

Review paper

Genetic diversity—seeing the forest through the trees

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Application. Processing biochemical markers from individual trees provides a means to resolve and measure genetic diversity. Identifying that variation and interpreting that rich source of genotypic information leads to effective means for managing populations to maintain forest health. Looking ahead, research in molecular forestry will extend toward discovering how the biology of forest species is effected by variation in individual genes.

Abstract. Forest trees, populations, races, species, and taxonomic groups above the species level display rich variation in biochemical markers. The variation stems from inherited modifications that trace back in time, through converging ancestries, towards common progenitors. Past movements of continents, mountain building events, and climate changes isolated forest populations one from another and provided critical challenges to the lineages that survived to the present day. A wealth of molecular variants in forest trees characterize these widely-distributed, large, long-lived, outbreeding, organisms. Forest trees have an abundance of rare variants and over one third of all the alleles (different forms of one gene) occur only rarely (<2% frequency) in a few trees of a species sample. Those rare alleles may either represent new variation or persistent forms of genes that have low adaptive value under present conditions. From another perspective, however, the largest share of genetic variation in forest trees is due to the presence of multiple alleles found at intermediate frequencies for only a small percentage of all the genes, and those alleles are commonly widespread throughout species areas. These common alleles may mark genes that track historical events in lineages or mark genes with adaptive significance in present populations. Evidence from enzyme studies supports the conclusion that highly comparable functional genes are common to different forest taxa. Future research will be toward understanding the phenotypic expression of particular genes and revealing the relative importance of genetic variants to adaptation and growth.

Introduction

“You can’t see the forest for the trees!” That familiar phrase is often intended as light hearted criticism of work on narrow problems. It carries a double meaning for forest geneticists who gathered at a symposium in

Corvallis, Oregon, to reflect, report, and speculate on a broad spectrum of information about forest diversity. This opening contribution to the symposium addressed a mix of three subjects ranging from the origins of forest taxa, through examination of diversity in a sample of species, to concluding remarks about the construct of forest species genomes. It covered general subject matter and intentionally avoided the specific themes in molecular forestry that were addressed by the other participants.

The timing of the symposium was appropriate because a sizable volume of findings had become available since July, 1979, when a similar group convened for discussions on isozymes of North American forest trees and insects (Conkle 1981). The presentations in 1979 demonstrated the high value of biochemical markers (primarily mobility variants for numerous, specific enzymes coded by nuclear genes) for evaluating variation in forest tree populations. Since then, the necessity to conserve and manage genetic variation has become a worldwide priority. And with few efficient means available to evaluate diversity, the rapid acquisition of precise genetic information using biochemical markers plays a central and critical roll in assessing populations and monitoring species in native forest ecosystems.

A complete consideration of forest diversity would include mention of variation in natural morphology, progeny growth in plantation trials, and survival responses in stress tests. Those measurements, trials, and tests are essential for evaluating whole plant responses but the differences observed in those trials are controlled by variation in unidentified genes. Here, consideration focuses on knowledge about variation in alleles of identified genes. Currently, these alleles and variation in these genes appear to have only minor predictive value of specific growth responses in whole plants.

Clues about diversity from the past

Skilled analysis of morphological characteristics has provided reasonably dependable and time proven methods for classifying organisms. Phylogenetic analyses of extant and fossil taxa when combined with events in paleohistory gives a basis for appreciating the ancestral relationships among modern species and speculating about the origins of their various adaptations. It is not trivial to observe that the relationships among forest species are best pictured as a tree with branches and twigs (clades) that trace back to a single root, a progenitor common to all plants.

Forest seed-plant origins are in the Coniferopsida (a subdivision of the gymnosperms which includes orders of ginkgos, conifers, and yews) and date from the Carboniferous, roughly 300 million years ago (Ma). Con-

stance Millar (Millar 1991; Millar and Kinloch 1991) evaluated information about key events in the history of pines, a genus with about 100 species, wide distribution in the northern hemisphere, and an informative example of forest tree species evolution. She used evidence from the oldest pine fossil (dated about 130 Ma) and from the wide abundance of other fossil pines shortly thereafter to estimate the derivation of the genus *Pinus* at about 200 Ma. The first pines evolved in the middle latitudes of the northern hemisphere at a time when continents formed a single great land mass (Pangaea) and ice blanketed extensive areas (Fig. 1a) of the south polar masses (Harrington 1987; Parrish 1987). Continental collisions during formation of that land mass raised mountains near points of impact (Appalachian and Atlas Mountains, and elevated topography in Greenland and Norway) that gave variety to habitats for the evolving lineages.

First angiosperm fossils appeared at about 120 Ma and differentiation

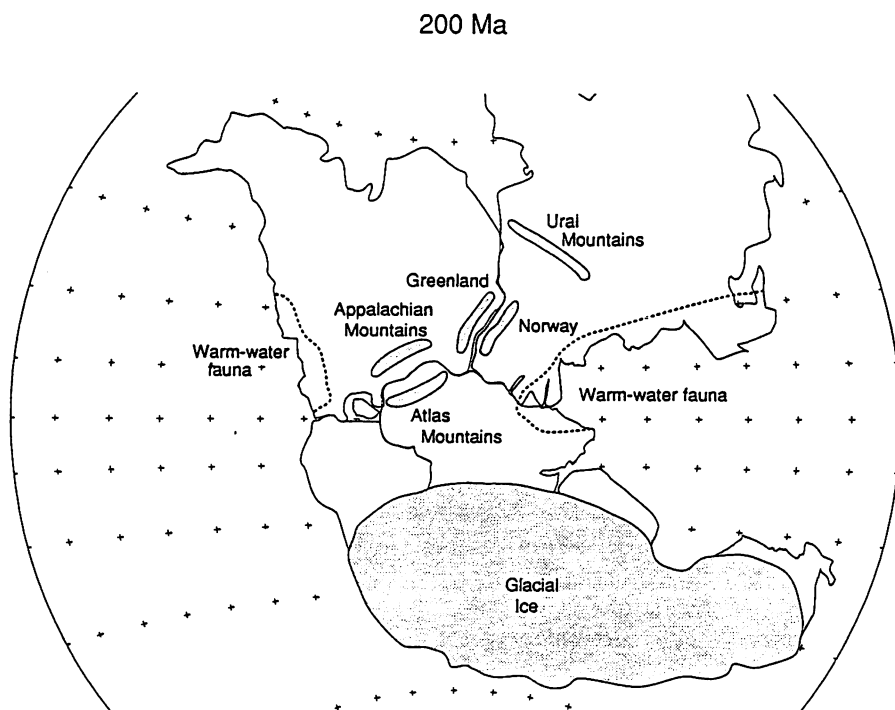


Fig. 1a

Fig. 1. Diagrammatic representation of land masses at three periods important to the history of temperate forest species, a) 200 million years ago (Ma), b) 54 Ma, and c) 18 thousand years ago (ka)(Newell 1972).

into dicotyledon and monocotyledon divisions virtually coincided with their first appearance (Friis et al. 1987). An explosive radiation of angiosperms from low latitudes established them world wide by 100 Ma as the overwhelming dominant land plant group (Crane 1987). Conifers, by that time, had spread east and west throughout North America and Eurasia (Laurasian land mass) and they equaled all the other land plants combined in absolute species diversity (Nichols et al. 1985).

At about 100 Ma a warm sea separated the northern from the southern land masses. The North American and Eurasian masses were drifting apart then, but they were still joined in their far northern areas. A group of continents (South America, Africa, India, and Australia joined to Antarctica) were drifting apart from the southern conglomerate (Gondwana). North-south interior seaways bisected North America (from the Gulf of Mexico to the Arctic) and Eurasia (from the eastern Mediterranean to the Arctic). Waters of those interior seaways isolated substantial regions of both of the northern land masses.

Events beginning about 54 Ma (Fig. 1b) profoundly influenced the

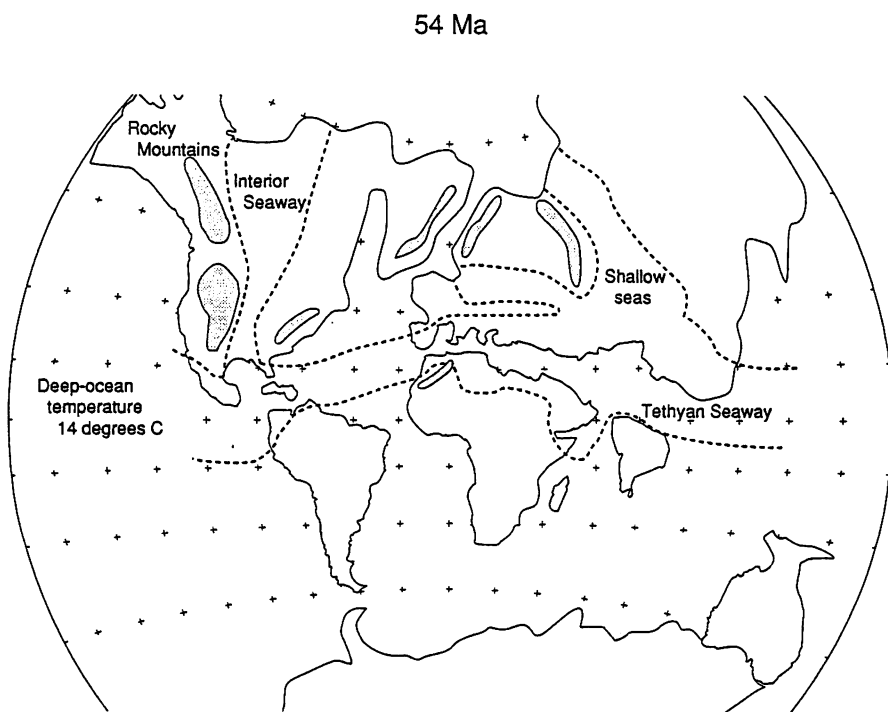


Fig. 1b

evolution of conifers (evidence from pines, Millar 1991) and deciduous forest genera (Critchfield 1980). During a period of warm humid climates, sub-tropical floristic associations became established in relatively high latitudes (centered 75 °N), fracturing and displacing the temperate species into either more northern, more southern, or more upland areas. Pine species adaptations and geographic patterns associated with modern subsections of *Pinus* largely reflect evolution in refugial regions for the period from about 54 Ma to 37 Ma (Millar 1991). Appalachian forests displayed a mix of moist tropical and deciduous hardwood vegetation suggesting that some seasonally dry and warm-temperate climates prevailed there during the same period (Critchfield 1980).

The continued westward drift of North and South Americas further isolated them from the eastern continents and their lineages from their eastern vegetation counterparts. The genetic differentiation of hard pines continued on separate continents and now genetic barriers to crossing precludes hybridizations between species native to the Eastern and Western hemispheres (Critchfield 1986).

Dramatic topographic and climatic events took place between 54 and 37 Ma. Rocky Mountain topography (with a 100 million year period of

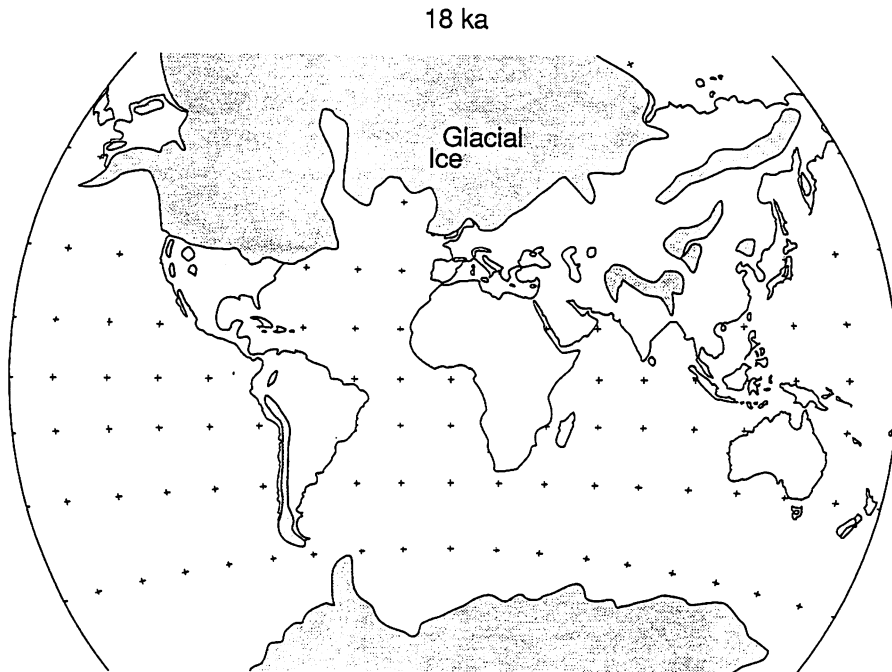


Fig. 1c.

uplift) emerged in full development. India collided with Asia and raised the Himalayas. Australia separated from Antarctica. Ocean currents, then shunted through cold polar seas in a new seaway south of Australia, brought about significant ocean cooling (Kennett 1977). A corresponding severe drop in temperatures on land (along with increased seasonality) brought the warm periods to a close. Tropical and subtropical plants were lost from mid and upper latitudes, and the previously displaced taxa reclaimed the temperate mid latitudes. The present differentiation among subsections of *Pinus* points to their evolution in the isolated refugial regions they occupied during the warm periods of the Eocene (Millar 1991). Further differentiation of lineages radiating from those refugia may have established many modern day species.

During a period between 37 and 2 Ma., the collision of Africa and Europe resulted in complete desiccation of the Mediterranean Sea (Hsu 1972) and development of the Pyrenees, Alps, and Carpathian Mountains. Continued uplift accompanied by volcanic activity along western margins of North, Central, and South America raised the Cascade-Sierra Nevada, elevated the Sierra Madre Occidental and the Transverse volcanic belt in Mexico, and formed the Andes in South America. Deep ocean cooling continued unabated. Deep ocean temperatures dropped from 14 °C, about 75 Ma, to 3 °C at the onset of the ice ages (Newell 1972).

Repeated cycles of the advance and retreat of glacial ice, beginning about 2.5 Ma, forced mid and upper latitude forest species into large scale migrations. The current ice ages began with about 40 full climatic cycles in 41 thousand-year rhythms. They then shifted to about 8 full cycles with long periods of glaciation, of 80 to 100 thousand years each, followed by short interglacial intervals, of 10 to 15 thousand years each (Ruddiman and Wright 1987). During each cycle, glacial ice advanced over polar and upper latitude areas and in high mountain terrain. Masses of ice at the culmination of the recent cold cycle, 18 thousand years ago, were the most extensive of all masses during the ice ages (Fig. 1c). Those great ice volumes lowered global seas about 130 m below the present levels (Cronin et al. 1981).

Forest species in mid and high latitudes shifted north and south, and higher and lower in elevation, in response to shifting habitats during the cold and warm periods of glacial cycles (Barnosky et al. 1987; Jacobson et al. 1987). The magnitude of these migrations can be appreciated by noting that northern boreal species of eastern North America were centered in southeastern United States about 18 thousand years ago (Webb et al. 1987). Vegetation in more stable environments (locations moderated by oceanic influences, arid rain-shadow locations, and equatorial regions) was effected less drastically. The migration of forest species revealed the limits of their respective adaptations. The near universal survival of forest

species plus their general lack of morphological change during extreme stress imposed by the ice age climates are evidence of their persistence and the well buffered genetic stability of their traits.

Critchfield (1984) used data from genetic markers to summarize several consequences of recent glacial events for North American conifers. He noted populations in previously-glaciated northern areas to be less variable than their southern counterparts; the migrating founders of some species carried forward only a limited sample of progenitor genomes. Critchfield identified extant species that lack or have distinctly low levels of variation, resulting presumably from being driven to near extinction. He identified geographic races that formed as populations radiated from different refugia. Some races had transient existence owing to introgression or fusion upon encountering compatible taxa. Other races, evolved during periods of isolation to become varieties, subspecies, or species. Critchfield also provided evidence that some species increased variation by gene exchange during periods of contact with compatible species.

Given the past histories of boreal and temperate lineages, taxa differ widely in their adaptations to different climatic conditions. An expected response of some successful species to repeated glacial cycles might be the accumulation of wide climatic tolerances. Since temperate forest species have long been mobile geographic units responding to climate change, they might be expected to have accumulated adaptations for colonizing new habitats along their leading fronts and for resisting elimination along their trailing borders. Each climatic cycle would result in shifts, from advance to retreat and from leading to trailing margins. Viewed today, some noteworthy species have extremely wide adaptations, they span significantly large ranges of elevations and latitudinal distances. Other species, presumably those for whom migration and survival were severe challenges, have narrow distributions. Some narrowly distributed taxa possess moderate variation, but others are known to be genetically depauperate and have little or no capacity remaining for significant evolutionary change. Biochemical markers, whether adaptive or neutral in present day environments, reveal amounts of allelic variation, identify genetic relationships, and track patterns of differentiation within and among populations.

Summary items for past evolution

- Forest species evolved from common ancestors;
- Conifers have about a 200 million year history that began when all continents were one land mass;
- Forest angiosperms evolved about 100 million years ago;

- Major differentiation of boreal and temperate forest groups occurred about 54 to 37 million years ago when warm climates favored development of subtropical vegetation in mid and upper latitudes, forcing temperate vegetation into cooler or drier refugia;
- Climatic cooling beginning about 37 Ma favored speciation of boreal and temperate forest taxa during periods of migration into new areas;
- Ice ages of recent history caused mass migration of species and contributed to further differentiation of lineages as populations repeatedly advanced and retreated, some into and out of restricted refugia, and others across latitudes and elevations that may have resulted in the development of wide environmental tolerances;
- Amounts and distribution patterns of variation in modern taxa reflect the molecular history of lineages as well as the disequilibrium inherent in mobile groups.

Profiles of species diversity

Diversity data from a number of species in California are available (Fig. 2). Species that are included all had genetic analyses performed using similar analytical and interpretative techniques and many grow together in natural mixtures (sympatry strengthens the comparisons among them). Wide spread species that span a notable range of elevations are: ponderosa (*Pinus ponderosa* Dougl. ex Laws.), Jeffrey (*P. jeffreyi* Grev. & Balf.), and sugar pines (*P. lambertiana* Dougl.), Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), incense cedar (*Calocedris decurrens* Torr.) and California white fir (*Abies concolor* var. *lowiana* [Grod.] Lemm.). Others — Bishop (*Pinus muricata* D. Don), knobcone (*P. attenuata* Lemm.), Monterey (*P. radiata* D. Don), Coulter (*P. coulteri* D. Don), and Digger (*P. sabiniana* Dougl.) pines — are restricted to relatively narrow elevational bands and occupy well defined habitats. A third group consists of Monterey cypress (*Cupressus macrocarpa* Hartw.), Torrey (*Pinus torreyana* Parry ex Carr.) and Washoe (*P. washoensis* Mason & Stockwell) pines that seem near extinction because they grow in only a few diminutive native populations.

Three pines in the California sample — ponderosa, Washoe, and Jeffrey — are members of the *Ponderosae* subsection and stem from a common lineage. All members of their subsection inhabit western North America, and differentiation of the lineage traces to the period between 54 and 37 million years ago. These three species have northern distributions and northern ecological affinities but they are close relatives with a large group of western yellow pines endemic to Mexico and Central America (Conkle and Critchfield 1988). Jeffrey pine crosses to a moderate extent with

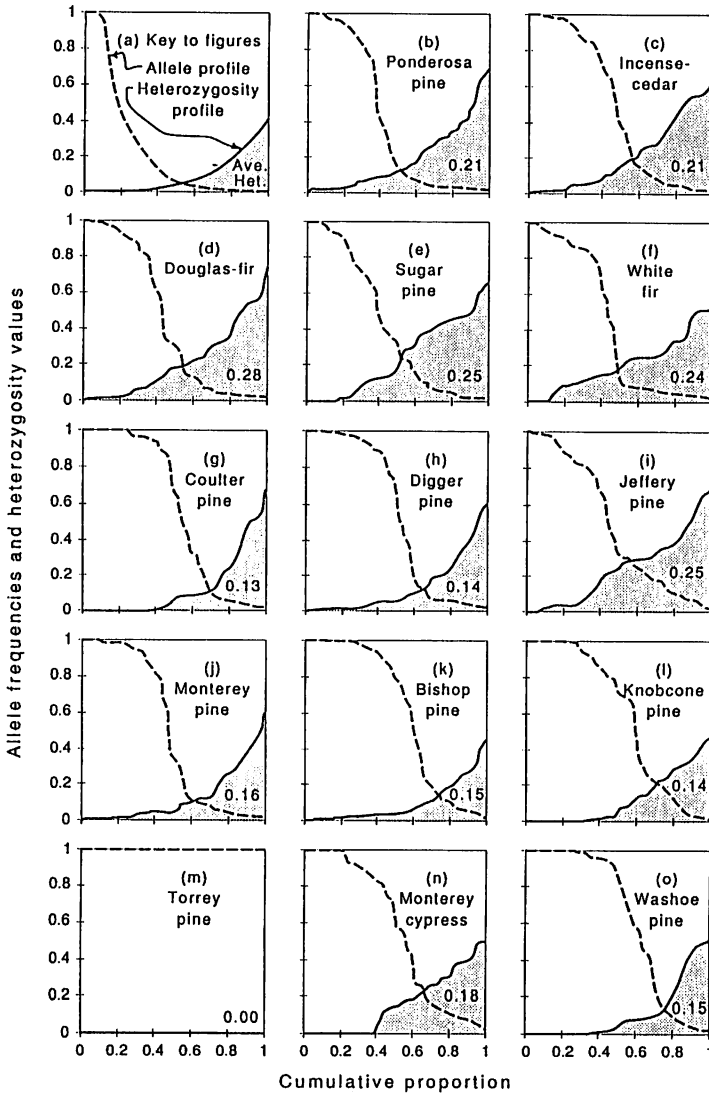


Fig. 2. Diversity profiles for 14 conifer species displaying cumulative distributions for: 1) alleles of different frequencies, irrespective of locus of origin, plotted in descending order, 2) expected heterozygosities by locus given in ascending order, and 3) mean expected heterozygosities (shaded areas under curves) whose species values are given: a) key to figures, b) ponderosa pine (*Pinus ponderosa*), c) incense-cedar (*Calocedris decurrens*, Harry 1984), d) Douglas-fir (*Pseudotsuga menziesii*), e) sugar pines (*Pinus lambertiana*), f) white fir (*Abies concolor*), g) Coulter pine (*Pinus coulteri*), h) Digger pine (*Pinus sabiniana*), i) Jeffrey (*Pinus jeffreyi*, Furnier 1984), j) Monterey pine (*Pinus radiata*, Millar et al. 1988), k) bishop pine (*Pinus muricata*, Millar et al. 1988), l) knobcone pine (*Pinus attenuata*, Millar et al. 1988), m) Torrey pine (*Pinus torreyana*, Ledig and Conkle 1983), n) Monterey cypress (*Cupressus macrocarpa* Conkle 1987), and o) Washoe Pine (*Pinus washoensis*, Niebling and Conkle 1990).

Coulter pine, and Jeffrey pine appears to be a species linking the *Ponderosa* and *Sabinianae* subsections (Conkle and Critchfield 1988). Coulter, Digger, and Torrey pines comprise the *Sabinianae* subsection. Their differentiation from yellow pines in Mexico and Central America traces to about 65 million years ago (Millar 1991). California closed cone pines — Monterey, Bishop, and knobcone — have historical differentiation times and geographic origins similar to the *Sabinianae*. They are the northern most members of the *Oocarpae* and are related to a group of extant species in Mexico and Central America. All pine species, just mentioned, are hard pines (subgenus *Pinus*).

Sugar pine is the only white pine (subgenus *Strobus*) in this sample. Its origins are obscure, but other members of subsection *Strobi* are distributed throughout North America and Eurasia. Douglas-fir, white fir, and Monterey cypress likewise are related to species distributed throughout North America and Eurasia. Northern hemisphere distributions for *Pinus*, *Pseudotsuga*, *Abies*, and *Cupressus* support the conclusion that these conifers evolved on northern land masses after separation from the southern land masses. Of the species sampled, only incense-cedar with its range centered in Oregon and California appears to have differentiated to a high degree prior to the break up of the universal land mass (Pangaea). Other Northern hemisphere incense-cedars (*Calocedrus*) are in China and Taiwan. Southern hemisphere incense-cedars (*Libocedrus*) are native to Chile in South America (also included under *Austrocedrus*) and to New Zealand and New Caledonia in the South Pacific.

Protein samples from forest trees, whether from seeds or vegetative tissues, when separated during electrophoresis and stained, reveal the presence of bands for specific enzymes. The majority of mobility variants found in enzyme studies of genetic diversity segregate in equal proportions with 50% of germ cells having one form and 50% having the alternative. Equal segregation of different alleles for genes tracing to egg or pollen cells provides strong evidence that they are alleles coded by nuclear genes (allozymes). Data from laboratory studies of allelic mobility variants for particular enzymes in species are often summarized in the form of allele frequencies and expected heterozygosities for numerous genes.

Species diversity characteristics can be generated from allozyme data by ignoring gene identities and by considering enzyme genes to be random samples of a large class of functional genes. Cumulative distribution frequencies characterize allele frequencies (frequency of occurrence) and provide a visual description of species heterozygosity (average proportion of loci with different alleles per gene in an individual, and also the average proportion of individuals in populations with different alleles per gene). Figure 2 pictures three comparisons for each species:

1. allele profiles based on a descending sorted sequence of allele frequencies for all genes;
2. heterozygosity profiles based on ascending sorted sequences of expected heterozygosities for all genes; and
3. average heterozygosity values (Ave. Het.).

The horizontal axis, marked in unit scale, is the same size as the vertical axis (allele frequencies and heterozygosity values) to make the figure a unit of area (the shaded area below the heterozygosity profile is equal to the heterozygosity for each species). Since alleles with frequencies less than 0.02 only have minor effects on heterozygosities, they were excluded from the allele frequency distributions (Fig. 2). For ponderosa pine, 20% of alleles have frequencies greater than 0.90, 50% of loci have expected values less than 0.10, and the average expected heterozygosity is 0.21.

Tree species commonly have minor proportions of fixed alleles (frequencies equal 1.00), and heterozygosities, when sorted, depict a linear to exponential increase in diversity from fixation (one allele, heterozygosity equals 0.00) to the most variable loci (heterozygosities > 0.6). The exception is Torrey pine populations (Fig. 2 [m]) which have all genes with fixed alleles (average heterozygosity equals 0.00).

The wide spread species — ponderosa pine, Fig. 2 (b), incense-cedar (c), Douglas-fir (d), sugar pine (e), white fir (f), and Jeffrey pine (i) — have a range of average heterozygosity values from 0.21 to 0.28 (mean 0.24). Species with somewhat limited distributions — Coulter pine (g), Digger pine (h), Monterey pine (j), Bishop pine (k), and knobcone pine (l) — on examination have comparatively more fixed alleles in their allele profiles and somewhat more concave than linear heterozygosity profiles. The latter species range from 0.13 to 0.16 (mean 0.14) for average heterozygosities.

The rare endemic species — Torrey pine Fig. 2 (m), Monterey cypress (n), and Washoe pine (o) — contrast with the previous species. Torrey pine has no measurable diversity, while Monterey cypress and Washoe pine have profiles and heterozygosities that are nearly comparable with species having large distributions. A rare event in Torrey pine's history, perhaps the present species establishment by one or only a few founders, or a near extinction drop in population size, presumably caused fixation in its genes (Ledig and Conkle 1983).

Genes in the sampled species differ in the numbers of alleles per locus (from 1 to about 10). Since rare alleles occur almost exclusively in the heterozygous condition and since alleles with intermediate frequencies are major contributors to population heterozygosities, it is informative to examine the percent of alleles at different frequencies for genes with differing number of alleles (Table 1). Table 1 was developed from allele

Table 1. Proportions of alleles at various frequencies grouped by the number of alleles that are detected for genes in a sample of 13 conifer species (see Fig. 2, and excluding Torrey pine)

Allele frequency	Number of alleles per gene					Total(%)
	1	2	3	4	> 4	
Number of alleles (% of total)						
	65 (6)	163 (15)	283 (26)	250 (23)	326 (30)	1,087 (100)
(%) in each class						
> 0.95	100	31	14	6	3	17
> 0.90	0	38	19	12	5	21
> 0.80	0	44	26	17	11	27
> 0.50	0	51	34	24	16	33
> 0.20	0	57	42	32	21	39
> 0.10	0	63	50	39	26	45
> 0.05	0	69	57	49	36	53
> 0.02	0	75	69	61	51	64
0.02 and lower	0	25	31	39	49	36

frequency data (total of 1,087 alleles) for all the sampled species except Torrey pine.

Overall, 6% of the genes had only one allele, 15% had 2 alleles, and close to 80% had 3 or more alleles (Table 1). About one third of all alleles had frequencies greater than 0.50. Thirty one % of genes with 2 alleles had frequencies in excess of 0.95 (>0.95), but genes with more the 4 alleles had only 3% of alleles with frequencies in excess of 0.95. Comparisons across other classes with different numbers of alleles per gene also show declining percentages; only about 20% of the genes with greater than 4 alleles have frequencies exceeding 0.20. Rare alleles, here noted as ones in frequencies equal to 0.02 and lower, by tally are 25% of the 2 allele per gene cases, but increase to near 50% for genes with more than 4 alleles. Rare alleles are abundant when expressed as a percent of all alleles, their percentages exceed one third of all alleles.

Heterozygosity values were also tabulated by the number of alleles per gene for all genes in the sample species (Table 2). Of a total of 360 loci, 82% were polymorphic. Genes with 2 and 3 alleles made up 52% of the total. Note in the right hand total column that 50% of the genes had heterozygosity values greater than 0.10. There is a positive relationship between the number of alleles per gene and the percent values in classes

for each row of cumulative heterozygosity values. Only 19% of genes with two alleles have heterozygosities greater than 0.20, while 70% of genes with greater than 4 alleles exceed 0.20 heterozygosity. It is noteworthy that the genes (including monomorphics) in these species had an average of 3 alleles each (1,087 alleles / 360 genes; Tables 1 and 2).

Table 2. Proportions of genes with various heterozygosity values grouped by the number of alleles that are detected for genes in a sample of 13 conifer species (see Fig. 2, and excluding Torrey pine)

Heterozygosity value	Number of alleles per gene					Total (%)
	1	2	3	4	> 4	
Number of genes (% of total)						
	65 (18)	93 (26)	93 (26)	56 (16)	53 (15)	360 (100)
(%) in each class						
> 0.50	0	0	13	14	19	8
> 0.20	0	19	36	50	70	32
> 0.10	0	41	62	71	85	50
> 0.05	0	52	78	84	98	67
> 0.02	0	63	89	96	100	69
0.02 and lower	100	37	11	4	0	31

Summary items for profiles of forest diversity

- A number of forest species in California, commonly exhibit high levels of diversity in biochemical markers;
- Wide spread species that span a significant range of latitudes and elevations have lower proportions of fixed loci (having just one allele) and higher average heterozygosities than more geographically restricted species;
- Rare endemic species vary from complete fixation and no detectable biochemical variation to relatively high levels of variation characteristic of more wide spread taxa;
- As the number of alleles per gene increases, alleles in high frequencies decrease;
- Rare (less than 0.02 frequency) alleles are abundant;
- About 85 % of all loci have 4 or fewer alleles;

- Diversity measured by allozyme heterozygosity traces to relatively few alleles with intermediate frequencies;
- Genes overall averaged 3 alleles each.

Biochemical markers and genomes

After noting long histories for forest lineages and monitoring some levels of diversity in allozymes, it is appropriate to briefly move somewhat closer to individual genes by considering their characteristics in cells and whole organisms.

The basic elements of gene action are well understood. The order of four different bases in DNA of chromosomes is transcribed and translated into the variety of molecules that sustain life processes. Higher plant cells carry three genomes in separate organelles: the nucleus contains several to many pairs of large homologous chromosomes (diploid with the alleles for similar gene regions on separate homologous chromosomes, and each chromosome consisting of one long strand of DNA) — chloroplasts and mitochondria (each having unique and numerous copies of small circular chromosomes, haploid in number).

Individual genes are regions of DNA with segments that regulate transcription and segments with the codes for functional molecules. Listing the many variations of DNA are beyond this discussion, but enzymes are a particularly important class of secondary DNA products having catalytic and metabolic functions needed for the vast majority of all cellular reactions. The enzymes included in most forest species studies average about 425 amino acid residues in length (range from about 325 to 700, Ledig and Conkle 1983), their corresponding DNA codes may approach several thousand bases in length. The relationship between enzymes and DNA is so close that enzyme genes, and alleles of enzyme genes are terms in common usage.

The complement of enzyme genes that are analyzed in different forest species appears common to them all. Most workers report that different species, even those from different forest genera, have similarities for the number of genes per enzyme system. Each set of bands stained for a particular enzyme have distinct phenotypes. Groupings of bands for each gene and overall patterns when several genes express on the same electrophoresis gel are similar in different taxa. The size, staining intensities, and occasional multiple patterns of bands of genes indicate their close homology in different taxa. And different genes have characteristic

phenotypes common across species when developmental patterns are compared and when different plant tissues are examined.

Several conifer enzyme systems may provide further evidence of wide interspecies gene homology through gene product compartmentalization. It is known that specific nuclear gene products associate with and are active only in specific cellular compartments: in chloroplasts, mitochondria, or cytoplasm. Reports on plants consistently validate that three genes are active in an enzyme system that different workers refer to as either GOT or AAT (glutamate oxaloacetate transaminase or aspartate aminotransferase). In corn, a fast mobility gene, stained for GOT on gels from electrophoresis, is expressed in mitochondria, another fast mobility GOT gene is expressed in plastids, and a third GOT gene with slow mobility is expressed in the cytoplasm (Goodman and Stuber 1983). The products of all three genes are independent, all only form intragene molecular associations (no intergene heterodimers are noted.)

GOT enzyme bands from dry and imbibed seeds of conifers similarly have an intensely staining high mobility gene with a single band in haploid tissues, a light staining high mobility gene (also single banded in haploids), and a consistently stained lower mobility gene (or perhaps duplicated genes), the latter commonly encodes for a characteristic stack of two or more bands on gels. The light staining high mobility bands increase in stain intensity as embryos enlarge and germination progresses.

Observations after separation and staining of enzymes from chloroplast enriched, mitochondria enriched, and cytoplasmic (mitochondria and chloroplasts removed) fractions of germinating conifer seeds in our laboratory support the conclusion that bands of the dark-staining fast-mobility GOT gene are associated with the mitochondrial fraction. Their dark stain reaction correlates with high levels of mitochondrial respiration expected in nutrient tissues during germination. Bands of the second GOT gene (often with mobilities slightly slower than the darker bands) are associated with the chloroplast enriched fraction. Their light stain reaction in early germination stages and darker stain in latter stages correlates with the onset of photosynthesis. Slow mobility GOT bands, commonly having a two banded haploid phenotypes, are associated with the cytoplasmic fraction. In conifers as in corn, the significant implication is that individual genes are unique in form, function, and site of action; many genes are homologous throughout all plants and must trace back through plant lineages to common progenitor codes.

While several lines of evidence support the hypothesis that forest trees in wide taxonomic groups share the same complement of genes, substantial numbers of reports on gene linkage (many based on recombination

ratios of segregating alleles in haploid tissues in conifer seeds) further indicate there is significant conservation of gene order on chromosomes of different species (Cheliak and Pitel 1985). Conserved linkage relationships extend across conifer genera in the case of closely linked gene pairs.

Summary items for organization of the genome

- Perhaps the majority of different functional genes are similar in different plant taxa, homologous gene products have specific sites of action in cells;
- Gene linkages on chromosomes appear to be moderately conserved even across diverse conifer genera;
- Individual gene similarities trace far back through branching lineages to common ancestors in all plants.

In conclusion, it is appropriate to note that new research on biochemical markers is unlimited in scope and importance due to techniques now available for decoding and manipulating DNA. All hereditary codes are open to investigation using enzymes that cut DNA strands at specific base sequences and use molecular tags that identify strands carrying codes of interest. Nuclear, plastid, and mitochondrial genomes can be tagged with numerous new markers. It seems safe to predict that a similar group of forest researchers gathering ten years from now will report on more precise phylogenetic relationships among taxa, provide more accurate information about the evolution of specific gene families, and discuss single gene regulation and expression in individuals and taxa. Reports may even provide information about phenotypic modifications of tree growth, pest resistance, and environmental tolerances induced by genetic manipulation. The unifying nature of biochemical and molecular research, that results from common phylogeny, means that findings on one plant, one family, a population, or taxon can be rapidly applied to a wide variety of other organisms.

References

- Barnosky, C. W., Anderson, P. M. and Bartlein, P. J. 1987. The northwestern U.S. during deglaciation; Vegetational history and palaeoclimatic implications, pp. 289–312. In: Ruddiman, W. F. and Wright, J. E., Jr. (Eds) North America and adjacent oceans during the last deglaciation. Boulder, Colorado, Geological Society of America, The Geology of North America, V. K–3.

- Cheliak, W. M. and Pitel, J. A. 1985. Inheritance and linkage of allozymes in *Larix laricina*. *Silvae Genetica* 34: 142—148.
- Conkle, M. T. 1981. Proceedings of the symposium on isozymes of North American forest trees and forest insects, July 27, 1979, Berkeley, California. Gen. Tech. Rep. PSW—48, Pacific Southwest Forest and Range Exp. Stn., Forest Serv., U.S. Dep. Agric., Berkeley, California, 64 pp.
- Conkle, M. T. 1987. Electrophoretic analysis of variation in native Monterey cypress (*Cupressus macrocarpa* Hartw.), pp. 249—256. In: Elias, T. S. (Ed) Conservation and Management of Rare and Endangered Plants. Proc. of a Conference held 5—8 Nov., 1986, Sacramento, California. California Native Plant Soc., Sacramento, California.
- Conkle, M. T. and Critchfield, W. B. 1988. Genetic variation and hybridization of ponderosa pine, pp. 27—43. In: Ponderosa Pine: The Species and its Management. Washington State Univ. Coop. Ext.
- Crane, P. R. 1987. Vegetational consequences of the angiosperm diversification, pp. 107—144. In: Friis, F. M., Chaloner, W. G. and Crane, P. R. (Eds) The Origin of Angiosperms and their Biological Consequences. Cambridge University Press.
- Critchfield, W. B. 1980. Origins of the eastern deciduous forest, pp. 1—14. In: Proceed. Dendrology in the Eastern Deciduous Forest Biome, September 11—13, 1979. Va. Polytech. Inst. and State Univ.
- Critchfield, W. B. 1984. Impact of the Pleistocene on the genetic structure of North American conifers, pp. 70—118. In: Lanner, R. M. (ed.), Proceed. 8th North America Forest Bio. Workshop. Logan, Utah.
- Critchfield, W. B. 1986. Hybridization and classification of the white pines (*Pinus* section *Strobus*). *Taxon* 35: 647—656.
- Cronin, T. M., Szabo, B. J., Ager, T. A., Hazel, J. E and Owens J. P. 1981. Quaternary climates and sea levels on the U.S. Atlantic coastal plain. *Science* 211: 579—584.
- Friis, E. M., Chaloner, W. G. and Crane, P. R. 1987. Introduction to angiosperms, pp. 1—15. In: Friis, F. M., Chaloner, W. G. and Crane, P. R. (Eds) The Origin of Angiosperms and their Biological Consequences. Cambridge University Press.
- Furnier, G. R. 1984. Population genetic structure of Jeffery pine. Dissertation, Doctor of Philosophy, Oregon State U., Corvallis.
- Goodman, M. M. and Stuber, C. W. 1983. Maize, pp. 1—33. In: Tanksley, S. D. and Orton, T. J. (Eds), *Isozymes in Plant Genetics and Breeding, Part B*. Elsevier Science Publishers B. V., Amsterdam.
- Harrington, J. B. 1987. Climatic change: a review of causes. *Can. J. For. Res.* 17: 1313—1339.
- Harry, D. E. 1984. Genetic structure of incense-cedar (*Calocedrus decurrens*) populations. Dissertation, Doctor of Philosophy, Un. of California, Berkeley.
- Hsu, K. J. 1972. When the Mediterranean dried up. *Sci. Amer.* 227: 26—36.
- Jacobson, G. L., Jr., Webb, T., III and Brimm, E. C. 1987. Patterns and rates of vegetation change during the deglaciation of eastern North America, pp. 277—288. In: Ruddiman, W. F. and Wright, J. E., Jr. (Eds) *North America and Adjacent Oceans During the Last Deglaciation*. Boulder, Colorado, Geological Society of America, The Geology of North America, V. K—3.
- Kennett, J. P. 1977. Cenozoic evolution of Antarctic glaciation, the circum-Atarctic Ocean, and their impact on global paleoceanography. *J. Geophy. Res.* 82: 3843—3860.
- Ledig, F. T. and Conkle, M. T. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). *Evolution* 37: 79—85.
- Millar, C. I. 1991. Impact of the Eocene on the evolution of pines. *Annals of Missouri Bot. Garden*, in press.
- Millar, C. I., Strauss, S. H., Conkle, M. T. and Westfall, R. D. 1988. Allozyme differentiation

- and biosystematics of the Californian closed-cone pines (*Pinus* subsect. *Oocarpae*). *Syst. Bot.* 13: 351—370.
- Millar, C. I. and Kinloch, B. B. 1992. Taxonomy, phylogeny, and coevolution of pines and their stem rusts. In: Hiratsuka, Y. (Ed) *Proceedings of the Third IUFRO Rusts of Pine Working Party Conference*. Northern Forestry Centre, Forestry Canada.
- Newell, N. D. 1972. The evolution of reefs. *Sci. Am.* 226: 54—65.
- Niebling, C. R. and Conkle, M. T. 1990. Diversity of Washoe pine and comparisons with allozymes of ponderosa pine races. *Can. J. For. Res.* 20: 298—308.
- Niklas, K. J., Tiffney, B. H. and Knoll, A. H. 1984. Patterns in vascular land plant diversification: a factor analysis at the species level, pp. 97—128. In: Valentine, J. W. (Ed) *Phanerozoic Diversity Patterns: Profiles in Macroevolution*. Princeton, Princeton University Press.
- Parrish, J. T. 1987. Global palaeogeography and palaeoclimate of the late Cretaceous and early Tertiary, pp. 51—73. In: Friis, F. M., Chaloner, W. G. and Crane, P. R. (eds), *The origins of angiosperms and their biological consequences*. Cambridge Univ. Press.
- Ruddiman, W. F. and Wright, H. E., Jr. 1987. Introduction. pp. 1—12. In: Ruddiman, W. F. and Wright, J. E., Jr. (Eds) *North America and Adjacent Oceans During the Last Deglaciation*. Boulder, Colorado, Geological Society of America, *The Geology of North America*, V. K—3.
- Webb, T., III, Bartlein, P. J. and Kutzbach, J. E. 1987. Climate change in eastern North America during the past 18,000 years; Comparisons of pollen data with model results, pp. 447—462. In: Ruddiman, W. F. and Wright, J. E., Jr. (Eds) *North America and Adjacent Oceans During the Last Deglaciation*. Boulder, Colorado, Geological Society of America, *The Geology of North America*, V. K—3.