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LOW GENIC DIFFERENTIATION AMONG ISOLATED POPULATIONS OF THE CALIFORNIA FAN PALM (*WASHINGTONIA FILIFERA*)

LEROY R. MCCLENAGHAN, JR. AND ARTHUR C. BEAUCHAMP
Department of Biology
San Diego State University
San Diego, CA 92182

Abstract.—The purpose of this study was to assess the relative roles of population size and geographic isolation in determining population-genetic structure. Using electrophoretic techniques to quantify allozymic variation at 16 genetic loci, we measured genic variation within and among 16 natural populations of the California fan palm (*Washingtonia filifera*). Genotypes were determined for every individual in each population so that parametric values rather than sample estimates for measures of genic variability were obtained. Palm populations displayed low levels of within-population variability. The proportion of polymorphic loci and observed heterozygosity were 0.098 and 0.009 per population, respectively. Population size displayed a significant positive correlation with proportion of polymorphic loci, but not with observed heterozygosity. Low levels of genetic differentiation among populations were demonstrated by an *F*-statistic analysis and the computation of genetic similarity values. A hierarchical analysis of gene diversity revealed that only about 2% of the total gene diversity in *W. filifera* resides as among-population diversity. Climatic and geological changes since the Pliocene have eliminated widespread palm populations, and the species is presently restricted to isolated locations around the Colorado Desert. Existing populations in southern California are either relicts or recent recolonizations resulting from the dispersal of seeds from a refugium population in Baja California, Mexico. The observed patterns of low within- and low among-population genic diversity seem most consistent with a recent colonization by fan palms. It is hypothesized that stochastic processes reduced levels of genic variability in this refugium population during its formation. Dispersal of seeds from this refugium into suitable habitats in the Colorado Desert would produce populations with low variability and high genetic similarity because of their common ancestry. However, low intrapopulation variability and genetic homogeneity across populations could be the product of uniform selection pressures favoring a narrow array of specialized genotypes in either relict or colonizing populations.

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Population size and gene flow are two potentially important factors influencing the genetic structure of natural populations. Theory predicts that large populations should generally maintain higher levels of within-population genetic variability than small populations (Wright, 1931; Kimura and Crow, 1964; Nei et al., 1975) and that spatially isolated populations should display greater between-population genetic heterogeneity than populations having high rates of gene exchange (Wright, 1943; Kimura and Weiss, 1964; Endler, 1977). It is assumed that large populations are less susceptible to the loss of genetic variability from stochastic processes (genetic drift) and that populations not exchanging genes may differentiate if selective regimes differ from population to population. Additionally, isolated populations may diverge more rapidly through random genetic changes if the populations are of small size. In practice, accurate determinations of population sizes and the extent to which gene flow occurs among populations are not often made.

Aspects of the natural history of the California fan palm (*Washingtonia filifera*) make it an attractive organism on which to conduct empirical studies exploring the influence of population size and geographic isolation on levels of within- and among-population genetic variability. *Washingtonia filifera* is a long-lived monocot that is endemic to the southwestern United States and northern Baja California, Mexico (Read, 1961; Munz, 1974; McClintock, 1978). While there is little published information on the biology of *Washingtonia filifera,* it appears that pollination is predominantly insect-mediated and that vegetative reproduction does not occur (J. Cornett, pers. comm.). Fossils of *Washingtonia* are known from the Miocene and Pliocene (Bailey, 1936; Vogl and McHargue, 1966), and palm populations were once widespread along the California coast and in what is now the Mojave Desert. Climatic and geological changes eliminated these widespread populations (Axelrod, 1950). Existing palm populations in the Colorado Desert of southern California are either relicts from when the species was more widely distributed or recent colonizations created by the movement of seeds from refugia populations in Baja California. Intermittent incursions of the Gulf of California and diversions of the Colorado River into the Salton Basin created favorable palm habitat by producing a series of seas and freshwater lakes from the Pliocene to the present (Jaeger, 1955; Norris and Webb, 1976; Oakeshott, 1978). Disjunct populations of fan palms ranging in size from 1 to 3,000 individuals presently exist in canyon bottoms, springs, and seeps at isolated locations in the Colorado Desert (Smith, 1958; Moran, 1977). There are an estimated 11,000 California fan palms north of the U.S.–Mexico border (Henderson, 1961, 1965). The locations of most fan palm populations have been mapped because of the palm's importance as an indicator of surface water in the desert. Thus, the spatial distribution of populations is well-known for this species. In addition, the conspicuousness of *W. filifera* makes it possible to count every individual present at a given location so that population sizes can be accurately determined and parametric values, rather than sample estimates, can be obtained for measures of genetic variability.

Given the existing information about the distribution and abundance of *W. filifera,* three a priori predictions about the genetic structure of this species were made. First, populations of *W. filifera* should, on the average, maintain lower levels of genetic variability than other plant species because of their potentially relictual nature and generally small size. Second, large fan palm populations should maintain higher levels of genetic variability than smaller populations. Third, the spatial isolation of these fan palm populations should produce substantial genetic differentiation among populations.

MATERIALS AND METHODS

California fan palms ($N = 446$) were sampled at 16 localities from the Colorado Desert of southern California (Fig. 1). Straight-line distances between palm populations ranged from less than 1 km to 75 km ($\bar{x} = 36.5$ km). Sampling was conducted from December 1981 through June 1982. Population size was determined by counting all the trees present at a locality, and a pruning saw with extension poles was used to remove a single green leaf from every tree in each population. Several leaflets were excised from each leaf with scissors and sealed in a plastic bag with a numbered tag identifying the source tree. These bags were kept on ice until they could be brought into the laboratory, where they were refrigerated at 2°C until they were electrophoresed.

Preparation of leaf tissue for electrophoresis followed the procedure of Torres and Tisserat (1980). Pieces of leaflets (1 cm^2) were placed in 2.3 × 2.3 cm plastic weighing boats and covered with two or three drops of a crushing buffer composed of 0.01 M pH 7.5 tris-citrate with 0.1 M 2-mercaptoethanol, 12% soluble polyvinylpyrrolidone (MW 40,000), 1 mM ethylenediamine tetraacetic acid and 3% bovine serum albumin. These pieces were simultaneously crushed and mixed with the buffer by placing them under the end of an aluminum rod (7.1 × 1.2 cm) and then striking the rod several times with a 454 g hammer. The crushate was then absorbed onto 5 × 10 mm wicks of Whatman chromatography paper.

Sample wicks were then placed into gels of 11.5% Electrostarch (Otto Hiller, Madison, WI). Lithium hydroxide and tris-citrate discontinuous gel buffers (Selander et al., 1971) were used. Lithium hydroxide gels were stained for leucine aminopeptidase (LAP), α-glycerophosphate dehydrogenase (α-GPD), 6-phosphogluconate dehydrogenase (6-PGD), malic enzyme (ME), fluorescent esterase (FEST), and alcohol dehydrogenase (ADH). Tris citrate gels were stained for glutamate oxaloacetate transaminase (GOT), glucosephosphate isomerase (GPI), phosphoglucomutase (PGM), and esterase (EST). Staining procedures followed Selander et al. (1971), with the exceptions of ME (Ayala et al., 1972), FEST (Mitton et al., 1979) and ADH (Torres, 1974). The most anodal form of each enzyme system was designated 1, with others numbered sequentially in order of decreasing anodal mobility. The most common allele at each lo-

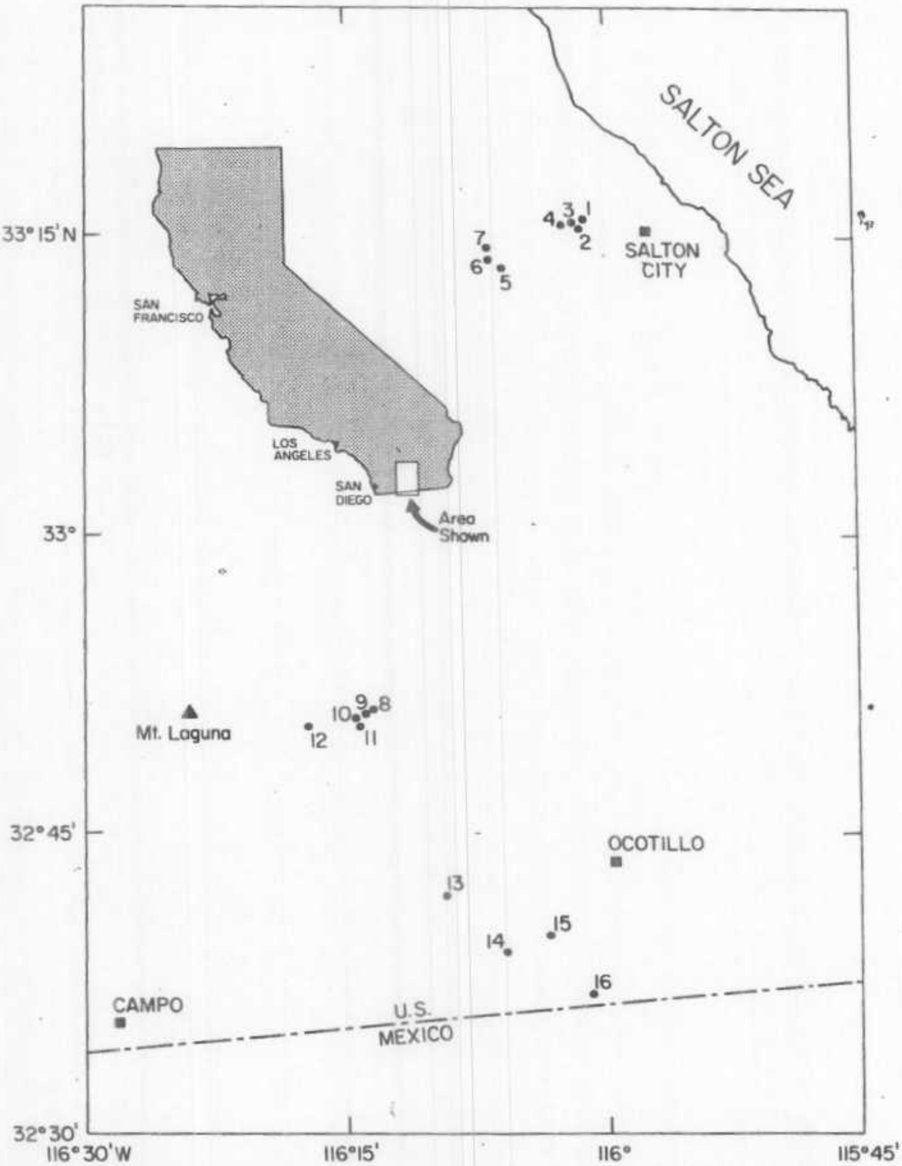

FIG. 1. San Diego and Imperial Counties, California, showing locations of California fan palm (*Washingtonia filifera*) populations utilized to assess levels of genic variability and differentiation. Numerical designations of populations correspond to those in Table 1.

cus was designated 100, with all other alleles designated according to the relative mobilities of their products compared to that of the common allele. Since all individuals in each population were counted and used in the electrophoretic analysis, the population sizes and the various genetic statistics obtained represent parametric values rather than sample estimates.

RESULTS

We were consistently able to resolve and score electromorph phenotypes at 16 genetic loci. Two enzyme forms were present for 6-PGD, PGM, GPI, ME, EST, and GOT. Single enzyme forms were seen for αGPD, FEST, LAP, and ADH. Polymorphisms were observed at eight of these 16 loci. Three alleles were detected at *Adh, Gpi-1, Est-1* and *Est-2,* while two alleles were segregating at *Got-2, Lap, Pgm-1* and *Me-1*. Observed genotype frequencies at these polymorphic loci were typically in close agreement with expected Hardy-Weinberg frequencies. Of 25 chi-square comparisons, only the observed *Adh* genotype frequencies in the Mortero Palms population were statistically different from Hardy-Weinberg expectations ($X^2 = 5.20$, $d.f. = 1$).

Parameters measuring levels of genic

TABLE 1. Population sizes (N), proportion of polymorphic loci (P), and observed (H_o) and expected (H_e) heterozygosities for 16 populations of the California fan palm, *Washingtonia filifera*. Population numbers correspond to localities in Figure 1.

Population	N	P	H_o	H_e
1. Palm Wash I	5	0.000	0.000	0.000
2. Four Palm Spring	9	0.125	0.014	0.013
3. Palm Wash II	1	0.000	0.000	0.000
4. Palm Wash III	2	0.000	0.000	0.000
5. Five Palms Oasis	2	0.063	0.031	0.023
6. Una Palma	1	0.000	0.000	0.000
7. Seventeen Palms	27	0.250	0.014	0.014
8. North Grove	35	0.063	0.013	0.011
9. Mary's Grove	76	0.188	0.006	0.006
10. Suprise Canyon	36	0.188	0.016	0.015
11. Pygmy Grove	45	0.188	0.008	0.008
12. Indian Canyon	7	0.125	0.018	0.016
13. Mortero Palms	82	0.063	0.004	0.005
14. Mountain Springs	11	0.000	0.000	0.000
15. Myers Creek	34	0.125	0.015	0.014
16. Pinto Canyon	73	0.188	0.006	0.006
Means	27.9	0.098	0.009	0.008

variability in each population of *Washingtonia filifera* are given in Table 1. No palm population was polymorphic for more than four loci (Seventeen Palms, $P = 0.25$), while five populations were monomorphic at all 16 loci surveyed. A mean proportion of polymorphic loci of 0.098 was observed across populations. Observed heterozygosities ranged from 0.031 in the Five Palm Oasis population to 0.000 in five of the fan palm populations. The mean for this parameter across all 16 populations was 0.009 (Table 1). Expected heterozygosities were generally equal to or less than observed heterozygosities.

The relationship between genic variability and population size was explored by correlation analysis (Zar, 1984). Population sizes for each palm population were first log-transformed and then correlated with the proportion of polymorphic loci and with the observed heterozygosity. Population size was found to be significantly correlated with P ($r = 0.718$, $d.f. = 14$) but not with H_o ($r = 0.118$, $d.f. = 14$). The two parameters of genic variability were not significantly associated with one another ($r = 0.441$, $d.f = 14$).

The results of a hierarchical analysis of gene diversity (Nei, 1973, 1975) are summarized in Table 2. Total species-wide gene diversities (H_T) ranged from 0.0022 for *Est-1* to 0.0799 for *Adh*. The mean H_T over all eight loci was 0.0170. Average gene diversities among populations (D_{ST}) only ranged from 0.0014 for *Gpi-1* to 0.0000 for *Est-1*. G_{ST} measures the relative diversity among populations ($G_{ST} = D_{ST}/H_T$). Values for G_{ST} varied from 0.0472 for *Gpi-1* to 0.0057 for *Est-1*. The average G_{ST} over loci was 0.0234. Thus, only about 2.3% of the total gene diversity observed in *W. filifera* is attributable to among-population diversity; the rest is due to within-population diversity.

The organization of genetic variation in fan palm populations was studied using the F-statistics of Wright (1965). The results of this analysis are given in Table 3. Although chi-square tests revealed significant spatial heterogeneity in allelic frequencies at four of the eight polymorphic loci (*Adh*, *Gpi-1*, *Est-2* and *Got-2*), levels of genetic subdivision as measured by F-statistics were low. F_{ST} values ranged from 0.0064 (*Est-1*) to 0.1364 (*Gpi-1*). The mean F_{ST} over all loci was 0.0383. Values of F_{IS} were typically small and negative ($F_{IS} = -0.0070$), while F_{IT} values were small and positive ($F_{IT} = 0.0167$).

A general lack of genetic differentiation among fan palm populations was also suggested when allelic frequencies at polymorphic loci were used to compute Nei's (1972)

TABLE 2. Estimates of gene diversity parameters for eight loci in the California fan palm.

Locus	Number of alleles	H_T	H_S	D_{ST}	G_{ST}
Adh	3	0.0799	0.0788	0.0011	0.0138
Gpi-1	3	0.0289	0.0275	0.0014	0.0472
Est-1	3	0.0022	0.0022	0.0000	0.0057
Est-2	3	0.0045	0.0043	0.0002	0.0349
Got-2	2	0.0045	0.0043	0.0002	0.0349
Lap	2	0.0067	0.0066	0.0001	0.0140
Pgm-1	2	0.0045	0.0044	0.0001	0.0256
Me-1	2	0.0045	0.0044	0.0001	0.0115
Means	20	0.0170	0.0166	0.0004	0.0234

normalized genetic identity (I). Values for I only varied from 0.9928 to 1.0000 for 120 pairwise comparisons of palm populations ($\bar{I} = 0.9989 \pm 0.0002$).

DISCUSSION

We initially predicted that populations of *Washingtonia filifera* should display relatively low levels of within-population genetic variability because of their generally small size and potentially relictual nature. The results of this study support that prediction (Table 1). The average palm population was polymorphic at only 0.098 of its loci examined, while the average individual was heterozygous at 0.009 of its loci. These values are substantially lower than those reported for plants in general and for long-lived perennials in particular. Hamrick et al. (1979) summarized the results from electrophoretic surveys of 113 plant taxa and found average values for the proportion of polymorphic loci and heterozygosity of 0.368 and 0.156, respectively. When these taxa were grouped according to generation length, long-lived perennials were the most variable class, polymorphic at nearly 0.66 of their loci and heterozygous for 0.27 of their loci. Guries and Ledig (1982) reported an average observed heterozygosity of 0.145 for five species of North American conifers. Low levels of genic variability comparable to those observed in this study of *W. filifera* have been reported for a few long-lived perennials, such as *Pinus resinosa* which displayed no electrophoretic polymorphisms in five populations surveyed for variation at nine loci (Fowler and Morris, 1977). Ledig and Conkle (1983) also observed total homozygosity at 59 loci in two populations of *Pinus torreyana,* which, like *W. filifera,* is a narrow endemic consisting of 2,000 individuals on Santa Rosa Island and another population of about 7,000 individuals near San Diego, California. Mashburn et al. (1978) reported no intraspecific polymorphisms within populations of *Typha latifolia* or *T. domingensis* surveyed for variation at 10 loci.

We also predicted that within-population variability in *W. filifera* would be some function of population size. In obtaining measures of population size and genetic variability, all individuals in each population were counted and included in the electrophoretic analysis. When correlation analysis was performed on the parameters, the results were somewhat ambiguous: the log of population size was significantly correlated with the proportion of polymorphic loci, but was not correlated with observed heterozygosity. This pattern may reflect differences in the properties of the parameters employed to estimate genic variability within palm populations. Perhaps the proportion of polymorphic loci more clearly reflects the influence of changes and bottlenecks in population size. Genetic drift in populations passing through bottlenecks primarily results in the loss of low frequency alleles (Nei et al., 1975), thus reducing the proportion of loci for which a population is polymorphic. However, the proportion of heterozygotes at polymorphic loci will be influenced not only by random changes in allele frequencies, but also by factors such as natural selection and inbreeding, whose effects may be independent of population size. For example, self-fertilization in a population would reduce the frequency of het-

TABLE 3. Estimates of F_{IT}, F_{IS}, and F_{ST} for eight loci in the California fan palm. Heterogeneity chi-square values (X^2) are also presented.

Locus	Number of alleles	F_{IT}	F_{IS}	F_{ST}	χ^2
Adh	3	0.0290	0.0154	0.0325	57.91**
Gpi-1	3	0.0253	−0.0231	0.1346	239.56**
Est-1	3	0.0057	0.0000	0.0064	11.43
Est-2	3	0.0349	0.0000	0.0419	74.51**
Got-2	2	0.0163	−0.0192	0.0348	30.97**
Lap	2	0.0064	−0.0077	0.0173	15.40
Pgm-1	2	0.0117	−0.0143	0.0261	23.22
Me-1	2	0.0046	−0.0069	0.0129	11.44
Means	20	0.0167	−0.0070	0.0383	464.45*

* $P < 0.05$.
** $P < 0.01$.

erozygotes regardless of the population's size. While the existence of hermaphroditic flowers in *W. filifera* suggests that self-fertilization is possible (Bailey, 1936; Moore, 1961; Read, 1961; Tomlinson, 1979), rates of selfing and outcrossing are presently unknown for this species. However, it appears from this study that inbreeding has had an insignificant effect on the genetic structure of palm populations. First, deviations of observed genotype frequencies from those expected on the basis of Hardy-Weinberg equilibrium were not statistically significant. Second, F_{IS} values (Table 3) were extremely small and usually negative, suggesting that inbreeding within subdivisions is negligible.

The results of the hierarchical analysis of gene diversity (Table 2), the *F*-statistic analysis (Table 3), and the computation of genetic similarity values all failed to support our prediction that fan palm populations would display extensive genetic differentiation because of their spatial isolation. Despite this isolation, only about 2% of the total gene diversity observed in *W. filifera* resides as among-population diversity (Table 2). Such low levels of among-population diversity are characteristic of species composed of large, relatively contiguous populations. For example, reported G_{ST} values for species in the genus *Pinus* include 0.023 for *P. rigida* (Guries and Ledig, 1982), 0.022 for *P. taeda* (Adams and Joly, 1980), and 0.041 for *P. contorta* (Yeh and Layton, 1979). Although significant spatial heterogeneity among populations was observed for allelic frequencies at four of the eight polymorphic loci (Table 3), levels of genetic subdivision among populations were low ($\bar{F}_{ST} = 0.038$; Table 3). In contrast, Levin (1978) reported mean F_{ST} values of 0.41 and 0.21 for *Phlox cuspidata* and *P. roemariana*, respectively, while Ellstrand and Levin (1980) observed values of 0.24 for *Oenothera laciniata* and 0.09 for *O. grandis*. Lastly, this relative lack of genetic divergence among palm populations is underscored by the high similarity displayed when Nei's (1972) genetic identity values are calculated for pairwise combinations of populations ($\bar{I} = 0.9989$).

The coincident patterns of low within-population variability and high among-population genetic similarity observed in this study can be accounted for by several models. First, according to the "niche width-variation hypothesis" (Van Valen, 1965; Soulé, 1973), there is an adaptive relationship between ecological amplitude and genetic variability. If the narrow habitat requirements and limited distribution of *W. filifera* can be equated with low ecological versatility, then this hypothesis predicts low levels of within-population variability, with natural selection favoring only a narrow array of specialized genotypes. Low genetic differentiation among populations would be expected if similar selective pressures were operating across palm populations. Babbel and Selander (1974) tentatively posed this mechanism as an explanation for low within- and among-population genetic diversity in *Lupinus subcarnosus*, an edaphically restricted legume from east-central Texas. However, it is difficult to assess the applicability of this hypothesis in explaining the observed patterns in *W. filifera,* since so little is known about the selective factors which might be operating on fan palms.

Alternatively, the patterns of genetic diversity observed in this study may reflect historical events associated with changes in the geographic distribution of *W. filifera* since the Pliocene. These patterns may also provide insights into the origin of the populations currently found in the Colorado Desert of southern California. If the palm populations sampled in this study are actually relicts, then stochastic processes occurring during the fragmentation of the species distribution should have reduced levels of genetic variability within these populations. However, these same stochastic processes would also be expected to produce substantial genetic differentiation among populations rather than the high levels of genetic similarity observed. A more plausible scenario would be one in which existing palm populations in the Colorado Desert are the products of seed dispersal from a source population having reduced genetic variability. It has been suggested that climatic changes may have completely eliminated fan palms from the Colorado Desert and restricted the species to small refugia populations in Baja California (J. Cornett, pers. comm.). The subsequent movement of seeds into southern California from a refugium population in northern Baja California would have resulted in these colonizing populations also having low variability and high genetic similarity because of their common ancestry. Gene flow among these colonizing populations would have reinforced their genetic similarity. The potential for palm seed dispersal even between spatially isolated populations may be considerable since coyotes, birds and Cahuilla Indians have all been reported as dispersal agents for palm seeds (McClintock, 1978; Bullock, 1980; Cornett, 1985).

The results of the present study suggest several avenues for future research that might yield further insights as to the origin of palm populations in southern California. First, more information concerning the colonizing abilities of *W. filifera* and levels of gene flow among palm populations should be obtained. Studies measuring effective seed-dispersal distances and rates of pollen flow among populations would be useful in assessing the extent to which populations exchange genes and the likelihood that seed dispersal has resulted in the colonization of suitable habitats in the Colorado Desert. Second, electrophoretic data from *W. filifera* populations in Baja California could be useful in determining whether those populations have served as sources of seed for colonizing populations in southern California. If so, then Baja populations should display low levels of within-population variability and high genetic similarity with populations in the Colorado Desert.

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