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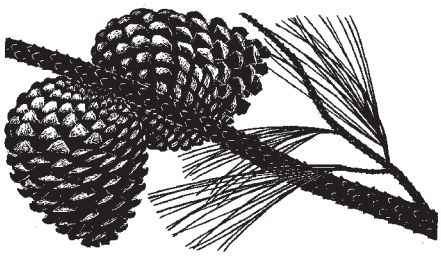
Current status of genetic information on Monterey pine



The genetic diversity information currently available for Monterey pine has been generated by four methodologies: 1) common-garden or provenance studies where differences in observable traits (e.g., phenotypic traits such as growth rate or insect resistance) are inferred to have a genetic basis because of the constancy of the environment (e.g., field or nursery trial) in which the plants are growing; 2) chemical composition studies, such as turpentine analysis; 3) allozyme studies; and 4) molecular studies of DNA or RNA. No matter which method has been used, there is much more information on genetic differences *among* the five populations than on genetic diversity *within* them. An additional source of information comes from descriptions of the pines *in situ*. Although phenotypic descriptions of trees in their native context do not necessarily reflect genetically based traits, a brief review of some phenotypic differences among the native populations is presented because these early observations provided clues about genetic differences which were often substantiated with subsequent genetic tests.

Different kinds of genetic information (or inferred genetic information) are appropriate for different issues or questions. For example, relationships between Monterey pine and other pine species (phylogeny) are perhaps better addressed by molecular and biochemical data than by morphological data (e.g., Wheeler and Guries 1982; Wheeler et al. 1983; Strauss and Doerksen 1990; Krupkin et al. 1996; Furman et al. 1997). Different DNA markers have differential strengths and weaknesses for the range of genetic questions. Microsatellite markers have proven useful in identifying among- and within-population genetic structure of forest tree species (e.g., Echt et al. 1998; Scotti et al. 1999); allozyme data continue to be useful in revealing levels of genetic diversity and genetic structure within and between populations. The great wealth of allozyme data for many forest tree species, particularly western conifers, allows the allozyme information on Monterey pine to be interpreted within an informed and comprehensive context. For some genetic questions—particularly those of amount or structure of genetic diversity—some context is required for interpretation such as the area of the genome that has been sampled and how genetic variability reflected by the particular method or marker varies across related taxa. Other questions, such as identification of foreign pollen or domestic cultivars, have a more restricted context for interpretation.

Phenotypic diversity

Phenotypic diversity—reflecting genetic and environmental influences and their interaction—is noted in early descriptions of Monterey pine. Considerable morphological diversity exists within Monterey pine, suggesting to some early taxonomists that these differences represented distinct species. One notable distinction between the island and mainland populations is the grouping of needles—generally grouped in fascicles of threes in the mainland trees and of twos in the island trees. The Cedros Island population, for example, was at one time called *Pinus muricata* D. Don or *P. muricata* var. *cedroensis* J.T. Howell (Moran 1996). Another characteristic with much variation within the species is cone morphology. Variation among populations is strikingly evident in average cone size (Figure 10). These differences have suggested several hypotheses. Population differences in cone size, cone symmetry, thickness of scales, and size and weight of seeds suggested that these characteristics are related to climate, and the length of the summer dry season in particular (Axelrod 1980). The differences among populations in the thickness of cone scales, cone attachment angles, and cone symmetry were suggested to be related to selective pressures from fire and squirrel predation (Linhart 1978).

Lindsay (1932) found the Cambria population to be distinguished from the other mainland populations in its larger

average cone size, faster height growth, better stem form, and tendency for foliage to be massed on the upper side of the side branches, giving a terraced appearance to the trees.

Considerable information is available on differences in needle and branch characteristics within and among the three mainland populations, based on trees directly sampled from the populations (Forde 1964b,c). Briefly, these studies showed that trees in the Cambria population, as compared with those in the Monterey population, have significantly longer and thicker needles with more widely spaced stomatal rows and marginal teeth. Trees from the Año Nuevo population are intermediate in these characteristics with the exception of the last characteristic: marginal teeth on needles of Año Nuevo and Monterey trees are significantly more narrowly spaced than on Cambria trees. Common-garden studies—that could differentiate between genetic and environmental effects—confirmed the existence of among-population differences in needle length and the observation that the Cambrian population has the longest needles. However, some disparities were noted between these results and the earlier field study. Specifically, the Monterey population was found to have longer needles than the Año Nuevo population, and there were no significant differences among populations in weight/length ratio of fascicles, indicating no population differences in needle thickness—in contrast to the findings of the earlier field study (Burdon and Low 1977).

Differentiation among populations of Monterey pine is also suggested by the geology and soils. Underlying the coastal California populations are different geologic substrates or soils that seem to confer some competitive advantage to the conifers over adjacent oak forests. The soils tend to be droughty or nutritionally poor. The unique substrates emphasize the fact that these populations represent island-like ecosystems, not just populations of trees (Barbour 1995).

Figure 10. Diversity in cone size among the five native populations of Monterey pine (Axelrod 1980, used by permission from University of California Press). Each cone represents the average size for that population. *Key:* 1 Cedros Island; 2 Guadalupe Island; 3 Monterey; 4 Año Nuevo; 5 Cambria.

Common-garden or provenance studies

Common-garden studies were established decades ago, many of them in Australia and New Zealand, and have since offered information on genetic diversity of many traits. There is evidence for substantial genetic differences among the five Monterey pine populations in their resistance to western gall rust, a disease caused by the fungus *Endocronartium harknessii* (Old et al. 1986). Specifically, the Guadalupe and Cedros Island populations are least susceptible. Of the three mainland populations, Año Nuevo is the most resistant. The island populations also are less susceptible to red band needle blight (Cobb and Libby 1968).

In glasshouse and field studies in Australia, considerable genetic variation in resistance to *Phytophthora cinnamomi* has been found both among and within populations of Monterey pine. Seedlings from the Cambria and Monterey populations showed the greatest degree of resistance. Seedlings from Año Nuevo and the two island populations generally were more susceptible (Butcher et al. 1984; Butcher and Stukely 1997). Also, there was large variation within the Monterey, Año Nuevo, and Guada-

lupe Island populations in family-level resistance (Butcher and Stukely 1997).

A California common-garden study containing clonal and seedling material from each of the three mainland populations showed that the Año Nuevo population suffered the least cold damage following an unusually cold 12-day period in December, 1972; the Cambria population showed the most damage; and the Monterey population had intermediate damage (Hood and Libby 1980). Subsequent studies have provided similar observations (Alazard and Destremau 1982; Burdon et al. 1992a).

The same common-garden study was also assessed for damage from black-tailed deer and porcupines—possibly a reflection of genetic differences in palatability. Significant differences in damage were seen among the three populations: the least porcupine damage was on trees from the Monterey population and the least deer damage (based on percentage of trees browsed) was to those from the Cambria population (Hood and Libby 1980).

Common-garden studies in New Zealand have been conducted since the 1950s, with significant new tests added in the mid-1960s and 1980. This long-term series allows insights into genetic differences of the populations expressed in a nonnative environment. In general, these studies suggest that the Año Nuevo and Monterey populations are better suited than the others to overall New Zealand conditions, with the caveat that Año Nuevo is much less adapted to phosphorus-deficient clay soils and better adapted to cold, snow-prone sites. In these same studies, the Cambria population has shown susceptibility to two foliage pathogens (*Dothistroma pini* and *Cyclaneusma minus*), shoot dieback, and frost and snow damage, but considerable tolerance to poor soils, and, in a Western Australian study, tolerance to *Phytophthora cinnamomi*. Trees from the Guadalupe Island population show modest overall adaptation to plantation conditions in New Zealand but have very straight stems and higher corewood density than the others. Similarly, trees from the Cedros Island population show less overall adaptation to these conditions than the mainland populations but interpopulation hybrids perform much better (Burdon et al. 1992a,b, 1997). The higher wood density of the Guadalupe Island population was also noted in a study in Australia—where island populations were noted to have higher wood density and thinner bark than mainland populations (Nicholls and Eldridge 1980). A summary of genetically based differences among populations as observed in these trials in New Zealand (with some supplementary information from other trials) is contained in Table 4. Note that the actual values for various populations (e.g., height superiority of one population versus another) are site dependent and thus may change were the trials to be located elsewhere. However, the fact of underlying genetic differences remains.

There are fewer reports of within-population genetic diversity but some of these show significant genetic differences among subpopulations. A physiological study suggests that, paradoxically, stands on coastal areas at Año Nuevo and Monterey have a lower salt tolerance as compared with inland stands in the same populations. No such differences were noted among the samples from the Cambria population but only three areas there were sampled. These results were explained as adaptations within subpopulations—the coastal areas at Año Nuevo and Monterey experiencing lower temperatures, lower evaporation, more frequent fog drip, and less salt accumulation within the soil profile than in areas further inland (Cromer et al. 1982). Common-garden studies in New Zealand show significant differences among five selected subpopulations of the Año Nuevo population in height growth (measured at 2.5 and 8 years), diameter, and incidence of forking (Burdon et al. 1992a). In this same study, genetic differences in branching pattern were noted among subpopulations of the Monterey population, and in 8-year height growth among subpopulations of the Cambria population. In this common-garden study, subpopulations were artificial groupings of sampled trees, based on locality, and hence may not have a clearly elucidated spatial genetic structure within populations. A series of eight provenance trials in New South Wales, Australia revealed significant height or basal area differences among some subpopulations within the three mainland populations (Johnson et al. 1997). Also, as noted above, there were considerable differences found within the populations (i.e., family-level) of Monterey, Año Nuevo, and Guadalupe Island in resistance to *Phytophthora cinnamomi* (Butcher and Stukely 1997). Genetic differences among families (i.e., open-pollinated progeny from the same female parent tree) have been noted for some seed and germination characteristics within the Cedros Island population (Quiroz V. 1998).

Chemical analyses

Additional genetic information comes from studies of turpentine composition (e.g., Bannister et al. 1962; Bannister and McDonald 1983) and seed protein (e.g., Murphy 1981) differences among the populations of Monterey pine. For both types of traits, considerable genetic diversity has been noted.

An early study of the turpentine composition of cortical oleoresin collected directly from trees of the three mainland populations showed differences in the proportion of alpha-pinene. Monterey and Cambria were quite similar in this feature, and different from the Año Nuevo sample (Bannister et al. 1962). However, because the trees were sampled directly from the forest, the results could not be strictly interpreted as genetic differences. In a later study, turpentine composition from samples from the two island populations showed differences between the two populations, as well as considerable differentiation from the mainland populations (Bannister and McDonald 1983). However, again, environmental effects could not be ruled out. More recently, Burdon et al. (1992d) provided more direct evidence of genetic differences in turpentine composition among populations with samples from planted trees in New Zealand field trials. In a comparison of Guadalupe Island material with that from the three mainland populations, clear differences were found among all populations in at least two of the monoterpenes assayed. When all monoterpenes were considered simultaneously, populations were again shown

as distinct, and Cambria and Monterey appeared the least different, consistent with the earlier (1962) observation. In a separate substudy within the same report, Cedros and Guadalupe samples were compared and strong differences were noted between the two island populations (Burdon et al. 1992d). Other studies have confirmed, using clonal material, the high degree of genetic control of monoterpene composition in this species (Burdon et al. 1992c).

Seed proteins from samples from each of the five Monterey pine populations have been compared using immunochemical assay techniques. Significant antigenic differences were noted between populations (Murphy 1981). These

Table 4. Summary of phenotypic characteristics of the native populations of Monterey pine in field trials in New Zealand (Burdon 1992). *Symbols:* + denotes superiority; – denotes inferiority; o denotes average; and • indicates no data were located.

		Population				
Attribute	Weight of evidence†	Año Nuevo	Monterey	Cambria	Guadalupe Island	Cedros Island
Growth rate	a	+	+	+	–	– –
Ease of transplanting	bc	+	o	–	+(+)	– –
Resistance/tolerance to:						
Frost	b	+ +	+	–	o?	– –
Snow damage	c	+	o	–	•	•
Boron deficiency	b	+	+	+	– –	– –
Phosphorus deficiency	b	–	+ +	+ +	–?	– –
Dothistroma pini	ab	+ +	+ +	– –	o	– –
Cyclaneusma minus	a	+	+ +	– –	–	– –?
Diplodia pinea	b	+ +	+ +	– –	– –	–
Phytophthora cinnamomi	b	– –	+	+ +	•	•
Endocronartium harknessii	b	+	–	– –	+ +	+
Pineus pini	c	+	+	–	– –	+
Damage by mammals:						
Deer/rabbit browse	bc	o	o	o	–	+
Deer browse	b	–	o	+	•	•
Porcupines	b	+	+	– –	—	—
Soil salinity	bc	o	+	+ +	– –	-
Tree form:						
Overall	a	– –	o	+	+	– –
Stem straightness	a	– –	o	+	+ +	+
Forking (lack):						
Early	a	– –	o	o	+ +	+
Later		–	+	+	–	–
Branch habit:						
Early	a	– –	o	o	+ +	+
Later		–	+	+	–	–
Butt straightness	a	– –	– –	+	+ +	+ +
Wood properties:						
Basic density	a	–	o	– –	+ +	+ +
Compression wood (lack)	c	–	o	+	o?	?
Grain spirality (lack)	c	–	+	+	–	?

†*Key:* **a** denotes a large body of solid experimental evidence (many sites); **b** denotes good experimental evidence but from limited number of sites/pot trials; **c** denotes slender evidence; and two letters denote intermediate weights of evidence.

differences strongly correlate with cone-length differences among populations. Also, these data suggest that the two island populations are more closely related to each other than to the mainland populations (Murphy 1981).

Allozyme diversity

The tremendous amount of allozyme literature for plant species indicates that pine species are among the most genetically diverse plants (e.g., Hamrick et al. 1979). Allozyme studies may reasonably be interpreted as reflecting relative levels of whole genome variation (e.g., Woodruff and Gall 1992). Not atypically, different statistics and different studies show somewhat different patterns (Table 5). For example, depending on which statistic and which study are considered, the Monterey, Cambria, or Cedros population has the highest diversity. For most allozyme measures of genetic diversity, though, the Monterey population shows the highest genetic diversity.

Compared with other western conifers, the genetic diversity of Monterey pine, as measured by certain allozyme statistics, is modest to average (Table 6). Overall genetic diversity (Nei 1973), including monomorphic loci, is estimated as H_t=0.117 (Moran et al. 1988). However, as compared with other western pine species, the within-species diversity is mid-range. As compared with other California closed-cone pines (knobcone, H_t=0.087, Strauss and Conkle 1986; bishop, H_t=0.085, Millar 1983), it is high.

Expected heterozygosity (H_e) under Hardy-Weinberg equilibrium ranges from 0 to approximately 0.33 for the 48 pine species for which data are available for 10 or more allozyme loci (Ledig 1998). The modal value lies between 0.13 and 0.16. The H_e values from studies of Monterey pine (0.098, Moran et al. 1988; 0.127, Plessas and Strauss 1986; 0.141, Millar et al. 1988) show both that genetic estimates can vary considerably depending on sampling design and that Monterey pine lies in the normal (modal) range for pine species generally. These values suggest that most individuals of Monterey pine are expected to be heterozygous at about 10 to 14% of their loci (not adjusting for population differences).

Table 5. Allozyme diversity for the native populations of Monterey pine from three studies: number of trees sampled per population (N), mean number of alleles per locus (A), percent polymorphic† loci (P), and expected heterozygosity (H_e).

Population	N	A	P	H_e
Moran et al. 1988				
Año Nuevo	50	1.47	33.1	0.088
Monterey	72	1.74	50.5	0.097
Cambria	50	1.58	38.7	0.110
Guadalupe Island	50	1.46	35.5	0.089
Cedros Island	50	1.56	38.7	0.092
Millar et al. 1988				
Año Nuevo	15	1.8	44	0.13
Monterey	15	2.4	91	0.15
Cambria	15	1.8	50	0.14
Guadalupe Island	15	1.7	47	0.13
Cedros Island	14	2.0	56	0.16
Plessas and Strauss 1986				
Año Nuevo	96	1.62	29.8	0.125
Monterey	77	1.87	32.4	0.122
Cambria	84	1.78	37.9	0.131

†With the exception of data from Plessas and Strauss, the criterion of polymorphism is 99%, meaning a locus must have a second allele with at least a frequency of 1% for that locus to be considered polymorphic. For the Plessas and Strauss data, the criterion is 95%, thus these data are an underestimate relative to the other data in the table.

Molecular diversity

Monterey pine has a fairly large genome of approximately 10^{10} bp (Smith and Devey 1994). As compared with 82 other pine species for which nuclear DNA content has been recorded, Monterey pine is average (Murray et al. 2001). For example, Monterey pine has considerably more nuclear DNA than pitch pine (*Pinus rigida*) but much less than sugar pine (*P. lambertiana*) (Dhillon 1980). Montery pine at 22.00 pg of nuclear DNA (Murray 1998) is intermediate to bishop pine (20.28 pg, Hall et al. 2000) and knobcone pine (25.05 pg, Murray 1998).

Many of the approaches for assessing diversity at the DNA molecular level have been employed with Monterey pine. Three of the most useful types of markers have been RAPDs (random amplified polymorphic DNA markers), RFLPs (restriction fragment length polymorphism markers), and microsatellites (simple sequence repeat markers). Genetic linkage maps have been constructed for Monterey pine using all three (Devey et al. 1996, 1999).

RAPD markers have revealed somewhat higher genetic diversity and stronger among-population differentiation than an analysis with allozyme markers carried out with the same populations (Wu et al. 1999; Table 7). In this study, only trees from Año Nuevo, Cambria, and Guadalupe Island were included. Other studies of different plant species support the observation that RAPD data reveal more genetic diversity than allozyme data. This may result from several conditions (reviewed by Aagaard et al. 1995), including the reasoning that allozyme data reflect only a very limited part of the plant genome and a part that may evolve more slowly or be under stronger selection pressures than the genome at large (Wu et al. 1999).

A study of microsatellite sequences confirmed the finding that dinucleotide repeats are abundant in the Monterey pine genome, albeit seemingly less frequent than has been reported for some other species (Smith and Devey 1994). The reasonably high levels of heterozygosity found in two microsatellite loci provide a basis for developing a fingerprinting strategy for Monterey pine.

No significant chloroplast DNA diversity was found among the populations (Hong et al. 1993). However, chlo-

roplast DNA in general is highly conserved and thus may not be a sensitive marker for distinguishing populations within species.

Population genetic structure

Genetic structure—the pattern of distribution of genetic diversity within and among populations—is important in conservation planning because it defines the rate and spatial scale at which populations can evolve in response to environmental perturbations (Porter 1999). Genetic structure is, to a large extent, spatial structure. Most plant populations have substantial spatial structure—limitations in the distances that individuals (or propagules) disperse will result in relatives mating due to close proximity and consequently the buildup of genetic isolation by distance (e.g., Epperson and Li 1997).

Genetic structure (often measured with the statistic F_{ST}) is generally increased by local selection and genetic drift and decreased by gene flow. Wright (1931) derived an equation to express the opposing relationship between gene flow and genetic drift ($F_{ST} = \frac{1}{4N_e m + 1}$). In this relationship, m is the number of migrants per generation (a measure of gene flow) and Ne is the effective population size. It can be seen that even a low amount of gene flow would greatly reduce the divergence among populations caused by genetic drift. However, this equation assumes that the populations are at equilibrium—a condition not often satisfied in nature. Others have attempted to evaluate the relative historical influences of gene flow and drift on regional population structure by comparing the relationship between genetic and geographic distances, with good success (e.g., Hutchison and Templeton 1999).

Genetic structure is often interpreted as a function of genetic and ecological processes including natural selection in local environments, mating system, geographic distribution, seed dispersal mechanism (e.g., Hamrick et al. 1993), successional status, population size, and natural disturbance regime of habitat. These generalizations are supported by population genetic theory (e.g., Mitton 1995) and much allozyme literature (e.g., Hamrick and Godt 1989). However, a review of genetic structure studies across a range of plant species reveals many exceptions to expected genetic structure based on genecology, suggesting that genetic structure may be more a reflection of the contingencies of evolutionary history than ecology, life form, distribution, or breeding system (Rehfeldt 1997). This view is supported by a review of genetic differences among the populations of Monterey pine, many of which are not well (or at least, not easily) explained by natural selection, but are more likely a result of founder effects from repeated local extinctions and re-colonizations (Burdon et al. 1992a). These lessons caution us about infer-

Table 6. Allozyme diversity in western conifer species native to California: mean number of alleles per locus (A), percent polymorphic† loci (P), and expected heterozygosity (H_e).

Species	A	P	H_e	Reference
Thuja plicata	1.0	—	—	Copes 1981
Pinus torreyana	1.0	—	0	Ledig and Conkle 1983
Cupressus macrocarpa	1.2	61	—	Conkle 1987
P. muricata	1.5	25	0.077	Millar 1989
Sequoiadendron giganteum	1.5	50	—	Fins and Libby 1982
P. attenuata	1.6	40†	—	Wu et al. 1999
P. radiata	1.8	46	0.098	**Moran et al. 1988**
***P. radiata*‡**	1.8	48	—	**Wu et al. 1999**
P. radiata	—	58	0.141	**Millar et al. 1988**
Larix occidentalis	1.8	58	—	Jaquish and El-Kassaby
Chamæcyparis lawsoniana	1.9	65	—	Millar and Marshall 1991
Taxus brevifolia	2.0	68	—	Wheeler et al. 1995
P. ponderosa§	—	68	0.155	Niebling and Conkle 1990
Calocedrus decurrens	2.5	50	—	Harry 1984
P. albicaulis	2.6	85	—	Jorgensen and Hamrick 1997
P. contorta	2.7	89	0.185	Conkle 1981
	—	66	0.17	Yang and Yeh 1993
P. lambertiana	2.8	80	0.275	Conkle 1981
Sequoia sempervirens	3.1	92	—	Rogers 2000
Pseudotsuga menziesii	3.9	74	—	Conkle 1981

†With the exception of data for *P. radiata* from Wu et al., the criterion of polymorphism is 99%, meaning a locus must have a second allele with at least a frequency of 1% for that locus to be considered polymorphic. For the Wu et al. data, the criterion is 95%, thus these data are an underestimate relative to the other data in the table.

‡Calculated from samples of Cambria, Guadalupe, and Año Nuevo populations only.

§*P. ponderosa* var. *ponderosa.*

Table 7. Comparison of RAPD and allozyme markers in a study based on three native populations of Monterey pine (Año Nuevo, Cambria, Guadalupe Island): mean number of alleles per locus (A), percent polymorphic† loci (P), expected heterozygosity (H_e), and among-population differentiation (G_{ST}, Nei 1986) (Wu et al. 1999).

Marker	A	P	H_e	G_{ST}
Allozyme	1.76	48	0.14	0.12
RAPD	1.50	50	0.17	0.26

†The criterion of polymorphism is 95%, meaning a locus must have a second allele with at least a frequency of 5% for that locus to be considered polymorphic.

ring cause and effect relationships where only correlations exist. Evolutionary history, geographic distributions, population demographics, and their associated features must all be considered to understand the basis for specific genetic structures and then interpret this pattern for conservation purposes.

Genetic structure is often used as a means of identifying unique populations for conservation attention. Thus, attention is given to such measures as the proportion of total genetic variation that is due to among-population differences (G_{ST}, Nei 1973) and Nei's genetic distance between individual populations and others within the species (e.g., Jaquish and El-Kassaby 1998).

Genetic differentiation among populations of Monterey pine (based on allozymes) has been estimated as 16.2% of the total genetic diversity (Moran et al. 1988)—a rather large proportion compared with other western North American pine species (Table 8). In fact, the value of 16.2% for Monterey pine is among the highest values presented by Hamrick (1983) for conifers or by Ledig (1998) for pine species. Using Nei's genetic distance measure, the Cedros Island population is most strongly differentiated from the others, and the Monterey and Año Nuevo populations are most similar to one another. Genetic isolation by distance is suggested (r=0.88, P=0.05) if the Guadalupe Island population is excluded from the analysis (Moran et al. 1988).

These interpretations are largely, but not completely, mirrored by a similar allozyme study (Millar et al. 1988). Here, the five populations were strongly differentiated (13% diversity among populations) and the Cedros Island population was found to be the most genetically distant from all others. However, the loci assayed in this studied suggested that Monterey and Cambria were the most closely related pair of mainland populations.

These studies underscore the distinctiveness of the island populations. Indeed, both have been given varietal names, prior to most of the genetic studies, based on their substantial morphological differences from the mainland populations and each other (see Taxonomy section in Chapter 2).

Table 8. Estimates of proportion of total genetic variation among populations (PGV), based on allozyme data, in rangewide studies of western North American pine species.

Species	PGV	Reference
	(%)	
Pinus albicaulis	3.4	Jorgensen and Hamrick 1997
P. longæva	3.8	Hiebert and Hamrick 1983
P. contorta	6.1	Wheeler and Guries 1982
P. attenuata	12.0	Millar et al. 1988
P. jeffreyi	13.8	Furnier and Adams 1986
P. monticola	15.0	Steinhoff et al. 1983
P. radiata	16.2	Moran et al. 1988
P. muricata	22.0	Millar et al. 1988
P. torreyana	100.0	Ledig and Conkle 1983

Additional perspectives on genetic structure come from studies of the cytoplasmic organelle genomes—mitochondrial and chloroplast DNA. We might expect studies based on mitochondrial DNA (mtDNA) to show stronger differentiation among populations possibly as a result of lower rates of sequence mutation, small effective population size, and limited gene flow for maternally inherited organelles (e.g., Birky 1988). Indeed, a recent study of mtDNA among the Año Nuevo, Cambria, and Guadalupe Island populations showed a strong level of population differentiation (G_{ST} = 0.79) (Wu, J. et al. 1998). This was considerably higher than a similar study conducted on Douglas-fir (Hong et al. 1995). Furthermore, this estimate of population differentiation for Monterey pine may have been an underestimate since the population that is apparently most strongly differentiated from the others—Cedros Island—was not included.

Fine-scale genetic structure, or genetic patterns *within* populations of Monterey pine, has not been well studied. The few available studies used few stratified samples per population and thus did not comprehensively explore possible structuring associated with local selection regimes (e.g., elevation, microclimate, and soil type). Strong local genetic structure associated with soil type has been noted in some other pine species. For example, abrupt changes in genetic variation are known in bishop pine due to changes in soil fertility (Millar 1983) and in ponderosa pine (*Pinus ponderosa*) due to serpentine/nonserpentine soils (Ledig 1998).

Some common-garden studies have, though, shown considerable differentiation among subpopulations of the natural populations. For example, significant differences in the incidence of stem forking among subpopulations were noted in a series of common-garden studies conducted in Chile (Jayawickrama and Balocchi 1993). Strong local differentiation based on monoterpene levels has been noted within the Año Nuevo population (Burdon et al. 1997a). The reason for this differentiation has not been determined. Some of the possible causes include local adaptation, genetic contamination from planted nonlocal trees, introgression with nearby knobcone pine, founder effects, or a combination of all of these. The study's authors favor the founder effect explanation. In this case, trees near the edge of the main population could have experienced a more restricted pollen cloud than those at the core, leading to some genetic differentiation over time (Burdon et al. 1997a).

Mating system effects

The mating system of plants usually refers to the level of inbreeding and outcrossing. Monterey pine is largely outcrossing, typical of the genus. Given that neither spatial nor temporal separation of the sexes is strong (e.g., placement of male and female structures on the tree and timing of pollen shed and seed cone receptivity), that related trees tend to be clustered, and that self-incompatibility seems to be lacking in most species of pines, the level of outcrossing must be maintained by some other mechanisms. Partial self-sterility resulting from inbreeding depression may be a major part of the explanation for many pine species (Ledig 1998). In-

breeding depression may be expected in pine species because of the high frequency of recessive lethal genes that are found throughout the genus (Ledig 1998). Of course, inbreeding depression can have a variety of expressions, not all of them the result of poor self-fertility due to recessive lethals. The expression of deleterious but nonlethal genes can be manifest as low viability in offspring that result from self-fertilization, for example (R.D. Burdon, pers. comm.).

A recent study suggests that the overall outcrossing rate for Monterey pine may actually be quite low relative to many other conifers. The overall rate estimated from samples from the five populations was 0.75, and for the island populations was even lower: 0.67 and 0.45. The lower outcrossing rates observed in the island samples could, in theory, be a result of less outcross pollen reaching the seed cones or lower numbers of embryonic lethal equivalents relative to mainland populations (Savolainen et al. 2002).

There is considerable evidence of inbreeding depression in Monterey pine, from lowered seed viability, to slower growth rates among seedlings, to smaller heights and diameters of more mature trees. For example, there is evidence of lower viability of selfed embryos relative to outcrossed—one study showing approximately 40% filled seed in self-pollinated cones versus 80% filled seed in open-pollinated cones (Griffin and Lindgren 1985). The reduction in proportion of full seed after selfing is due to embryonic mortality, because conifers have no self-incompatibility system (Savolainen 1994). However, the selfing effect shown in that study is less dramatic than that found in some other pine species. For example, viability is reduced from 90.5% (open-pollinated) to 14.4% (self-pollinated) in piñon pine (*Pinus edulis*) (reviewed in Lanner 1998). In a controlled-pollination study, there were significantly fewer selfed seedlings produced than expected. This could have been the result of either lowered fertilization success with self pollen or reduced survival of inbred embryos (Matheson 1980). More recently, a single recessive lethal allele, associated with the death of Monterey pine seedlings (progeny of selfing) in their first month after germination, has been identified (Kuang et al. 1998).

Nursery and field studies conducted in New Zealand, in which comparisons were made between artificially cross-pollinated and selfed progeny of Monterey pine, provided further evidence that selfing can be detrimental. Selfed progeny were generally slower growing, had more crooked stems, displayed less desirable branching habit, and were more susceptible to needle diseases as compared with the cross-pollinated progeny (Wilcox 1983).

More evidence of selection against selfed genotypes in Monterey pine is apparent from a study that compared genotypes from embryos, seedlings, and more mature trees. In this allozyme study, homozygosity (i.e., indicative of selfing) is highest at the embryo stage, less at the sapling stage, and least in mature trees (Plessas and Strauss 1986). In another comparison of inbred (selfed) and outcrossed seedlings, the inbred genotypes grew only 80 to 90% as well as the outcrossed genotypes over an 8-year period (Pawsey 1964). The effects of inbreeding on growth were measured over 13 years in a field study of pedigreed Monterey pine in Australia (Wu, H.X. et al. 1998). Outcrossed material was compared with full siblings, half-sib matings, and first- and second-generation selfs. Inbreeding depression was shown to be a dynamic process, being greatest at the initial stage of stand development (four years), lessening for several years, and then increasing again with a secondary peak at the end of the study (13 years). In summary, in pines in general and Monterey pine more specifically, there is a fairly high expected true genetic load—which would tend to lead to inbreeding depression and drive the species towards outcrossing.

Understanding the genetic basis of inbreeding depression is important for appropriate conservation decisions. For Monterey pine, we do not know how many loci mutate to lethals. We do not know whether inbreeding depression is due to a few highly deleterious alleles or a large number of less deleterious alleles. If the former, then the unfavorable alleles could be quickly purged; if the latter, they could become fixed in the population (Savolainen 1994). Understanding the nature of the genetic load in Monterey pine, then, is critical to choosing the appropriate management response for inbred populations and for managing risk in the others.