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A comparison of *Hemizonia conjugens* (Otay tarplant) with two closely related tarplant species using enzyme electrophoresis and soil textural analysis

FINAL REPORT

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Jonathan Snapp-Cook provided oversight of the soil textural analysis as well as doing most of the tedious labor related to that. He also mounted and produced labels for the herbarium specimens, used a GPS locator to describe precisely our collection sites, created all the computer-generated maps, prepared soils data for analysis, and contributed to sections of the report. Garret Williams helped with collection of soils, mapping of site locations, and preparation of soils for analysis; James Lawson and Brenda McMillan went to Skunk Hollow and collected soil and plants; and Jonathan Dunn participated in the collection of voucher specimens and soil samples. Paul Detwiler and Jennifer Harris worked on the soil textural analysis. Bengt Allen cultivated the *Hemizonia* seedlings and made the drawings of leaves. Juda Sakrison wrote the progress reports and dealt with permit and budget matters. Jim Zimmer, the SDSU technician for the Ecology Program Area, helped with supplies and technical support. Though a small project, it took the contributions of many to bring to completion, and we appreciate everyone's efforts.

ABSTRACT

Hemizonia conjugens Keck (Otay tarplant) is a narrowly distributed member of the Asteraceae family restricted to southwestern San Diego County (Keck 1958) and northwestern Baja California, Mexico (Tanowitz 1978). Loss of habitat, combined with threats to remaining populations, led to listing as an endangered species by the State of California in 1979 (CDFG 2000) and placement on the threatened species list of the US Fish and Wildlife Service in 1998 (Federal Register 1998). It is considered a "covered" species under the Multiple Species Conservation Program Subregional Plan (City of San Diego 1998). The State and Federal listings, combined with the MSCP "covered" status, make this species a focus of conservation and management efforts.

Three species of *Hemizonia* are reputed to occur in southwestern San Diego County (Beauchamp 1986). They are *Hemizonia conjugens* (Otay tarplant), *H. fasciculata* , and *H. paniculata.*

The goals of this project are to: 1) characterize the genetics, using allozyme electrophoresis, of the three species of *Hemizonia* believed to occur in southwestern San Diego County; 2) determine through population genetic analysis the species status of the *Hemizonia* populations on San Miguel Ranch, particularly in relation to tarplant populations on the southern portion of the property (Proctor Valley) versus those on the northern portion; and 3) determine the textural attributes of soils adjacent to each *Hemizonia* species population sample.

Results of our limited study indicate that the three species were clearly distinguished from each other using allozyme electrophoresis. Substantial genetic diversity resides within individual populations of *Hemizonia conjugens* , making each remaining population of *H. conjugens* potentially valuable for this diversity. Hybridization between the co-occurring species was not indicated. Mechanisms of isolation between *fasciculata* and *conjugens* could be genetic, temporal, or spatial. *H. paniculata* was not observed at any of the southern San Diego County sites.

Earlier observations of a close association of *H. conjugens* with clayey soils are substantiated. This would pose limitations for expansion of populations or altering the known distribution of the species.

CHAPTER 1. INTRODUCTION

1.1. PURPOSE OF THE PROJECT

Hemizonia conjugens Keck (Otay tarplant) is a narrowly distributed member of the Asteraceae family restricted to southwestern San Diego County (Keck 1958) and northwestern Baja California, Mexico (Tanowitz 1978). Urbanization, agricultural development and degradation of habitat by various disturbances has reduced the United States populations to about 22, all within a 240 km^2 area. Loss of habitat, combined with threats to remaining populations, led to listing as an endangered species by the State of California in 1979 (CDFG 2000) and placement on the threatened species list of the US Fish and Wildlife Service in 1998 (Federal Register 1998). It is considered a "covered" species under the Multiple Species Conservation Program Subregional Plan (City of San Diego 1998). The State and Federal listings, combined with the MSCP "covered" status, make this species a focus of conservation and management efforts.

Three species of *Hemizonia* are reputed to occur in southwestern San Diego County (Beauchamp 1986). The three species are *Hemizonia conjugens* (Otay tarplant), *H. fasciculata*, and *H. paniculata*. Baldwin (1999) recognizes all three as members of the segregate genus *Deinandra*. For purposes of this report, the widely used *Hemizonia* will be used. *H. conjugens* is clearly distinguishable from *H. fasciculata*, based on floral and other morphological characteristics. Due to character overlap, *H. conjugens* is not so easily distinguished from *H. paniculata*. At least two of the above species (*H. fasciculata* and *H. conjugens*) are known to co-occur in the general area of a proposed development known as San Miguel Ranch (P and D 1999), and there are also records suggesting that *H. paniculata* may occur in the area (Beauchamp 1986). Correct identification of *H. conjugens* is crucial to assessing its overall status, determining its remaining distribution, and outlining appropriate management actions.

The goals of this project are to: 1) characterize the genetics, using allozyme electrophoresis, of the three species of *Hemizonia* believed to occur in southwestern San Diego County; 2) determine through population genetic analysis the species status of the *Hemizonia* populations on San Miguel Ranch, particularly in relation to tarplant populations on the southern portion of the property (Proctor Valley) versus those on the northern portion; and 3) determine the textural attributes of soils adjacent to each *Hemizonia* species population sample.

1.2. LOCATION OF THE STUDY

The sampling sites for *Hemizonia conjugens* and *H. fasciculata* , along with associated soils, were all located in southern San Diego County (Figures 1 and 2). *Hemizonia paniculata* plants and associated soil were sampled from extreme northwestern San Diego County and southern Riverside County (Figure 1). Details regarding the locations are given in Section 2.1 (Methods: Collection of soil and plant material).

1.3. BACKGROUND ON THE SPECIES

1.3.1. General

Hemizonia conjugens Keck (Otay tarplant) is a rare and poorly understood annual of restricted distribution. Seedlings form rosettes, and as the season progresses and flowering approaches, the plants bolt to 1-4 dm in height, branching above the middle. The lower leaves are oblanceolate and lobed while upper leaves are entire to toothed. Foliage is glandular above and bristly below. Flowering occurs late in the growing season, peaking in early summer, but often extending into early fall. Heads contain 13-21 fertile or staminate yellow disk flowers, with the 8-10 ray flowers having yellow ligules about 3-6 mm long.

The species type specimen was collected from *"River bottom land near Otay, San Diego County, California, Abrams 3521, UC!* " (Tanowitz 1978). In the United States, Otay tarplant occurs as far north as the northern edge of the Sweetwater Reservoir and south nearly to the Mexican border (Figure 3). Populations are also known from northwestern Baja California, Mexico. *H. conjugens* appears to be confined to clayey soils with grassland or coastal scrub vegetation, generally below 300 m.

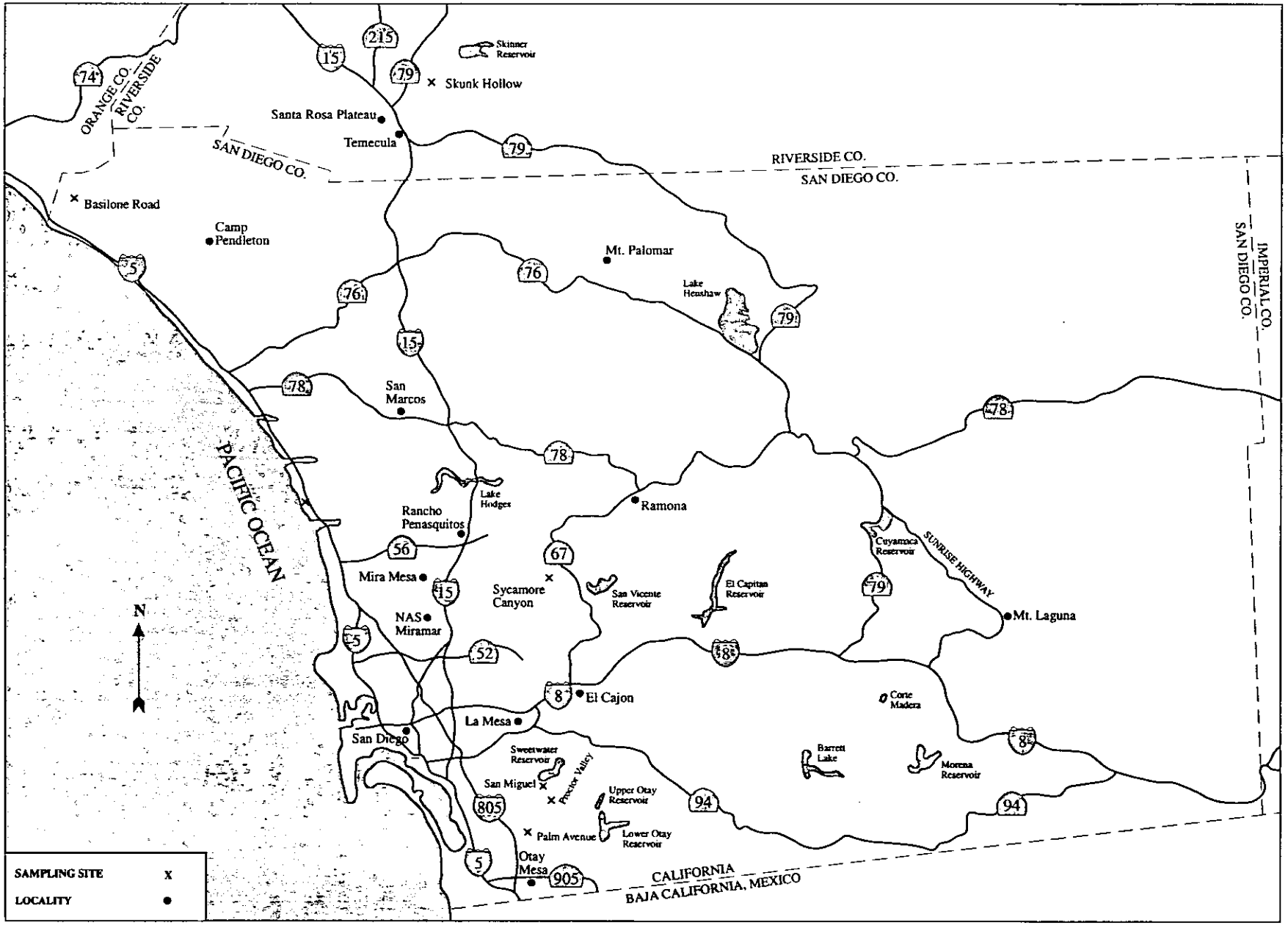

Figure 1. Sampling sites for the study of three *Hemizonia* species in southern California.

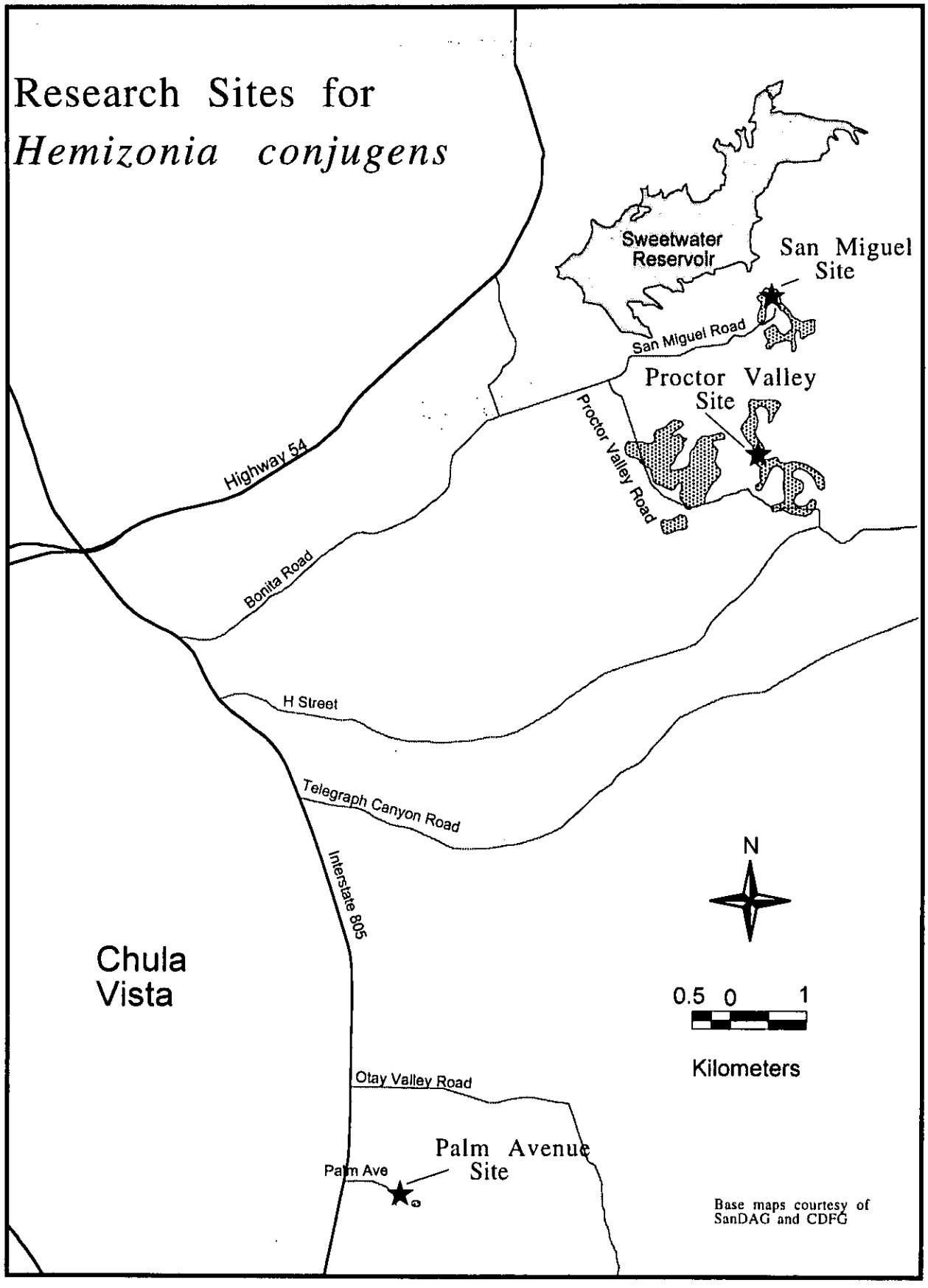

Figure 2. Locations of southernmost research sites.

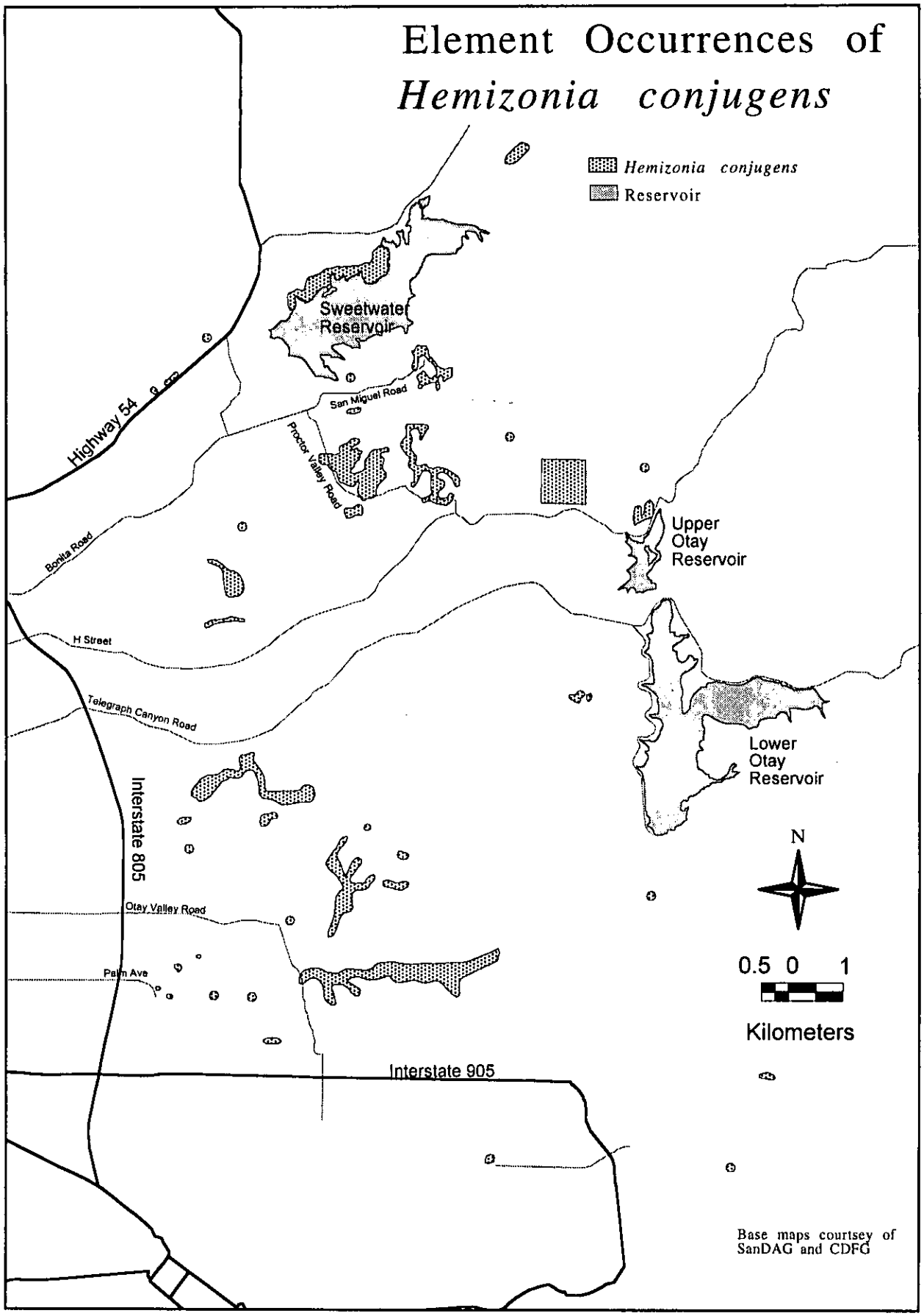

Figure 3. Location of *Hemizonia conjugens* Element Occurrences (CDFG) in south San Diego County.

1.3.2. Systematics of the genus *Hemizonia*

Hemizonia (Asteraceae: Heliantheae-Madiinae) is a group of 33 species (27 annuals and 6 perennials) which is the largest genus comprising approximately 26% of the subtribe Madiinae, the tarweeds (Kyhos et al. 1990). The name tarweed refers to the sticky leaf exudates produced on the surface of these plants. The exudates consist mainly of flavonoids and related compounds (Bohm and Fong 1990). There appear to be two centers of diversity for the group: the Hawaiian Islands (insular taxa) and the continental United States (mainland taxa). The mainland tarweeds occur almost exclusively in the western United States, with their distributional center in the central valley of California (Kyhos et al. 1990). Several species present in California extend into Mexico, with several others being restricted to the Mexican mainland or offshore islands. Two highly disjunct species are found in Chile and Argentina. In general, the mainland group appears adapted or restricted to lowland, often quite xeric habitat.

The *Hemizonia* are among the most diverse in chromosome number within the entire subtribe (Kyhos et al. 1990), and few plant groups have been as intensively studied cytologically. *Hemizonia* includes a continuous series of gametic chromosome numbers from n = 9 to n = 14, modally centered at n = 12 (Kyhos et al. 1990). When the distribution of gametic chromosome numbers for other genera in the subtribe are considered, the distribution in *Hemizonia* appears to indicate that the genus is primitively polyploid, with gametic numbers above and below the mode (n = 12) representing aneuploid derivatives from this polyploid mode (Kyhos et al. 1990). The species of *Hemizonia* appear to form four natural species groups or sections based on chromosome number. Clausen and his colleagues (Clausen 1951, Clausen et al. 1945) conducted extensive crossing studies within the group, and found that, generally, crosses within the genus produced hybrids with low-to-extremely low fertility. More than half of the more than 55 interspecific combinations produced were highly sterile hybrids, indicating strong reproductive barriers among most *Hemizonia* species.

Hemizonia conjugens has a gametic chromosome number of n = 12 (2n = 24). Keck (1958), based on the species annual habit and a variety of morphological characters, considered *H. conjugens* to be most closely related to *H. fasciculata* Torrey and A. Grey (n = 12) and *H. paniculata* A. Grey (n = 12). Because *H. conjugens* displays intermediacy for several morphological traits, and given the geographical distribution of the three *Hemizonia* species, Keck (1958) postulated that *H. conjugens* was an amphidiploid (i.e. allopolyploid) derivative of a hybrid between *H. fasciculata* and *H. paniculata*. This does not appear to be the case, as the gametic chromosome number is the same in all three species, though there is still a possibility

of a homoploid hybrid origin (Baldwin personal communication). However, the cytological, morphological, and geographic data, coupled with preliminary flavonoid analyses noted in Tanowitz (1978), strongly indicate that the three species may be closely related and may have arisen from the same ancestral stock.

Baldwin (1999) revised the taxonomy of the tarweeds to reflect current knowledge of phylogenetic relationships. In his scheme, *H. conjugens*, *H. fasciculata* and *H. paniculata* become *Deinandra conjugens*, *D. fasciculata* and *D. paniculata*. *Deinandra* comprises all of the (annual) members of *Hemizonia* sect. *Madiomeris* and the perennials constituting the informal "Fruticosae" or "Zonamra" group of *Hemizonia*. *Centromadia* encompasses the spikeweeds and *Hemizonia* in the new sense is restricted to the hayfield tarweeds, i.e. *Hemizonia congesta* (Baldwin 1999).

CHAPTER 2. METHODS

2.1. COLLECTION OF SOIL AND PLANT MATERIAL

Leaf tissue and associated soil samples were collected from four localities within San Diego County and one within Riverside County, representing typical habitat for the three species of *Hemizonia* covered by this report (Tables 1 and 2)(Figures 1 and 2). Collections were made in the spring of 1998 and 1999. The San Miguel Ranch property is part of the San Diego National Wildlife Refuge, the Proctor Valley site is south of the Refuge and within the proposed San Miguel Ranch development, Skunk Hollow in Riverside County is within the Barry Jones Mitigation Bank, the Pendleton site is on Marine Corps Base Camp Pendleton just south of Basilone Road, the Palm Avenue collections were made on the proposed open space within the Hidden Trails project on west Otay Mesa, and the Sycamore Canyon collections were made in the Goodan Ranch Preserve (CDFG EO 32). The two San Miguel Ranch NWR collections were within 50 meters of each other.

Using a Scoutmaster GPS (Trimble/Navigation Model #17319-45), latitude and longitude were determined for each collection site (Table 2).

Tissue specimens used for allozyme electrophoresis were collected by Dave Truesdale. Species type was confirmed in the field by floral morphology. The plants were chosen from each population in a dispersed pattern based on the location of plants with new green growth. The

Population Name	Population Designation	Species	n (plants)	n (soil)
Camp Pendleton	CPPANIC	paniculata	45	10
Skunk Hollow	SHPANIC	paniculata	15	10
San Miguel Ranch	SMFASCIC	fasciculata	45	30
Palm Avenue	PAFASCIC	fasciculata	30	--
Sycamore Canyon	SCFASCIC	fasciculata	20	20
San Miguel Ranch	SMCONJU	conjugens	45	--
Palm Avenue	PACONJU	conjugens	30	30
Proctor Valley Road	PVCONJU	conjugens	45	30

Table 1. Population name, population designation for genetical analysis, species collected and sample size for the 8 populations analyzed for genetic variation in *Hemizonia spp.* and for texture of associated soils. Population designations indicate collection site and associated species.

Population Name	Species	Latitude (N)	Longitude (W)
Camp Pendleton	paniculata	33° 23' 12"	117° 34' 09"
Skunk Hollow	paniculata	33° 33' 21"	117° 06' 07"
San Miguel Ranch	fasciculata	32° 41' 19"	116° 58' 30"
Sycamore Canyon (EO 32-1)	fasciculata	33° 56' 06"	116° 58' 57"
Sycamore Canyon (EO 32-2)	fasciculata	33° 56' 11"	116° 58' 35"
San Miguel Ranch	conjugens	32° 41' 21"	116° 58' 33"
Palm Avenue	conjugens	32° 34' 54"	117° 01' 23"
Palm Avenue	fasciculata	same	same
Proctor Valley Road	conjugens	32° 39' 53"	116° 59' 16"

Table 2. Latitude and longitude for each sampling site.

new growth was cut from the plant and placed in a sealed plastic bag with a few drops of water. Tissue was transported to the lab on ice and extracted within 24 hours of collection. Tissue from plants grown from seed was used as well. For details of post-collection treatments, see Section 2.5.

At the base of each plant or plant cluster, two soil samples were collected. One 100-g soil sample was collected to a depth of 10 cm and the other one was taken 10-20 cm from the surface. Soils were stored at room temperature and allowed to dry until the textural analysis was completed.

Whole plants were collected for voucher specimens. In spring of 1998, Jonathan Dunn collected *Hemizonia conjugens* plants at the Proctor Valley and San Miguel Ranch locations, and *H. fasciculata* from the San Miguel Ranch location only. Jonathan Snapp-Cook collected *H. paniculata* from Skunk Hollow and Camp Pendleton, and *H. fasciculata* from the two sites at Sycamore Canyon in the spring of 1999. Three plants of each species were collected at each site. No specimens were collected at the Palm Avenue site.

2.2. PREPARATION OF HERBARIUM SPECIMENS

Jonathan Snapp-Cook mounted the specimens and prepared the herbarium labels according to standard practices. The identification of the specimens was verified by Dr. Jon Rebman, Curator of Botany, San Diego Natural History Museum. Dr. Bruce Baldwin also verified the identifications while they were at the Museum.

2.3. LEAF MORPHOLOGY

Seeds of all three *Hemizonia* species were placed in pony packs of soil, moistened and placed on an outdoor bench at the San Diego State University greenhouse in the summer of 1999. As seedlings developed, Bengt Allen harvested plants and measured and drew individual leaves.

2.4. SOILS ANALYSIS

The soil samples were broken up using a mortar and pestle and then passed through a 2-mm soil sieve prior to analysis.

Textural analysis was performed in the summer of 1999 using the hydrometer method of Gee and Bauder (1986). Tests on subsamples indicated oven drying did not reduce moisture significantly. Each replicate used 50 g of soil in 125 ml of sodium hexametaphosphate (50g/L), and hydrometer readings were recorded at 30 seconds, 60 seconds, 1.5 hour, and 24 hours. Correction factors were taken from Brower and Zar (1977). USDA particle size classifications were used (Bowman 1973). Data were analyzed using ANOVA with arcsine square root transformation of proportion data, with the exception of the pH's which were analyzed without transformation. Pair-wise comparisons are tested using Fisher's PLSD (protected least significant difference) test. Results were compared with the more conservative Scheffe's F test and the Bonferroni/Dunn procedure.

Soil pH was determined using a standard pH probe calibrated against standard solutions of known pH. The test was done using a soil paste rather than a solution because this was the most efficient method for a preliminary analysis.

2.5. ENZYME ELECTROPHORESIS

Approximately 0.5 g of leaf tissue was homogenized in an 1.5 ml Eppendorf tube with freshly prepared 0.2 M phosphate buffer (pH 7.5) containing 0.20 M sodium tetraborate, 0.01 M sodium metabisulfite, 0.015 M diethyldithiocarbamic acid sodium salt, 2% (w/v) L-ascorbic acid sodium salt, 6% (w/v) PVP-40, 2% (w/v) bovine serum albumin (Fraction V), 0.5% (v/v) 2- mercaptoethanol and 10 mg/25 ml NADP. Due to the close proximity of *H. fasciculata* and *H. conjugens* at San Miguel (< 50 m) and Palm Avenue (intermixed), we germinated seed from each of these species at these localities and the Proctor Valley *conjugens* population to determine if these species hybridize naturally.

Crude extracts were absorbed onto filter paper wicks and placed into 12% horizontal starch gels composed of the following buffers: lithium hydroxide (pH 8.3; May, 1994), histidine-citrate (pH 6.2; May, 1994), and tris-versene-borate (pH 8.6; May, 1994). A total of 21 loci could be reliably scored of which 9 were polymorphic. The *Hemizonia fasciculata* sample from San Miguel Ranch was chosen as the reference population due to the large number of alleles present at the leucine aminopeptidase locus (7), and individuals of known electrophoretic mobility (i.e. genotype) from this population were included on all gels to facilitate scoring and insure internal consistency. The most anodal form of each enzyme system was designated A, with others lettered sequentially in order of decreasing anodal mobility.

Allele frequencies were calculated using Biosys-1 (Swofford and Selander 1981) and Genestrut (Constantine et al. 1994). Genetic variability parameters estimated included the mean number of alleles per locus (A), percentage polymorphic loci (P), the observed heterozygosity (H_o) and the expected heterozygosity based on Hardy-Weinberg assumptions (H_e). Conformity to Hardy-Weinberg expectations, based on chi-square expectations using Levene's (1949) correction for small sample size and Yate's correction for continuity, were utilized. Workman and Niswander's (1970) formulae were used for contingency chi-square testing of hypotheses of homogeneity in allele frequencies.

F-statistics (Wright 1978) were calculated using the methods of Weir and Cockerham (Weir and Cockerham 1984; Weir 1990). Significance of the departure of F-statistics from zero and 95% confidence intervals were calculated by bootstrapping over populations and jackknifing over loci respectively. Hierarchical analyses of gene diversity followed the methods of Chakraborty (1980).

Nei's (1978) unbiased genetic distance and identity were calculated with PHYLIP (Felsenstein 1993) and were used to determine relationships among populations. Multiple allele frequency data sets (100) were generated with PHYLIP SEQBOOT. SEQBOOT is a general "bootstrapping" program that generates resampled versions of the original data set by sampling characters randomly with replacement. The random variation of the results from analyzing the bootstrapped data sets have been shown to be typical of the variation expected from collecting new data sets (Felsenstein 1993). Genetic distance matrices were generated using the GENDIST option of PHYLIP. Multiple trees were generated using the Neighbor-Joining and UPGMA methods. The NEIGHBOR-JOINING method constructs a tree by successive clustering of lineages, setting branch lengths as the lineages join. This method does not assume an evolutionary clock, so that, in effect, it is unrooted. The UPGMA method constructs a tree by agglomerative (successive) clustering using an average-linkage method of clustering. The PHYLIP CONSENSE program was utilized to construct a majority rule consensus tree of relatedness between populations. Basically, a consensus tree consists of groups that occur as often as possible in the data. The majority rule consensus tree consists of all groups that occur more than 50% of the time and has at each fork a number indicating how many times the group which consists of the species to the right of the fork occurred.

CHAPTER 3. HERBARIUM SPECIMENS AND LIVE PLANT MATERIAL

3.1. DISPOSITION OF HERBARIUM SPECIMENS

One copy of each specimen was left at the SD Natural History Museum to be entered into their Herbarium collection. The other two copies were returned to San Diego State University, where one copy will be entered into the SDSU Herbarium and the other copy will be temporarily housed in Ellen Bauder's personal plant collection until studies on *Hemizonia* are completed. Those specimens will then either be placed in the SDSU Herbarium or another suitable location.

3.2. MORPHOLOGICAL COMPARISON OF LIVE MATERIAL

In the vegetative, rosette stage, leaves of *Hemizonia fasciculata* and *Hemizonia conjugens* were indistinguishable, but those of *H. paniculata* had more prominent indentations along the leaf margins (Figure 4).

CHAPTER 4. RELATIONSHIP OF SPECIES TO SOIL TEXTURE

4.1. RESULTS

The surface soil (0-10 cm) clay fraction was greatest at the Proctor Valley (*Hemizonia conjugens*) and Sycamore Canyon (*H. fasciculata* and *Acanthomintha ilicifolia*) sampling sites, and nearly as great at the San Miguel Ranch site associated with *H. conjugens* (Tables 3 and 4, Figure 5). The adjacent San Miguel Ranch site (c. 50 distant) that supports *H. fasciculata*, has a significantly lower surface clay fraction compared to the nearby *conjugens* site ($p < .0001$)(Figure 6)(Table 3). All pair-wise comparisons of sites show significant differences in clay content ($p < .0001$), except the Proctor Valley/Sycamore Canyon comparison ($p = .9663$). The Pendleton/Skunk Hollow comparison was non-significant ($p = .0285$) by the Scheffe and Bonferroni/Dunn tests.

The Basilone Road/Camp Pendleton site, and the Skunk Hollow location had the lowest percentage of clay and the highest sand fraction (Tables 3, 4 and 5) (Figure 5). These sites support populations of *H. paniculata.* Of the two San Miguel Ranch sites, the one associated with *fasciculata* had a significantly higher percentage of sand ($p <.0001$)(Tables 4 and 5).

Hemizonia

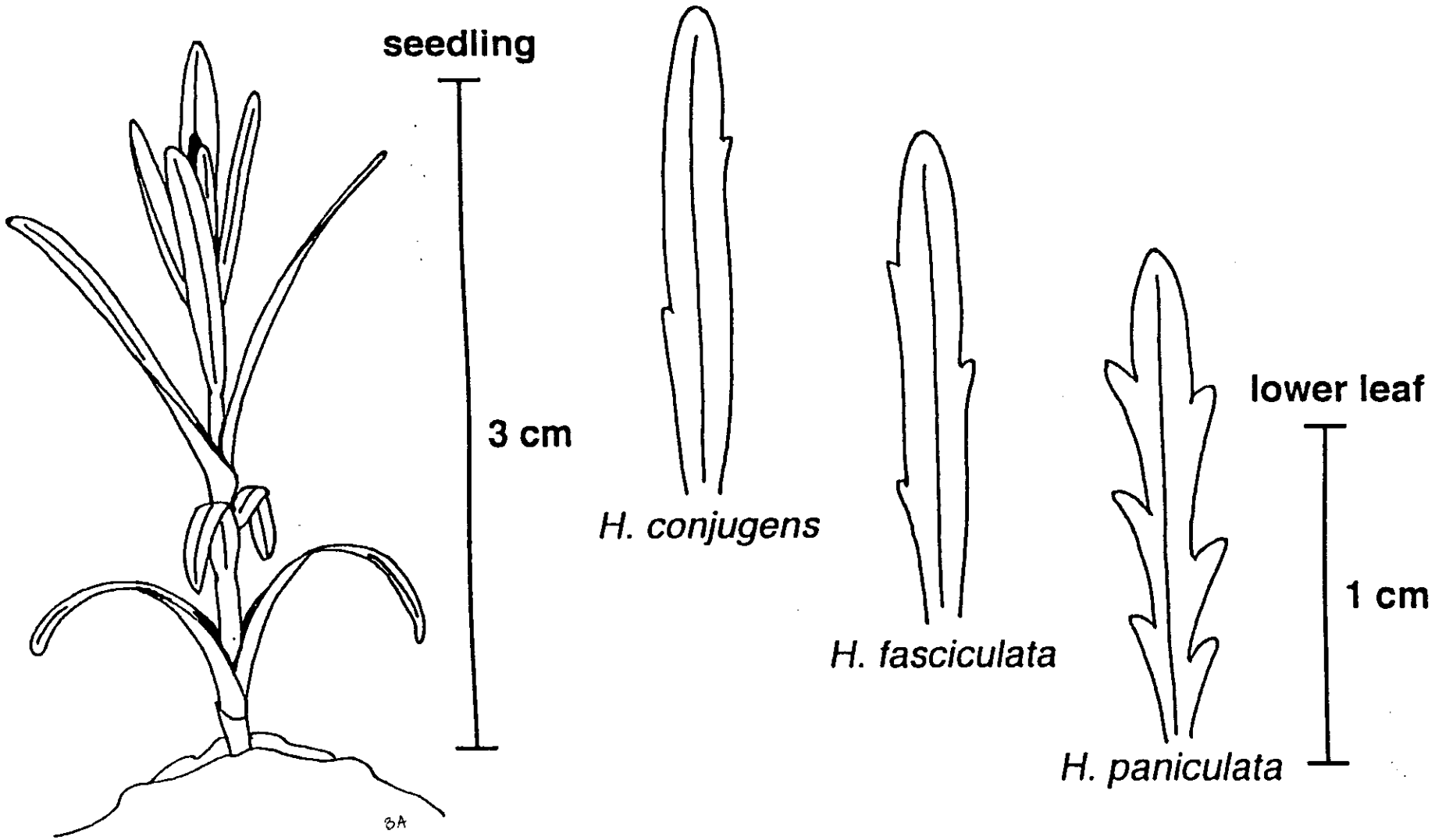

Figure 4. Leaves of three species of *Hemizonia* seedlings.

ANOVA Table for Arcsqrtclay

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Site	5	2.375	.475	116.638	<.0001	583.188	1.000
Residual	124	.505	.004				

Means Table for Arcsqrtclay
Effect: Site

	Count	Mean	Std. Dev.	Std. Err.
San Miguel-conjugens	30	.559	.069	.013
San Miguel-fasciculata	30	.419	.040	.007
Proctor Valley	30	.636	.083	.015
Pendleton	10	.229	.008	.002
Skunk Hollow	10	.293	.020	.006
Sycamore Canyon	20	.635	.078	.017

Fisher's PLSD for Arcsqrtclay
Effect: Site
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
San Miguel-conjugens, San Miguel-fasciculata	.140	.033	<.0001	S
San Miguel-conjugens, Proctor Valley	-.077	.033	<.0001	S
San Miguel-conjugens, Pendleton	.329	.046	<.0001	S
San Miguel-conjugens, Skunk Hollow	.266	.046	<.0001	S
San Miguel-conjugens, Sycamore Canyon	-.077	.036	<.0001	S
San Miguel-fasciculata, Proctor Valley	-.218	.033	<.0001	S
San Miguel-fasciculata, Pendleton	.189	.046	<.0001	S
San Miguel-fasciculata, Skunk Hollow	.126	.046	<.0001	S
San Miguel-fasciculata, Sycamore Canyon	-.217	.036	<.0001	S
Proctor Valley, Pendleton	.407	.046	<.0001	S
Proctor Valley, Skunk Hollow	.344	.046	<.0001	S
Proctor Valley, Sycamore Canyon	.001	.036	.9663	
Pendleton, Skunk Hollow	-.063	.056	.0285	S
Pendleton, Sycamore Canyon	-.406	.049	<.0001	S
Skunk Hollow, Sycamore Canyon	-.343	.049	<.0001	S

Table 3. ANOVA table and results of PLSD test for proportion of surface clay (arcsine square root transformed data) in soil at six sites.

Sampling Site	Percent			pH
	Sand	Silt	Clay	
San Miguel Ranch (*conjugens*)				6.22
Top	40.6	34.3	25.1	
Bottom	37.3	32.7	30.0	
San Miguel Ranch (*fasciculata*)				6.35
Top	51.7	32.3	16.0	
Bottom	49.3	31.6	19.1	
Proctor Valley (conjugens)				6.98
Top	48.4	19.4	32.2	
Bottom	46.9	17.2	35.9	
Camp Pendleton (*paniculata*)				7.29
Top	87.3	7.8	4.9	
Bottom	88.3	6.5	5.1	
Skunk Hollow (*paniculata*)				6.14
Top	72.3	18.5	9.2	
Bottom	74.6	16.5	8.9	
Sycamore Canyon (*fasciculata*)	40.0	24.6	35.4	6.64

Table 4. Mean pH and mean percentage of sand, silt, and clay in the soil at six *Hemizonia* sampling sites.

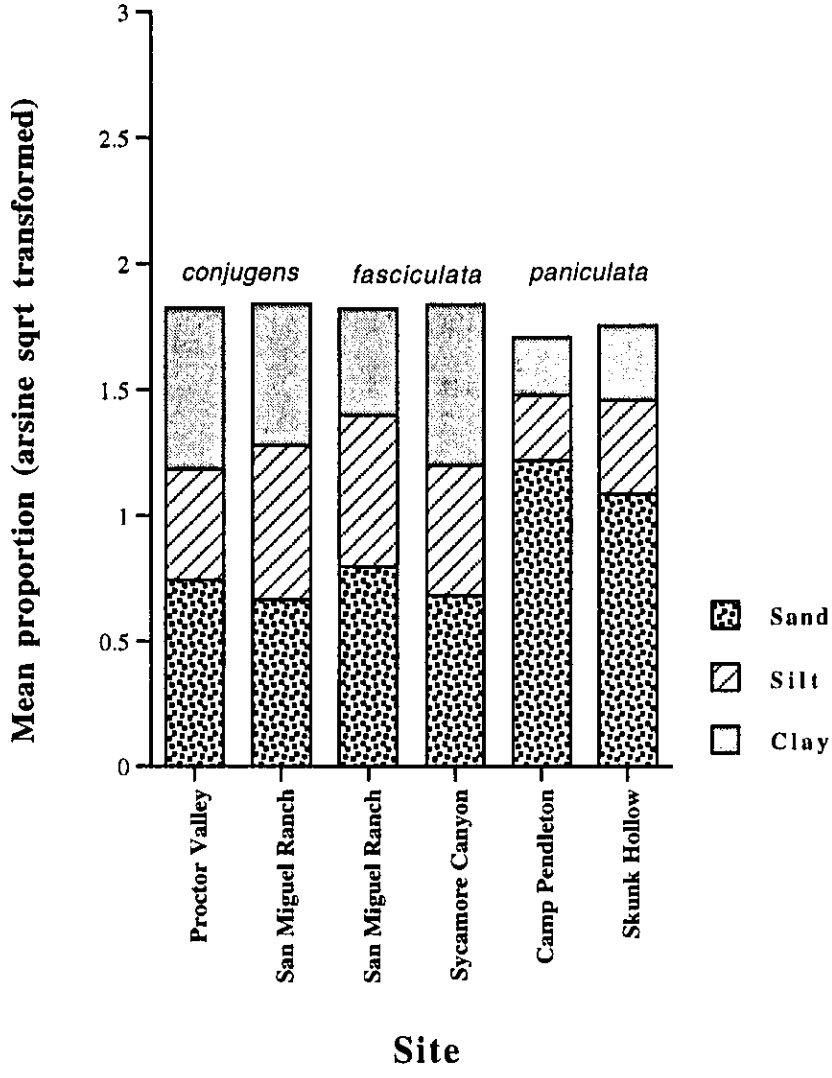

Figure 5. Mean proportion of sand, silt, and clay in the top 10 cm of soil at each of six *Hemizonia sites.*

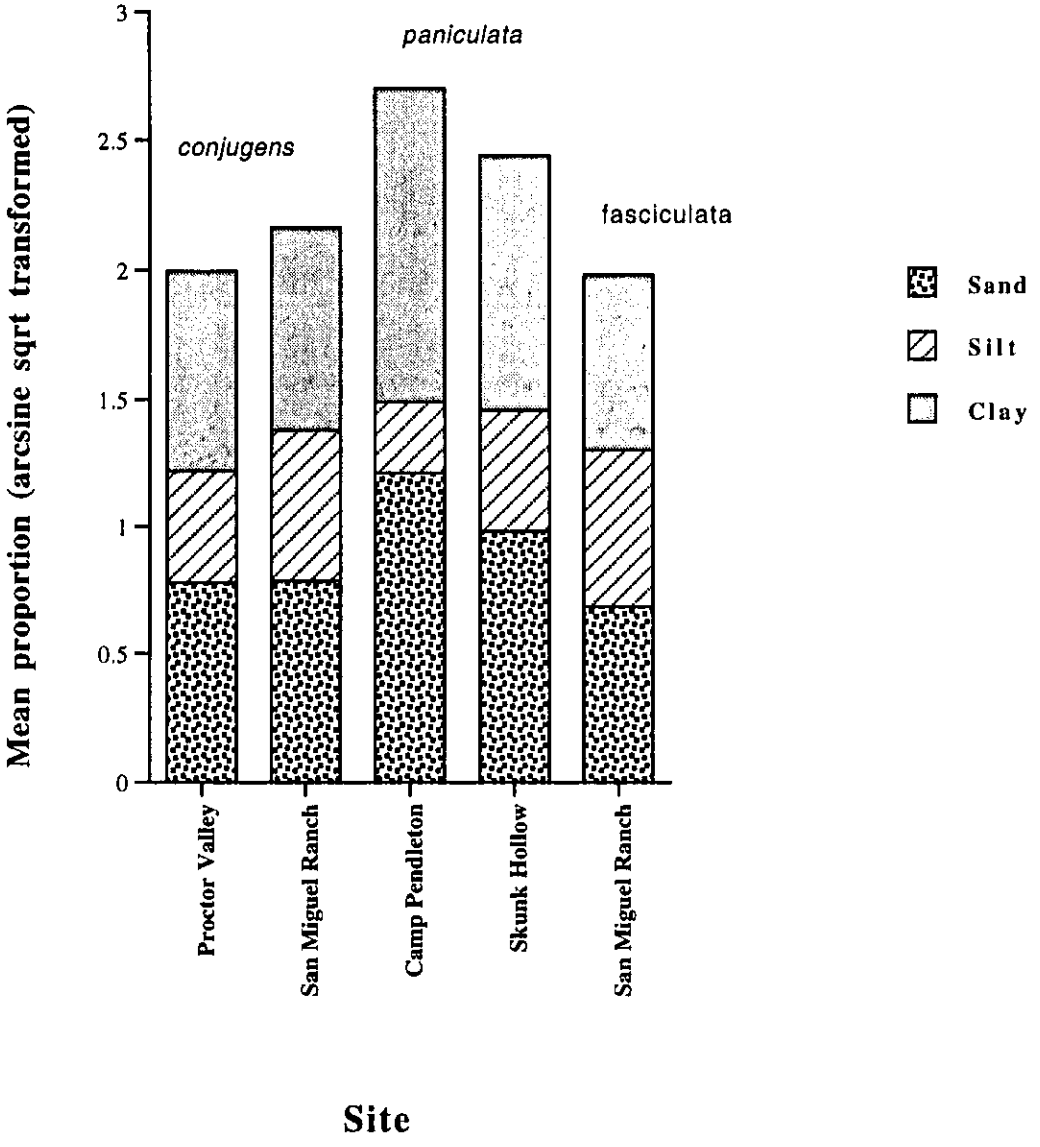

Figure 6. Mean proportion of sand, silt, and clay 10-20 cm below the surface at each of five *Hemizonia* sites.

ANOVA Table for Arcsqrtsand

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Site	5	3.480	.696	185.084	<.0001	925.419	1.000
Residual	124	.466	.004				

Means Table for Arcsqrtsand
Effect: Site

	Count	Mean	Std. Dev.	Std. Err.
San Miguel-conjugens	30	.666	.068	.012
San Miguel-fasciculata	30	.797	.042	.008
Proctor Valley	30	.745	.082	.015
Pendleton	10	1.220	.014	.004
Skunk Hollow	10	1.087	.045	.014
Sycamore Canyon	20	.684	.059	.013

Fisher's PLSD for Arcsqrtsand
Effect: Site
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
San Miguel-conjugens, San Miguel-fasciculata	-.131	.031	<.0001	S
San Miguel-conjugens, Proctor Valley	-.079	.031	<.0001	S
San Miguel-conjugens, Pendleton	-.554	.044	<.0001	S
San Miguel-conjugens, Skunk Hollow	-.421	.044	<.0001	S
San Miguel-conjugens, Sycamore Canyon	-.018	.035	.2986	
San Miguel-fasciculata, Proctor Valley	.052	.031	.0015	S
San Miguel-fasciculata, Pendleton	-.424	.044	<.0001	S
San Miguel-fasciculata, Skunk Hollow	-.290	.044	<.0001	S
San Miguel-fasciculata, Sycamore Canyon	.112	.035	<.0001	S
Proctor Valley, Pendleton	-.475	.044	<.0001	S
Proctor Valley, Skunk Hollow	-.342	.044	<.0001	S
Proctor Valley, Sycamore Canyon	.061	.035	.0008	S
Pendleton, Skunk Hollow	.133	.054	<.0001	S
Pendleton, Sycamore Canyon	.536	.047	<.0001	S
Skunk Hollow, Sycamore Canyon	.403	.047	<.0001	S

Table 5. ANOVA table and results of PLSD test for proportion of sand (arcsine square root transformed data) in surface soil at six sites.

The percentage of silt was highest at the three sites with high clay content (the two San Miguel Ranch sampling sites and Sycamore Canyon), but did not differ between the two San Miguel Ranch sites ($p = .1271$)(Table 6). Again, the two sites with *paniculata* were more similar to each other than to the other sites, but all the pair-wise comparisons were significant ($p < .0001$), except the above-mentioned one between the San Miguel Ranch sites. Scheffe's F test indicated no significant difference between San Miguel-*fasciculata* and Proctor Valley ($p = .0678$).

There was no significant difference between the top 10 cm and the next 10 cm in the sand and clay fractions (ANOVA: $p < .0847$, $F = 3.000$, $df = 4, 210$ and $p < .9379$, $F = .006$, $df = 4, 210$), although the site x layer (depth) interaction term was significant for both the sand and silt fractions ($p = .0005$ and $p < .0001$, respectively). This reflects the greater disparity between soil layers at the Skunk Hollow site compared to the other sites.

Soil pH was highest at Camp Pendleton (mean pH = 7.29) and lowest at Skunk Hollow (mean pH = 6.14) (Table 4). All pair-wise comparisons of sites were significant ($p = .0004$ to $p < .0001$), except the comparison of the two San Miguel Ranch sites ($p = 1660$), San Miguel Ranch-*conjugens* vs. Skunk Hollow ($p = .5627$), and San Miguel Ranch-*fasciculata* vs. Skunk Hollow ($p = .1204$).

4.2. SUMMARY AND DISCUSSION

The soils of the two San Miguel Ranch sampling sites, though immediately adjacent to each other and separated by no more than 50 m, were significantly different in texture (Tables 3-6). The soil associated with *H. conjugens* had a higher percentage of clay at the surface (25.1 % vs 16.0 %) and lower percentage of sand (40.6 % vs 51.7 %) than the nearby site supporting *H. fasciculata*. The surface soil pH at the two sites did not differ.

Sites associated with *H. paniculata* were more similar to each other than to the other four sites. They were higher in sand and lower in clay and silt content.

The association of *H. fasciculata* with soil texture is less clear. It occurs at one of the three clayeyest sites (Sycamore Canyon) but not at the adjacent, more clayey San Miguel Ranch site or at the Proctor Valley site. At Sycamore Canyon, it co-occurs with *Acanthomintha*

ANOVA Table for Arcsqrtsilt

	DF	Sum of Squares	Mean Square	F-Value	P-Value	Lambda	Power
Site	5	1.549	.310	314.371	<.0001	1571.857	1.000
Residual	124	.122	.001				

Means Table for Arcsqrtsilt
Effect: Site

	Count	Mean	Std. Dev.	Std. Err.
San Miguel-conjugens	30	.616	.037	.007
San Miguel-fasciculata	30	.604	.023	.004
Proctor Valley	30	.443	.029	.005
Pendleton	10	.260	.016	.005
Skunk Hollow	10	.373	.041	.013
Sycamore Canyon	20	.518	.037	.008

Fisher's PLSD for Arcsqrtsilt
Effect: Site
Significance Level: 5 %

	Mean Diff.	Crit. Diff	P-Value	
San Miguel-conjugens, San Miguel-fasciculata	.012	.016	.1271	
San Miguel-conjugens, Proctor Valley	.173	.016	<.0001	S
San Miguel-conjugens, Pendleton	.356	.023	<.0001	S
San Miguel-conjugens, Skunk Hollow	.243	.023	<.0001	S
San Miguel-conjugens, Sycamore Canyon	.098	.018	<.0001	S
San Miguel-fasciculata, Proctor Valley	.160	.016	<.0001	S
San Miguel-fasciculata, Pendleton	.344	.023	<.0001	S
San Miguel-fasciculata, Skunk Hollow	.231	.023	<.0001	S
San Miguel-fasciculata, Sycamore Canyon	.086	.018	<.0001	S
Proctor Valley, Pendleton	.183	.023	<.0001	S
Proctor Valley, Skunk Hollow	.070	.023	<.0001	S
Proctor Valley, Sycamore Canyon	-.075	.018	<.0001	S
Pendleton, Skunk Hollow	-.113	.028	<.0001	S
Pendleton, Sycamore Canyon	-.258	.024	<.0001	S
Skunk Hollow, Sycamore Canyon	-.145	.024	<.0001	S

Table 6. ANOVA table and results of PLSD test for proportion of silt (arcsine square root transformed data) in surface soil at six sites.

ilicifolia which is known to exist at several sites in the Proctor Valley area (CDFG EO 10 and S. McMillan, pers. comm.), but EO 10 is recorded as extirpated.

Sycamore Canyon and Proctor Valley are nearly identical in the percentage clay in the surface 10 cm (35.4 % and 32.3 %, respectively). They differ in the sand and silt fractions, but these differences are generally smaller than those between other sites. This leaves the interesting question: Why is *H. conjugens* absent from most *A. ilicifolia* sites in San Diego County? Possible explanations are differences in dispersal or chance dispersal events, local extinctions, other restricting soil factors, weather (temperature maxima and minima and precipitation) or relationships with native or exotic vegetation.

CHAPTER 5. ENZYME ELECTROPHORESIS

5.1. RESULTS

5.1.1. Isozyme variation

Twenty-one putative electrophoretic loci were interpreted. All loci could be reliably scored for all populations and patterns conformed to those generally reported for subunit structure and number of loci typically observed in plant species. Nine loci were polymorphic in at least one species (Table 7). No significant differences in allele frequency or allelic composition were found among the original samples collected and those germinated from seed collected at San Miguel, Palm Avenue or Proctor Valley (based on Monte Carlo sampling procedures) and data sets were combined in all subsequent analyses.

5.1.2. Genetic Diversity Within Populations

The percentage of polymorphic loci detected ranged from 33 to 43% with a mean of 39% (Table 8). All variable loci (9) were polymorphic for two or more alleles. The average number of alleles per locus ranged from 1.43 to 1.86 with a mean of 1.70. The average genetic diversity (H_e) based on all 21 loci was 0.175 for all populations surveyed. The observed heterozygosity values ranged from ranged from 0.136 (Skunk Hollow paniculata) to 0.197

		Population							
Locus	Allele	CPPANIC	SHPANIC	SMFASCIC	PAFASCIC	SCFASCIC	PACONJU	SMCONJU	PVCONJU
Lap	A	0.000	0.000	0.550	0.467	0.500	0.083	0.250	0.550
	B	***1.000***	***1.000***	0.000	0.000	0.000	0.000	0.000	0.000
	C	0.000	0.000	0.383	0.483	0.500	0.550	0.667	0.267
	D	0.000	0.000	0.067	0.050	0.000	0.317	0.017	0.000
	E	0.000	0.000	0.000	0.000	0.000	**0.050**	0.000	0.000
	F	0.000	0.000	0.000	0.000	0.000	0.000	***0.067***	***0.183***
Pgi 2	A	0.000	0.000	0.150	0.017	0.133	0.050	0.117	0.133
	B	0.883	0.867	0.583	0.900	0.600	0.500	0.200	0.250
	C	0.117	0.133	0.267	0.083	0.267	0.367	0.600	0.567
	D	0.000	0.000	0.000	0.000	0.000	***0.083***	***0.083***	***0.050***
Pgm 1	A	0.100	0.067	0.250	0.883	0.325	0.033	0.000	0.133
	B	0.317	0.333	0.067	0.067	0.050	0.000	0.000	0.000
	C	0.583	0.600	0.150	0.033	0.145	0.917	0.950	0.700
	D	0.000	0.000	0.533	0.017	0.525	0.050	0.050	0.167
Pgm2	A	0.033	0.000	0.000	0.017	0.000	0.000	0.067	0.097
	B	0.483	0.433	0.950	0.550	0.950	0.033	0.633	0.726
	C	0.317	0.367	0.050	0.367	0.050	0.950	0.267	0.145
	D	0.167	0.200	0.000	0.067	0.000	0.017	0.033	0.032
Sod	A	0.433	0.400	0.150	0.367	0.125	0.667	0.833	0.800
	B	0.567	0.600	0.850	0.633	0.875	0.333	0.167	0.200

Locus	Allele	CPPANIC	SHPANIC	SMFASCIC	PAFASCIC	SCFASCIC	PACONJU	SMCONJU	PVCONJU
Mdh	A	0.450	0.433	0.483	0.267	0.525	0.067	0.050	0.000
	B	0.550	0.567	0.450	0.683	0.475	0.267	0.367	0.650
	C	0.000	0.000	0.067	0.050	0.000	0.667	0.583	0.350
Gdh	A	0.717	0.800	0.650	0.645	0.640	0.683	0.600	0.550
	B	0.283	0.200	0.350	0.355	0.360	0.317	0.367	0.317
	C	0.000	0.000	0.000	0.000	0.000	0.000	***0.033***	***0.133***
Dia	A	1.000	1.000	0.983	1.000	1.000	0.700	0.767	0.900
	B	0.000	0.000	0.017	0.000	0.000	0.300	0.233	0.100
Skdh	A	0.100	0.120	0.667	0.600	0.650	0.350	0.417	0.367
	B	0.900	0.880	0.233	0.183	0.300	0.483	0.200	0.600
	C	0.000	0.000	0.100	0.217	0.050	0.167	0.383	0.033

Table 7. Allele frequencies at 9 polymorphic loci detected in *Hemizonia* populations. The locality abbreviations are given in Table 1. Those frequencies which are bold and underscored indicate private alleles—those detected in only one population. Those confined to one varietal type are bolded and italicized. There was no indication of deviation from Hardy-Weinberg expectations at any locus within any population.

Population Designation	H_o	H_e	A	$P_{(99\%)}$
CPPANIC	0.141(0.049)	0.144(0.050)	1.48(0.18)	33.33
SHPANIC	0.136(0.048)	0.141(0.049)	1.43(0.15)	33.33
SMFASCIC	0.173(0.054)	0.176(0.055)	1.71(0.21)	42.86
PAFASCIC	0.164(0.051)	0.167(0.051)	1.76(0.24)	38.10
SCFASCIC	0.162(0.051)	0.166(0.053)	1.52(0.16)	38.10
PACONJU	0.183(0.054)	0.186(0.055)	1.81(0.24)	42.86
SMCONJU	0.190(0.054)	0.193(0.055)	1.86(0.25)	42.86
PVCONJU	0.197(0.054)	0.200(0.055)	1.81(0.24)	42.86
Means	0.172(0.022)	0.175(0.16)	1.70(0.17)	39.29

Table 8. Genetic variability measures: observed heterozygosity (H_o); gene diversity (H_e); mean number of alleles per locus (A) and percent polymorphic loci (P) for *Hemizonia* populations. All estimates of variability measures are based on all 21 loci scored with standard errors in parentheses. Means for variability measures are weighted with standard deviations in parentheses. Population designations are detailed in Table 1.

(Proctor Valley *conjugens*). When the data across all populations within each group defined by Nei's (1978) unbiased genetic distance were pooled, the most diverse group was that containing the *conjugens* populations, and the least diverse was that containing *paniculata* populations (Figure 7). There were no significant deviations from Hardy-Weinberg expectations ($p > 0.05$) at any locus in any population sampled.

5.1.3. Genetic Diversity Between Populations

F_{ST} compares the ratio of the between population component of diversity to the total diversity. In this survey, F_{ST} for all populations was estimated as 0.304, which indicates that the between-population component accounts for ≈ 30% of the detected variation (Table 9). Gene diversity was apportioned hierarchically according to the methods of Chakraborty (1980) (Table 10). This analysis revealed that ≈ 76% of gene diversity was maintained within the populations sampled, with ≈ 7% and 17% being attributable to differences among populations within species and among the species sampled respectively.

5.1.4. Genetic distance and identity

A dendrogram based on Nei's (1978) unbiased genetic distances is shown in Figure 7. Based on individual pairwise comparisons, the mean genetic distance (D) was 0.082 with a range of 0.000 (CPPanic to SHPanic and SMFascic to SCFascic) to 0.133 (PAConju to SCFascic). The dendrogram defines three main clusters: with one group being composed of the *H. paniculata* populations sampled; another of the *H. fasciculata* populations and the last of *H. conjugens* populations. The mean genetic identity (I) among all pairwise comparisons was 0.922. However, pairwise comparison among the species sampled revealed that mean genetic identity between *paniculata-fasciculata* = 0.907 ± 0.002, *paniculata-conjugens* = 0.897 ± 0.001, *fasciculata-conjugens* = 0.907 ± 0.02. Among populations within species, mean genetic identity was 1.000, 0.978 ± 0.02 and 0.961 ± 0.02 for *paniculata*, *fasciculata* and *conjugens*, respectively. F statistics were also calculated for the population groupings defined by Nei's genetic distances (Table 9). There was no differentiation indicated among H. paniculata populations ($F_{ST} = 0$). This was an order of magnitude lower—though still highly significant—among *H. fasciculata* and *H. conjugens* populations ($F_{ST} = 0.067$ and 0.097, $p < 0.01$; respectively) than that indicated when all populations were considered together ($F_{ST} = 0.304$).

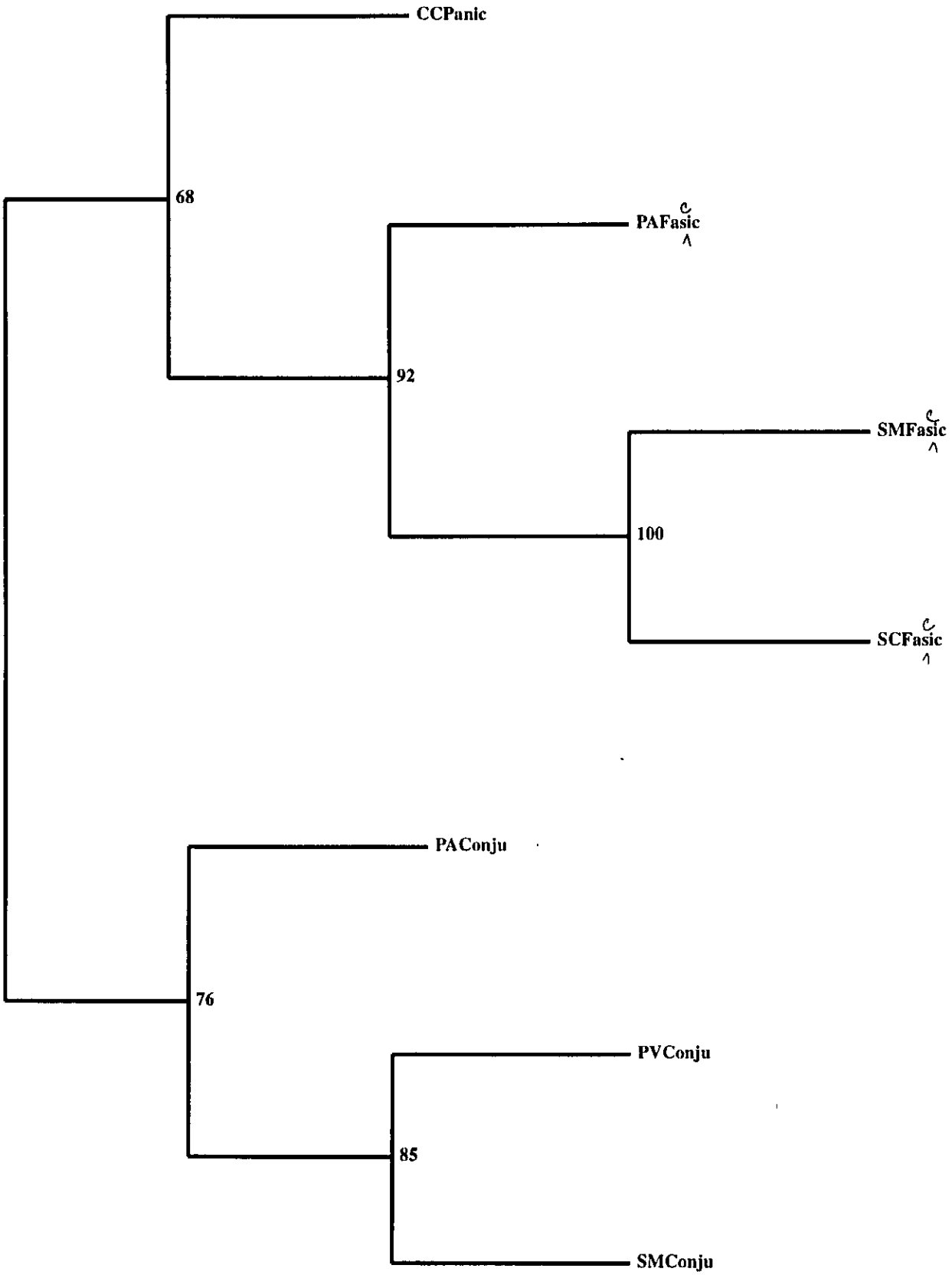

Figure 7. Majority rule consensus tree (see text) obtained from 100 bootstrap samples of the allozyme data for the *Hemizonia* species surveyed. Data from the two *H. paniculata* samples was combined (genetic identity = 1.0) and is designated CCPanic. Numbers indicate the number of times out of 100 a node occurred.

Locus	H_T	H_S	D_{ST}	F_{ST}	F_{IS}
Lap	0.711	0.424	0.287	0.404**	0.046
Pgi 2	0.577	0.470	0.106	0.184**	0.018
Pgm 1	0.614	0.353	0.261	0.425**	0.019
Pgm 2	0.560	0.399	0.161	0.287**	0.043
Sod	0.498	0.368	0.130	0.261**	0.026
Mdh	0.624	0.488	0.136	0.218**	0.020
Gdh	0.618	0.507	0.111	0.179**	0.047
Dia	0.483	0.481	0.002	0.004	0.114
Skdh	0.194	0.151	0.025	0.218**	0.175
Mean	0.542	0.405	0.135	0.304**	0.049
SE	0.049	0.037	0.031	0.012	0.005
95% CI	0.498-0.586	0.372-0.438	0.107-0.163	0.252-0.377	0.024-0.067
Species					
paniculata	0.428	0.423	0.005	0.012	0.071
fasciculata	0.418	0.390	0.028	0.067**	0.051
conjugens	0.500	0.452	0.049	0.097**	0.027

H_T, total gene diversity; **H_S**, averaged gene diversity; **D_{ST}**, gene diversity among populations; **F_{ST}**, proportion of interpopulation gene differentiation; **F_{IS}**, deviation from Hardy-Weinberg proportions within populations; **SE**, jacknifed estimate of standard error; **95% CI**, bootstrapped estimate of 95% confidence interval.

** = $p < 0.01$

Table 9. Measures of genetic diversity, substructuring and differentiation among *Hemizonia* populations.

Level	Percent diversity		
	Among Species	Among Populations/ Within Species	Within Populations
Pooled total for the 8 California populations	17	7	76
Individual Species			
H. paniculata		1.1	98.9
H. fasciculata		9.0	91.0
H. conjugens		8.2	91.8

Table 10. Apportionments of gene diversity (Chakraborty1980) among species, among populations within species, and within populations for the 8 populations of *Hemizonia* surveyed in this study.

5.2. SUMMARY AND DISCUSSION

This study provided evidence for substantial genetic differentiation in isozymes among the three species sampled. The genetic variability measures detailed in Table 8, as well as the values of H_T and H_S (Table 9), are consistent with those presented by Hamrick and Godt (1990) for 187 and 146 taxa of annual plants, respectively. They are significantly below the values reported by Warwick and Gottlieb (1985) for the mainland Madiinae complex of *Layia* ($p \approx 67\%$; $A \approx 3.4$ alleles per locus). The patterns detected in this study more closely conform to those reported for endemic insular forms of Madiinae (Witter 1990 and references therein). One of the surprising results of this study is that populations of *H. conjugens*, though apparently restricted to clayey type soils, maintain higher levels of allozymic polymorphism than the more widespread *H. fasciculata* and *H. paniculata*. However, our results must be interpreted with caution because of the relatively low number of polymorphic loci, the small number of populations surveyed, and the restricted sampling of the more widespread species.

Population genetics theory predicts that, as a consequence of genetic drift, inbreeding and restricted gene flow, small, edaphically restricted populations should show lower levels of genetic variation and higher genetic differentiation among populations than more widespread, larger populations (Barrett and Kohn 1991, Ellstrand and Elam 1993). However, historical factors such as the age of the species and past changes in its distribution may also affect levels of genetic variation both within and among populations. A species of recent origin may have a restricted distribution and may maintain low levels of polymorphism due to a more recent genetic bottleneck associated with speciation. Likewise, a more relictual species may have existed long enough to accumulate variation (in the form of mutations), however, genetic bottlenecks associated with anthropogenically mediated events may have reduced current levels of diversity. Genetic erosion in small populations has been demonstrated for several species of plants (Van Treuren et al. 1991, Raijmann et al. 1994). Our data for the *Hemizonia* species included in this study are not in agreement with these findings, as no major effect of species distribution on the extent and structure of genetic variation was detected. Similar results have been reported in other small disjunct populations of plant taxa (Prentice and White 1988, Dolan 1994). What is clear is that rare species such as *H. conjugens* are particularly susceptible to stochastic changes in allele frequency (reviewed in Barrett and Kohn 1991 and Ellstrand and Elam 1993) and to strong selection that may reduce levels of genetic diversity across populations of a species or to elimination of rare alleles due to increased inbreeding in small populations. We emphasize that without further sampling and study of additional populations and species within this complex these results are somewhat speculative.

Mean genetic identity between all *Hemizonia* populations (I = 0.922) was below the value reported by Gottlieb (1977) for conspecific populations of 22 species. Genetic identity values ranged widely among pairs of populations and significant differences in allele frequencies were found for most polymorphic loci surveyed. Considered separately, mean genetic identity between populations of the same species were 1.000, 0.978, and 0.961 for *H. paniculata*, *H. fasciculata*, and *H. conjugens*, respectively. These values are similar to those reported by Gottlieb (1977) and are, likewise, congruent with those reported by Witter (1990) for species of *Wilkesia* and *Dubautia* though they are substantially above the values presented by Witter for species of *Layia* (Witter 1990).

The relatively high level of genetic differentiation (F_{ST} = 30.4%) found among *Hemizonia* populations was due primarily to major allele frequency shifts at most polymorphic loci (Table 7). For example, both *paniculata* populations sampled were monomorphic for allele B at the Leucineaminopeptidase (Lap) locus, while *fasciculata* and *conjugens* were highly polymorphic, though they displayed significant differences in both allelic frequency and composition. Neither *fasciculata* nor *conjugens* contained the monomorphic allele found in *paniculata*. Similar frequency and composition differences are found at most loci listed in Table 7. Given the smaller—but still significant differentiation among populations of *fasciculata* and *conjugens*—these patterns suggest that both species are derived from a more highly polymorphic ancestor and that genetic drift plays a major role in the population dynamics of these species. Furthermore, samples examined from seed germinated from sites where *fasciculata* and *conjugens* are found less than 50 m apart, did not indicate that these species hybridize naturally.

CHAPTER 6. GENERAL DISCUSSION

The results of our limited population genetics study indicate that the three *Hemizonia* species can be distinguished from each other using allozyme electrophoresis. There is a high level of genetic differentiation among the *Hemizonia* populations, resulting primarily from allele frequency shifts at most polymorphic loci. The northern part of the San Miguel Ranch property (within the US Fish and Wildlife Service Refuge) has stands of *Hemizonia conjugens* and *H. fasciculata* adjacent to each other. At Palm Avenue, *fasciculata* and *conjugens* were growing together. In the field, these two species cannot be distinguished from each other except at the flowering stage. Hybridization between them was not indicated by the allozyme analysis. *H. paniculata* was not observed at any of the southern San Diego County sites.

Analysis of soils associated with five of the six *Hemizonia* populations studied indicates that *H. conjugens* was associated with soils having a high clay content, higher than soils supporting the two populations of *H. paniculata,* both of which occurred on sandy soil. Clay content ranged more widely were *H. fasciculata* was sampled, consistent with its wide geographic distribution.

Conclusions suggested by our data are:

- Substantial genetic diversity resides within individual populations of *Hemizonia conjugens* , making each remaining population of *H. conjugens* potentially valuable for this diversity.

- To verify unequivocally that the plants occurring at these sites are *H. conjugens* it would be advisable to sequence the ITS (internally transcribed spacer) and ETS (externally transcribed spacer) regions, which, according to Baldwin (pers. comm.), display distinct differences between all three species.

- Mechanisms of isolation between *fasciculata* and *conjugens* could be genetic, temporal or spatial, among many possibilities.

- Earlier observations of a close association of *H. conjugens* with clayey soils are substantiated. This would pose limitations for expansion of populations or altering the known distribution of the species.

LITERATURE CITED

Baldwin, B. 1999. New combinations and new genera in the North American tarweeds (Compositae-Madiinae). Novon 9:462-471.

Barrett, S.C.H. and J.R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pages 3-30 *in* D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, New York, NY.

Beauchamp, R. M. 1986. A flora of San Diego County, California. Sweetwater River Press, National City, CA.

Bohm, B.A. and C. Fong. 1990. Nonpolar flavonoids of *Wilkesia* and *Argyroxiphium*. Phytochemistry 29:1175-1177.

Bowman, R. H. 1973. Soil survey of the San Diego area, California. Soil Conservation Service, U.S. Department of Agriculture, Washington, D. C.

Brower, J.E. and J. H. Zar. 1977. Field and laboratory methods for general ecology. W.C. Brown Co., Dubuque, IO.

California Department of Fish and Game. 2000. State and federally listed endangered, threatened, and rare plants of California. California Natural Diversity Database, Sacramento, CA.

Chakraborty R. 1980. Gene diversity analysis in nested subdivided populations. Genetics 96:721-726.

City of San Diego. 1998. Final multiple species conservation program: MSCP plan. San Diego, CA.

Clausen, J. 1951. Stages in the evolution of plant species. Cornell University Press, Ithaca, New York.

Clausen, J., D.D. Keck, and W.M. Heust. 1945. Experimental studies on the nature of species II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Publ. Carnegie Inst. Wash. 564:1-174.

Constantine C.C., R.P. Hobbs and A.J. Lymbery. 1994. Fortran programs for analyzing population structure from multilocus genetypic data. Journal of Heredity 85:336-337.

Dolan, R.W. 1994. Patterns of isozyme variation in relation to population size, isolation, and phytogeographic history in royal catchfly (*Silene regia*; Caryophyllaceae). American Journal of Botany 8:965-972.

Ellstrand, N.C. and D.R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Ann. Rev. Ecol. Syst. 24:217-242.

Felsenstein J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c distributed by the author. Department of genetics, University of Washington, Seattle.

Federal Register. 1998. 50 CFR Part 17, pages 54938-54956.

Gee, G.W. and J.W. Bauder. 1986. Pages 383-410 *in* Klute, A., editor. Methods of soil analysis. American Society of Agronomy: Soil Science Society of America, Madison, WI.

Gottlieb L.D. 1977. Electrophoretic evidence and plant systematics. Annuals of the Missouri Botanical Garden 64:161-180.

Gottlieb, L.D., S.I. Warwick. and V.S. Ford. 1985. Morphological and electrophoretic divergence between *Layia discoidea* and *L. glandulosa*. Systematic Botany 10:484-495.

Hamrick, J.L. and M.J.W. Godt. 1990. Allozyme diversity in plant species. Pages 43-63 *in* A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir, editors. Plant population genetics, breeding, and genetic resources. Sinauer, Sunderland, MA.

Keck, D.D. 1958. Taxonomic notes on the California flora. Aliso 4: 101-114.

Keil, D. J. 1993. Asteraceae[Compositae]. *Hemizonia*. Pages 280-284 *in* Hickman, J.R., editor. The Jepson manual: Higher plants of California. University of California Press, Berkeley, CA.

Kyhos, D.W., G.D. Carr, and B.G. Baldwin. 1990. Biodiversity and cytogenetics of the tarweeds (Asteraceae: Heliantheae-Madiinae). Ann. Missouri Bot. Gard. 77:84-95.

Levene H. 1949. On a matching problem arising in genetics. Annals of Mathematical Statistics 20:91-94.

May B. 1994. Starch gel electrophoresis of enzymes. Pages 1-27 *in* Hoelzell, A.R., editor. Molecular genetic analysis of populations: A practical approach. IRL Press, New York.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

P and D Environmental. 1999. Draft subsequent environmental impact report EIR 97-02 (Third Tier EIR), San Miguel Ranch sectional planning area plan and tentative maps, City of Chula Vista, California. Chula Vista, CA.

Prentice, H.C. and R.J. White. 1988. Variability, population size and isolation: the structuring of diversity in Oland *gypsophila fastigiata*. Acta Oecologia/Oecologia Plantarum 9: 19-29.

Raijmann, L.E.L., N.C. Van Leeuwen, R. Kersten, J.G. Oostermeijer, H.C.M. Den Nijs, and S.B.J. Menken. 1994. Genetic variation and outcrossing rate in relation to population size in *Gentiana pneumonanathe* L. Conservation Biology 8:1014-1026.

Swofford D L and Selander RB. 1981. BIOSYS-1. A computer program for the analysis of allelic variation in genetics. Release 1. Urbana: University of Illinois.

Tanowitz, B. 1978. *Hemizonia conjugens.* Distribution, chromosome number, and relationships. Madrono 25:159.

_____. 1982. Taxonomy of *Hemizonia* sect. Madiomeris (Asteraceae: Madiinae). Systematic Botany 7:314-339.

Van Treuren, R., R. Bijlsma, W. van Delden and N.J. Ouborg. 1991. The significance of genetic erosion in the process of extinction. I. Genetic differentiation in *Salvia pratensis* and *Scabiosa columbaria* in relation to population size. Heredity 66:181-189.

Warwick, S.I. and L.D. Gottlieb. 1985. Genetic divergence and geographic speciation in *Layia* (Compositae). Evolution 39:1236-1241.

Weir B.S. 1990. Genetic data analysis. Sinauer Associates, Inc., Sunderland, MA.

Weir B.S. and Cockerham C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Witter, M.S. 1990. Evolution in the Madiinae: evidence from enzyme electrophoresis. Ann. Missouri Bot. Gard. 77:110-117.

Workman PL and Niswander JS. 1970. Population studies on southwestern Indian tribes II. Local gene differentiation in the Papago. American Journal of Human Genetics 41:225-233.

Wright, S. 1978. Evolution and the Genetics of Populations. Vol. 4: Variability within and among Natural Populations. University of Chicago Press, Chicago.

LITERATURE CONSULTED

Beardmore, J.A. 1983. Extinction, survival, and genetic variation. Pages 125-151 *in* C.M. Schonewald-Cox, S.M. Chambers, B. MacBryde, and W.L. Thomas, editors. Genetics and conservation: a reference for managing wild animals and plants. Benjamin/Cummings, Menlo Park.

Chapman, H. and P. Pratt. 1961. Methods of analysis for soils, plants and waters. Division of Agricultural Sciences, University of California Davis, Davis, CA.

Falk, D.A. and K.E. Holsinger, editors. 1991. Genetics and conservation of rare plants. Oxford University Press, Oxford.

Godt, M.J.W., J. Walker, and J.L. Hamrick. 1997. Genetic diversity in the monotypic endangered lily *Harperocallis flava*, and a close relative, *Tofieldia racemosa*. Conservation Biology 11:361-366.

Hamrick, J.L., J.B. Mitton, and Y.B. Linhart. 1979. Levels of genetic variation in trees: Influence of life history characteristics. Pages 35-41 *in* M.T. Conkle, editor. Isozymes of North American forest trees and forest insects. Pacific Southwest Forest and Range Exp. Stn., Berkeley, CA.

Hamrick, J.L., M.J.W. Godt, D.A. Murawski, and M.D. Loveless. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. Pages 75-86 *in* D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford.

Karron, J.D. 1987. A comparison of levels of genetic polymorphism and selfcompatibility in geographically restricted and widespread plant congeners. Evol. Ecol. 1:47-58.

_____. 1991. Patterns of genetic variation and breeding systems in rare plant species. Pages 87-98 *in* D.A. Falk and K.E. Holsinger, editors. Genetics and conservation of rare plants. Oxford University Press, Oxford.

_____, Y.B. Linhart, C.A. Chaulk, and C.A. Robertson. 1988. Genetic structure of populations of geographically restricted and wide-spread species of *Astragalus* (Fabaceae). American Journal of Botany 75:1114-1119.

Lesica, P., R.F. Leary, F.W. Allendorf, and D.E. Bilderback. 1988. Lack of genetic diversity within and among populations of an endangered plant, *Howellia aquatilis*. Conserv. Biol. 2:275-282.

Levy, M. and D.A. Levin. 1975. Genic heterozygosity and variation in permanent translocation heterozygotes of the *Oenothera biennis* complex. Genetics 79:493-512.

Lewis, P.O. and D.J. Crawford. 1995. Pleistocene refugium endemics exhibit greater allozymic diversity than widespread congeners in the genus *Polygonella* (Polygonaceae). Am. J. Bot. 82:141-149.

Linhart, Y.B. and C. Premoli. 1993. Genetic variation in *Aletes acaulis* and its relative, the narrow endemic *A. humulis* (Apiaceae). Am. J. Bot. 80:598-605.

Loveless, M.D. and J.L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15:446-461.

Purdy, B.G. and R.J. Bayer. 1996. Genetic variation in populations of the endemic *Achillea millefolium* ssp. *megacephala* from the Athabasca sand dunes and the widespread ssp. *lanulosa* in western North America. Can. J. Bot. 74:1138-1146.

Schwartz, O.A. 1985. Lack of protein polymorphism in the endemic relict *Chrysosplenium iowense* (Saxifragaceae). Can. J. Bot. 63:2031-2034.

Soltis, P.S., D.E. Soltis, T.L. Tucker, and F.A. Lang. 1992. Allozyme variation is absent in the narrow endemic *Bensoniella oregona*. Conserv. Biol. 6:131-134.

Waller, D.M., D.M. O'Malley, and S.C. Gawler. 1987. Genetic variation in the extreme endemic *Pedicularis furbishiae* (Scrophulariaceae). Conserv. Biol. 1:335-340.