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# GENE DIVERSITY AND GENETIC STRUCTURE IN A NARROW ENDEMIC, TORREY PINE (*PINUS TORREYANA* PARRY EX CARR.)

F. THOMAS LEDIG AND M. THOMPSON CONKLE
*Institute of Forest Genetics, Pacific Southwest Forest and Range Experiment Station, USDA Forest Service, P.O. Box 245, Berkeley, California 94701*

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Recent reviews have suggested that tree species are the most variable of organisms, as measured by proportion of polymorphic loci or average heterozygosity (Hamrick, 1979; Hamrick et al., 1979). Average heterozygosity is greater than 0.30 for several conifers. By contrast, annual herbaceous species have a mean heterozygosity of 0.13 (Hamrick et al., 1979).

Conifers have several mechanisms that promote outcrossing, and are expected to maintain high levels of genetic variation. Despite these mechanisms, it is uncertain whether the breeding system is capable of maintaining variability in small populations and in the absence of migration. While the genetic consequences of reduced population size have long been understood in theory, empirical evidence is scarce in plants, particularly in tree species. The potential to maintain high levels of genetic variation in reduced and scattered populations is important because of its implications for genetic resource conservation, and in fact, for the ability of species to respond to environmental change and avoid extinction. Because most conifers are commercially valued and exploited for lumber and paper products, much of the original forest in North America has been destroyed, and the tendency under management will be to reduce native populations to scattered relicts.

One way of forecasting the fate of species reduced in numbers is to make use of natural experiments, by examining the genetic structure of species that occur in disjunct populations (Shaffer, 1981). We chose to investigate how much variation might be preserved in conifers by observing a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr.). The investigation also contributes to an understanding of the biogeography of the California Channel Islands.

Torrey pine has the smallest population of any known pine. The 1973 count in the 445 ha (1,100 acre) Torrey Pines State Reserve on the Pacific Coast at San Diego, California was 3,401 mature trees (Calif. Dept. Parks Rec., 1975). Including seedlings, the Reserve's naturalist estimated ca. 7,000 individuals in 1979 (H. Nicol, pers. comm.). The population includes only two other small stands, contiguous with the Reserve. Another population occurs on Santa Rosa Island, one of the Northern Channel Islands off the California coast near Santa Barbara. There may be 2,000 individuals on the northeast coast of the island. Climatic, edaphic, and floristic characteristics of the two sites were summarized by Haller (1967). The San Diego and Santa Rosa Island populations are separated by 280 km and the island is 40 km from the mainland. It is highly unlikely that there has been any significant opportunity for gene exchange within recent centuries. Nor is Torrey pine likely to exchange genes with its closest relatives, digger pine (*Pinus sabiniana* Dougl.) and Coulter pine (*Pinus coulteri* D. Don), both of which are allopatric. Controlled crosses with Digger pine succeed only with difficulty and there have been no hybrids with Coulter pine despite several attempts (Critchfield, 1966).

## MATERIALS AND METHODS

Old cones, some in excess of 5 years old, were collected from stands in the Torrey Pines Reserve and on Santa Rosa Island in spring 1980. Cones had opened on the trees and most seed was shed; but in Tor-

rey pine, cone scales reflex upon opening to such a degree that some seed is trapped in the basal scales several years after the bulk of the seed cohort has dispersed.

We obtained viable seed from four areas within the Reserve: from 37 contiguous trees on the windward side of North Grove overlooking the ocean; 25 trees on High Point (113 m above sea level) where some of the oldest Torrey pine (about 125 years old) occur; and 40 trees on two adjacent spur ridges along with three from an intervening valley in the Extension Area. Seed was also collected from the largest native Torrey pine (ca. 100 cm dbh), located in the Extension Area. The groves in the Extension Area were ca. 1.75 km NNE of the North Grove, and the North Grove is ca. 450 m NW of High Point. On Santa Rosa Island we collected sound seed from 25 trees on the side of one canyon facing Beecher's Bay and 26 more from another canyon ca. 400 m east.

We used starch gel electrophoresis (Conkle et al., 1982) to process seed from 106 trees in the Reserve and 51 from the island. Seeds were stratified under cold-moist conditions for 30 days and then germinated to the stage where the emerging radicals extended 2–4 mm beyond the seed coat. The first analyses used one seed of each parent tree. Samples from the Reserve were co-electrophoresed with samples from the island to maximize the potential for identifying differences in isozyme mobility. Later we examined up to five additional seed per tree for 32 trees (18 from the Reserve and 14 from the island).

The female gametophytes and embryos of each seed were analyzed separately at adjacent positions on the starch gel. Isozyme bands from the gametophytes gave genetic information on the seed parent. Pollen parent genotypes were inferred by comparing the diploid pattern from an embryo with the pattern from the corresponding female gametophyte. When taken together, the female gametophyte and pollen analyses served to evaluate 246 haploid genomes from the Reserve and 132 haploid genomes from the island.

The gels were stained to produce isozyme bands in 25 enzyme systems (Table 1). We used experience gained from our studies of the inheritance of allozymes in other conifer species (Conkle, 1981) to estimate the number of loci needed to account for the isozymes within each enzyme system. We estimated that a total of 59 loci were required to account for the patterns in these 25 enzyme systems.

## Results and Discussion

Every individual from which seed was analyzed at the Torrey Pines State Reserve was identically homozygous to all others at each of the 59 gene loci. This includes trees growing on the coast and those inland, as well as some of the oldest and the largest native Torrey pines. Similarly, every individual sampled on Santa Rosa Island was identically homozygous.

However, the trees on Santa Rosa Island differed from those on the mainland at two, or 3.4%, of the 59 loci. One was a malic dehydrogenase locus and the other, shikimate dehydrogenase. Because all allelic variation is not detectable by electrophoresis, the two populations undoubtedly differ at more than 3.4% of their loci. Based on the proportion of amino acid substitutions producing differences in electrostatic charge, only 27.56% of all alleles should be detectably different (Shaw, 1965), but it has become generally accepted that electrophoretic techniques can detect amino acid changes which produce no change in net charge. Based on empirical studies of known forms of hemoglobin, 40% of all amino acid substitutions may be detectable by electrophoresis under standard conditions (Ramshaw et al., 1979). Using Ramshaw et al.'s estimates, we expect the mainland and island populations to differ at $0.34/0.4 = 0.085$ or 8.5% of their gene loci.

The observed pattern, all variation between populations and little or none within, is unknown for conifers. For all previously reported species, genic variation within stands has ranged from 88% to 97% of the total variation within the species (O'Malley et al., 1979; Yeh and Layton,

TABLE 1. *Enzyme systems and estimates of the number of loci analyzed in Torrey pine and the number of amino acid residues as calculated from molecular weights in Table 6 of Harris and Hopkinson (1976).*[1]

| Enzyme system | Number of loci | Molecular weight × 10 | Number of amino acid residues per locus |
|---|---|---|---|
| Acid phosphatase | 6 | 41 | 381 |
| Aconitase | 1 | 74 | 687 |
| Alcohol dehydrogenase | 2 | 40 | 371 |
| Aldolase | 2 | 39 | 362 |
| Catalase | 1 | 60 | 557 |
| Diaphorase | 3 | — | — |
| Esterase | | | |
| Colorimetric | 5 | 35 | 325 |
| Fluorescent | 1 | 35 | 325 |
| Glutamate dehydrogenase | 1 | | |
| Glutamate oxalacetic transaminase | 3 | 46 | 427 |
| Beta-glucosidase | 3 | 72 | 668 |
| Glycerate dehydrogenase | 2 | — | — |
| Glucose-6-phosphate dehydrogenase | 2 | 53 | 492 |
| Hexoseaminidase | 2 | — | — |
| Isocitriate dehydrogenase | 1 | 48 | 446 |
| Leucine aminopeptidase | 3 | 54 | 501 |
| Maliate dehydrogenase | 3 | 35 | 325 |
| Menadione reductase | 4 | — | — |
| Peptidase | 1 | 54 | 501 |
| Peroxidase | 2 | — | — |
| Phosphoglucomutase | 2 | 55 | 511 |
| 6-phosphogluconate dehydrogenase | 2 | 52 | 483 |
| Phosphoglucoseisomerase | 2 | 62 | 576 |
| Shikimate dehydrogenase | 1 | — | — |
| Superoxide dismutase | 4 | 17 | |

[1] Molecular weight divided by 107.7, the average weight per amino acid residue in Table 6.2 of Harris and Hopkinson (1976).

1979; Yeh and O'Malley, 1980; Guries and Ledig, 1982). Neither is any other conifer so invariable. Even the relatively uniform species, red pine (*Pinus resinosa* Ait.) and western redcedar (*Thuja plicata* Donn ex D. Don), have expected heterozygosities of 0.002 and 0.04, respectively (Guries and O'Malley, pers. comm.; Yeh, pers. comm.).

Allozyme results agree with other information. Measured in the field, the mean difference between populations for a composite score based on cone size, cone and cone scale shape, and foliage color was twice as large as the spread within populations, and in a uniform garden trial there were significant differences between the populations for height growth, tree form, and needle length (Haller, 1967). Oleoresin content was "remarkably similar between individuals" within populations, but populations were distinct for limonene and β-phellandrene concentrations, although percentages varied less than between populations of many other pines (Zavarin et al., 1967). Thus, population structure as revealed by morphological, biochemical, and growth traits supports the contention based on genetic analysis that there is little genic diversity within populations but minor differences between them.

The most likely explanation for the lack of variants is genetic drift. Apparently, both populations were reduced in size in the recent past to even smaller numbers (much less than 50) than now occur. There have been several climatic fluctations which would have influenced the distribution and abundance of Torrey pine. In particular, the Xerothermic period 8,500 to 3,000 years B.P. may have reduced Torrey pine at San Diego to a few individuals and eliminated intermediate populations. The presence of a disjunct So-

noran desert association at the Torrey Pines State Reserve indicates a more arid climate in the Holocene (Axelrod, 1967). As few as 20 to 30 generations may have elapsed since the end of the Xerothermic, leaving little opportunity for accumulation of genetic variants by mutation. With a mutation rate of $10^{-6}$ per gene per generation only $59/10^6$ new variants per individual would be expected in the most recent generation, and even in 50 generations (perhaps 5,000 years in Torrey pine), it would not be unusual to find no new variants in a sample the size of ours.

Lack of variation in the population on Santa Rosa Island could also be attributed to chance acting through the founder effect. To accomodate the presence of fossil mammoth remains on Santa Rosa, it was assumed that a land bridge connected the Northern Channel Islands to the mainland (Valentine and Lipps, 1967). Seismic reflection profiles and bathymetric maps now indicate a land bridge was unlikely (Junger and Johnson, 1980; Vedder and Howell, 1980), but about 18,000 years B.P. when sea level was ca. 117 m lower than now (Nardin et al., 1981), all the Northern Channel Islands were one large island, Santarosae, separated from the mainland by only 6 to 7 km (Vedder and Howell, 1980; Wenner and Johnson, 1980). If there was no land bridge, Torrey pine must have reached the island as seed stored in a floating cone or in the beak of a seed-caching bird. Clark's nutcrackers are reported to cache seed of pinyon pine (*Pinus edulis* Engelm.) more than 22 km from the source (Vander Wall and Balda, 1977). While there is no evidence that birds now cache Torrey pine seed, its seed is nearly wingless, not adapted to wind dispersal, and suggestive of bird dispersal (Lanner and Vander Wall, 1980). Transport by rafting or by a bird might establish Torrey pine on the island. The depauperate and unbalanced Channel Islands fauna support the view of "sweepstakes" dispersal over a barrier rather than migration over a bridge (Wenner and Johnson, 1980). It is virtually impossible for seed to have been rafted from the vicinity of San Diego because the longshore current along the California coast runs north to south, so Torrey pine on Santa Rosa Island must have originated on either the Santa Barbara coast or further north.

Even a single founder had to originate from a relatively invariable parental population or have suffered subsequent genetic erosion to explain the lack of variability in Santa Rosa Island Torrey pine. A naturalized population of pitcher plant (*Sarracenia purpurea* L.), although known to be established from only a single founder, nevertheless had an average heterozygosity of 0.042 compared to a mean of 0.095 for ten other populations (Schwaegerle and Schaal, 1979). Guries and Ledig (1982) found that heterozygosity in a marginal isolate of pitch pine (*Pinus rigida* Mill.), probably a relict from the Xerothermic 3,000 years B.P., was only slightly less than that in central populations (0.120 vs. 0.156).

Another possibility is that Torrey pine on Santa Rosa Island and the mainland at San Diego were part of the same population 20 million years B.P. and were separated by tectonic movement. Paleomagnetic data indicate that the Northern Channel Islands were rotated about an axis near the eastern end of the Santa Monica Range and translated northward during the Miocene (Kamerling and Luyendyk, 1979; Luyendyk et al., 1980). Santa Rosa Island was apparently part of the coast near San Diego in the mid-Miocene (B. P. Luyendyk, pers. comm.). Eocene gravels on Santa Cruz Island, part of the Northern Channel Islands chain, have correlated with the Poway Conglomerate near San Diego (Abbott and Smith, 1978). But estimates of time since genetic divergence are an order of magnitude too small to accommodate this possibility.

Using observed protein differences and length of time since divergence in the fossil record, Prager et al., (1976) estimated that it would take 7.5 to 11 million years to accumulate a 1% difference in amino acid sequencing between plant populations. They used 9 million years in their calculations. We estimate 24,689 amino acid

residues in the 59 loci we examined (Table 1). With two detectable isozyme variants and a detection rate of 40%, the populations differ at five, or 0.02%, of the amino acid residues. If divergence between insular and mainland populations of Torrey pine proceeded at the rate of 1% per 9 million years, then these populations have been separated 180 thousand years.

Another method of calculating time since divergence uses Nei's (1975 p. 177) standard genetic distance, $D$. For Torrey pine, $D = 0.0862155$. Time ($t$) since divergence (Nei, 1975 p. 192) is $t = D/2\alpha$, in which $\alpha$ is the mutation rate. The best estimate of mutation rates in higher organisms is $10^{-5}$ to $10^{-7}$ (Nei, 1975 p. 28), so time since isolation has been between 4.3 to 430 thousand years.

We invoke two rare events to explain population structure in Torrey pine: the reduction of a local San Diego population to such low numbers that all genetic variation was eliminated and the dispersal of a founder to Santarosae across the Santa Barbara Channel. Present populations must have been derived from a somewhat polymorphic ancestral population, because they are fixed for different alleles. There has not been time since the bottleneck of the Xerothermic or since the colonization of Santarosae Island to fix two novel mutations. If there had been time for the fixation of mutation, there would also have been time for the accumulation of other polymorphic loci.

For Torrey pine, growing at the edge of aridity, restricted to coastal pockets where fog and high humidity are crucial to its survival and reproduction, xerothermic intervals (Axelrod, 1967, 1981) may have fractured the species and created subpopulations that fluctuated within the demographic limits in which drift and fixation become the predominant driving force of evolution. There is no reliable fossil record of Torrey pine. Needle fascicles from an Oligocene formation in Oregon attributed to Torrey pine (Mason, 1927) are really indistinguishable from those of five-needle, hard pines from Mexico such as Montezuma pine (*Pinus montezumae* Lamb.) and its allies. The paucity of fossils is consistent with the picture of a species that may always have been relatively restricted in range to a progressively shrinking belt of temperate climate along the coastal strip (Axelrod, 1981).

## Summary

Early investigations suggested that genetic variability might be associated with longevity, and that tree species were the most heterozygous of organisms. However, these ideas may be modified as species representing a wider range of population structure are investigated. Torrey pine, a California endemic, occurs in only two populations, one on the coast at San Diego and one 280 km northwest on Santa Rosa Island. Populations number only ca. 7,000 and 2,000 individuals, respectively. Each population is composed of identical homozygous genotypes at 59 isozyme loci. The island population is fixed for alternate alleles to those in the coastal population at two of the 59 loci. These results agree with other lines of evidence that suggest Torrey pine is characterized by minor differences between relatively uniform populations.

Drift is the most likely explanation for lack of variability in Torrey pine. It probably experienced one or more reductions in population size, most recently during the Xerothermic period 8,500 to 3,000 B.P. The founder effect may have operated during colonization of Santa Rosa Island ca. 18,000 B.P. Apparently, the Northern Channel Islands to which Santa Rosa belongs were not connected to the adjacent mainland in recent geologic history, and colonization would require transport across at least a 6- to 7-km channel.

Red pine and western redcedar, conifers with greater ranges than Torrey pine, are only slightly more variable. Thus, despite the presence of mechanisms to maintain high levels of outbreeding, it appears the conifer breeding system can survive inbreeding with its consequence of genetic depauperization.

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Corresponding Editor: S. Jain

## ANNOUNCEMENT

The Department of Philosophy and the Institute for Molecular Biology at California State University, Fullerton announce the Thirteenth Annual Philosophy Symposium "BIOLOGY AND PHILOSOPHY." Invited papers include those of: Dr. Montgomery Furth, UCLA; Dr. Francisco Ayala, UC Davis; Dr. Larry Wright, UC Riverside; Dr. Gunther Stent, UC Berkeley; Dr. John Campbell, UCLA; Dr. J. William Schopf, UCLA; Dr. Ernst Mayr, Harvard University; Dr. Richard Burian, Drexel University; Dr. Marjorie Grene, UC Davis (Emeritus); Dr. Morton Beckner, Pomona College. The Symposium will be held March 8–11, 1983.