation with 46 (less than 0.31)
47 - slight correlation with 29,31,36 (less than 0.31)
16 - slight correlation with 15,47 (less than 0.32)

APPENDIX IV

RESULTS OF DISCRIMINANT ANALYSIS

IV.A. Discriminant analysis results+ for 21 populations, using the direct procedure in SPSS DISCRIMINANT.

FUNCT.	EIGEN-VALUE	% OF VARIANCE	CUMUL. %	CANON. CORR.	WILK'S LAMBDA	CHI-SQUARED	D.F.	SIG.
Before discriminating power of the first function was removed:					0.0001	1648.00	480	
1	8.825	41.30	41.30	0.9477	0.0006	1260.80	437	0.000
2	4.737	22.17	63.47	0.9087	0.0034	964.66	396	0.000
3	1.477	6.91	70.39	0.7722	0.0084	810.92	357	0.000
4	1.290	6.04	76.42	0.7505	0.0191	670.48	320	0.000
5	1.128	5.28	81.71	0.7281	0.0407	542.46	285	0.000
6	0.817	3.82	85.53	0.6705	0.0740	441.26	252	0.000
7	0.663	3.10	88.63	0.6313	0.1231	355.07	221	0.000
8	0.572	2.67	91.30	0.6029	0.1934	278.49	192	0.000
9	0.476	2.23	93.53	0.5680	0.2855	212.47	165	0.000
10	0.300	1.40	94.94	0.4805	0.3712	167.98	140	0.054
11	0.274	1.28	96.22	0.4634	0.4727	127.00	117	0.249
12	0.249	1.16	97.38	0.4464	0.5904	89.33	96	0.672
13	0.207	0.97	98.35	0.4139	0.7124	57.49	77	0.953
14	0.113	0.53	98.88	0.3181	0.7927	39.38	60	0.982
15	0.086	0.40	99.28	0.2808	0.8605	25.46	45	0.992
16	0.075	0.35	99.63	0.2642	0.9251	13.20	32	0.999
17	0.043	0.20	99.83	0.2040	0.9653	5.99	21	0.999
18	0.022	0.10	99.94	0.1460	0.9863	2.34	12	0.999*
19	0.010	0.05	99.98	0.0992	0.9961	0.67	5	0.985
20	0.004	0.02	100.00	0.0626				

+ All 20 canonical discriminant functions were used in the subsequent discriminant analyses.

* Significance values start to decrease again.

IV.B. Discriminant analysis results for 11 populations consolidated by location, standardized by sample size, using the direct procedure in SPSS DISCRIMINANT.

FUNCT.	EIGEN-VALUE	% OF VARIANCE	CUMUL. %	CANON. CORR.	WILK'S LAMBDA	CHI-SQUARED	D.F.	SIG.
Before discriminating power of the first function was removed :					0.0043	951.84	240	
1	4.566	46.60	46.60	0.9057	0.0238	652.27	207	0.000
2	1.683	17.17	63.77	0.7920	0.0639	480.08	176	0.000
3	1.098	11.21	74.98	0.7235	0.1340	350.76	147	0.000
4	0.733	7.48	82.46	0.6504	0.2322	254.80	120	0.000
5	0.589	6.01	88.47	0.6089	0.3690	173.95	95	0.000
6	0.371	3.79	92.26	0.5202	0.5059	118.89	72	0.000
7	0.354	3.61	95.87	0.5112	0.6849	66.05	51	0.077
8	0.169	1.72	97.59	0.3800	0.8005	38.83	32	0.189
9	0.147	1.50	99.09	0.3583	0.9184	14.85	15	0.462
10	0.089	0.91	100.00	0.2856				

IV.C. Discriminant analysis results for 11 populations consolidated by location and standardized by sample size, using the the stepwise procedure in SPSS DISCRIMINANT.

1. Sequential assessment of the usefulness of the 24 variables.

STEP	VARIABLE ENTERED	REMOVED	WILK'S LAMBDA	STEP	VARIABLE ENTERED	REMOVED	WILK'S LAMBDA
1	36		0.3659	13	52A		0.0112
2	5A		0.2043	14	7		0.0099
3	34		0.1243	15	53		0.0089
4	45B		0.0894	16	46		0.0079
5	48		0.6658	17	2		0.0070
6	23A		0.0505	18	18		0.0062
7	29		0.0388	19	35		0.0056
8	6		0.0302	20	4A		0.0051
9	13		0.2334	21	19		0.0047
10	15		0.0176				
11	14		0.0150	21 Steps were performed of a possible 30;			
12	47		0.0125	8A, 31 and 16 were left out of the analysis.			

2. Discriminant Functions.

FUNCT.	EIGEN-VALUE	% OF VARIANCE	CUMUL. %	CANON. CORR.	WILK'S LAMBDA	CHI-SQUARED	D.F.	SIG.
Before discriminating power of the first function was removed:					0.0047	943.71	210	
1	4.521	46.99	46.99	0.9049	0.0259	642.99	180	0.000
2	1.679	17.45	64.44	0.7917	0.0694	469.53	152	0.000
3	1.048	10.89	75.34	0.7154	0.1422	343.35	126	0.000
4	0.722	7.50	82.84	0.6475	0.2448	247.71	102	0.000
5	0.584	6.07	88.91	0.6073	0.3878	166.73	80	0.000
6	0.357	3.71	92.62	0.5128	0.5261	113.03	60	0.000
7	0.328	3.41	96.03	0.4970	0.6987	63.10	42	0.019
8	0.160	1.65	97.68	0.3704	0.8098	37.13	26	0.073
9	0.142	1.48	99.16	0.3526	0.9248	13.76	12	0.317
10	0.081	0.84	100.00	0.2742				

APPENDIX V

ULTRAVIOLET LIGHT WAVELENGTH MAXIMA FOR EACH ISOLATED COMPOUND

V. Ultraviolet spectral maxima in nanometers (nm). Band II, 240 to 280nm / Band I, 300 to 380nm. ds=decomposing slowly; s=shoulder rather than peak. MeOH=methanol; NaOMe=sodium methoxide; $AlCl_3$=aluminum chloride; HCl= hydrochloric acid; NaOAC=sodium acetate; H_3BO_3=boric acid. Compounds printed in boldface type are variable compounds that were used in the taxonomic analyses. (For techniques, see Mabry,T.J., Markham,K.R. and Thomas,M.B. 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York.)

	REAGENTS ADDED					
COMPOUND	MEOH	MeOH+NaOMe	MeOH+$AlCl_3$	MeOH+$AlCl_3$+ HCl	MeOH+NaOAc	MeOH+NaOAc+ H_3BO_3
1/3A	271/314	273/310, 364ds	278/310,424	278/310,401	278/298s, 313,374	268/312,370
3	270/300s, 314,364	274/310s, 362	279/302s, 312,442	279/302s, 312,390	277/300s, 315,374	266/300s, 312,374
5/5+	226/301s, 312	274/310s, 365	227,277/ 308,399	229,281/ 308,398	276/310,366	268/312
5A	**270/300s, 314**	**276/312, 364d**	**278/309, 400**	**280,310, 408**	**276/302s, 313,365**	**268/300sh, 316**
9	244s,268/ 301s,314	274/311s, 366	228,278/ 308,396	228, 279/ 308,317,396	275,300s/ 312,368	269,301s/315
10	269,300s/ 316,360s	275/310,370	276/306, 320s,400	277/306, 321s,399	275/312,358	269/315,370
11	269/300s, 312	274/310s, 368ds	274/306, 320,406	275/306, 320,402,410	275/311,366	267/314
12	270,282s/ 314	273/366ds	275/302,402	276/302,402	274/314, 374s	266,317s
13	**268,290/ 314**	**273/308s 366ds**	**277/300 326,398**	**277/300 326,394**	**276,299/ 310s,370**	**268,294/ 314**
14	**268,282 330,322s**	**276/330, 402**	**274/302s, 348,398**	**274/302s 348,390**	**276/374**	**268,382s 350**
15	**268,282s/ 326s**	**275/370ds**	**275,398s**	**275,396s**	**274/374s**	**268,282/ 358s**
15A	268/334	281/329,390	275/303s, 348,400	275/304s, 348,400	274/370	268/340

V. (Continued).

COMPOUND	REAGENTS ADDED: MEOH	MeOH+NaOMe	MeOH+$AlCl_3$	MeOH+$AlCl_3$+ HCl	MeOH+NaOAc	MeOH+NaOAc+ H_3BO_3
18A	272,280s/ 310	276/342s	276/312	274/312	277/318	272/310
19	**270/300s 322**	**274/312s, 378**	**277/307 330,398**	**277/306 328,398**	**276/315, 372s**	**269/320**
20	241s,252/ 298s,362	270/390ds	240s,268/ 314,432	263/308s, 362s,406	273s/332, 366s,376	285s/382
21	268/302,344	216/330,404	274/304, 350,398	274/304, 347,400	275/306,378	267,298/350
24	267/310, 348	274/318,378	273/306, 349,400	276/308, 348,400	274/306,370	264/306s,346
25	267/305s, 350	275/326,402	276/304, 350,399	276/304, 348,398	274/306,382	267/303s,350
32	254s,266/ 312s,352	274/321s, 402	275/310, 354,404	278/310, 354,404	276/312,380	254s,267/ 300s,314,353
32A	266/305s, 346	274/309s, 316,376	275/308, 352,399	273/306 345,396	276/310,374	266/304s,346
36	**266/306s, 346**	**274/314, 382**	**273/308 352,398**	**273/306, 346,398**	**275/306, 374**	**266/306s, 350**
39	255,270/346	273/330s, 415	268/306s, 362,402	270/306s, 357,394	275/318, 372,388s	256/355
45A	284/338s	286/420	309/389	307/389	282/330s	284/330s
45	270/348s, 354	273/322s, 416	275/310, 370s,405	278/309, 367,403	278/314s, 390	270/310s,355
46	**286/388s**	**242s,288/ 361**	**311/390**	**310/389**	**286/334s**	**286/330**
48	**282/375s**	**290/330s, 434**	**289/312s, 391,413**	**284/312 376**	**282/334s, 432**	**286/397s 438s**