

Development of demographic and genetic conservation strategies for maintenance of an endangered annual plant.

Running title: Conservation strategies for an endangered plant

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Abstract:

Cordylanthus palmatus is an annual plant that inhabits seasonally flooded wetlands with saline and alkaline soils in California, USA. In 1986, the plant was listed as endangered due to the small size or threats to its five remaining populations. The goal of our research was to inform conservation strategies by examining genetic diversity and structure and their potential response to demographic and environmental stochasticity. We used nuclear DNA markers to measure genetic characteristics. To explore potential influences on genetic variation and structure we assessed fluctuations in population size and interspecific hybridization between *C. palmatus* and *C. mollis* ssp. *hispidus*.

We found geographically separate populations of *C. palmatus* were genetically distinct, with a F_{ST} of 0.23, indicating substantial genetic structure among populations. Within populations, there was no evidence of isolation- by- distance. However, two adjacent vernal pools contained genetically distinct sub-populations. Overall, the pattern of genetic variation within populations supported the hypothesis that the historical frequency and extent of seed dispersal by overland flooding has influenced population genetic structure strongly. Despite founder effects and population bottlenecks, small populations maintained levels of genetic variation comparable to large populations. We found no evidence of hybridization between the congeners.

All extant populations of *C. palmatus* are genetically variable and distinct. We recommend that hydrologic connectivity be considered in collection and sowing of seeds intended to increase the abundance of *in situ* populations or to create experimental *ex situ* populations.

INTRODUCTION

Declines in occurrence and abundance of most species are due to habitat loss and degradation (Foin et al. 1998). Often these changes are accompanied by genetic changes, but focusing on genetics as a means to maintain or restore plant populations is relatively uncommon (Hufford and Mazer, 2003). However, when habitat is highly limited and cannot be restored or reconstructed, genetic considerations, including the scale at which genetic variation occurs, may be important for in situ conservation efforts such as increasing population sizes and seed banking, particularly for annual plants (Ramp et al., 2006). Many plants, especially rare taxa, exhibit microhabitat preferences (Maliakal-Witt et al, 2005). When these microhabitats occur in the landscape in discrete and small-scale patches, they create opportunities for genetic divergence at a small spatial scale. If genetic data are assessed relative to potential influences on genetic structure (the differential distribution of genetic variation among populations), results can be used to inform efforts to maintain or simulate ecological phenomena that have played a role in the microevolutionary development of the species.

Genetic structure in plants develops over ecological and evolutionary time. When gene flow is reduced, populations can diverge due to the random processes of mutation and drift, or through directional processes such as natural selection. Conversely, development of divergent genetic structure can be forestalled when gene flow is frequent and widespread. By combining molecular determinations of genetic structure with data on population size over time, breeding system, and field observations of potential mechanisms of gene flow, we can develop an understanding of biotic and abiotic drivers of genetic structure.

Cordylanthus palmatus is an annual plant that inhabits seasonally flooded wetlands with saline and alkaline soils in California's Central and Livermore valleys. The plant survives the summer dry season through hemiparasitism (Chuang and Heckard 1971), its ability to excrete salt crystals through its leaves, and deep roots (USFWS 1998). The species was listed as endangered under state and federal endangered species acts in 1984 and 1986, respectively, due to the small population size or threats to the five remaining populations. Geographic distance among populations exceeds the probable dispersal ability of the species, and virtually no other habitat remains. Accordingly, understanding of genetic structure may be a substantial asset to *in situ* and *ex situ* conservation actions for the species.

Preliminary experiments in which enclosed flowers on individual plants were hand pollinated with pollen from the same plant suggested that *C. palmatus* is self-compatible (CCB 1993). *C. palmatus* is believed to be insect pollinated because the flowers are closed at anthesis, and therefore wind pollination is unlikely (Iwanami et al. 1988). Early experiments demonstrated that there was no fruit set or seed set in the absence of pollinators (CCB 1993). A few studies found that *Bombus californicus* (CCB 1994) and *B. vosnesenskii* (Lee and Associates 2002) pollinate *C. palmatus*. However, *C. palmatus*, like other plants with relatively long flowering periods, may have a succession of pollinators. In addition to *Bombus*, these pollinators appear to include solitary bees, such as *Halictus* spp. and *Lasioglossum* sp. (CCB 1992, Lee and Associates 2002). Scanning electron micrographs of the legs of Halictid bees collected at flowers of *C. palmatus* confirmed that the bees can carry *C. palmatus* pollen (CCB 1992). Nonetheless, the low fruiting rate of plants from which *Bombus*, but not *Halictus*, experimentally were excluded suggested that these smaller bee species did not play a significant role in pollination

(CCB 1994).

In general, bees begin to collect pollen at the lowest flower of a columnar inflorescence, such as the spike in *C. palmatus*, and move upward (Proctor and Yeo, 1972). Bees then fly to inflorescences on neighboring plants, with occasional longer-distance flights. Previous observations suggested that *B. californicus* transports considerable amounts of *C. palmatus* pollen within and among plants, potentially facilitating both inbreeding and outcrossing (CCB 1993, 1994). Further work suggested that that *Bombus* are faithful to particular patches of *C. palmatus* during the period in which they are foraging on that plant species (CCB 1994). A potential consequence of this behavior is limited transfer of pollen among patches of *C. palmatus*. If seed dispersal is limited, the net result of self-compatibility and limited pollen movement is development of genetic structure on a scale commensurate with pollination distance.

Small size, crested coats, and hair-like wax extrusions (Chung and Heckard, 1972) suggest that *C. palmatus* seeds are dispersed by water (Howe and Smallwood, 1982). A preliminary laboratory study showed that fresh seed can float well for six days (Ayres, unpublished). However, earlier field and greenhouse studies suggested that gravity also was a key dispersal agent for *C. palmatus* seed (CCB 1993, 1994). Because *C. palmatus* occurs in areas with anthropogenic impediments to the flow of surface water, we aimed to determine whether genetic structure within populations could be attributed to hydrologic isolation. Long distance dispersal of buoyant seeds by surface-water flow in wetlands might prevent development of genetic structure within populations, while restricted surface-water movement or gravity dispersed seeds might promote fine-scale genetic structure. The wetland patches of *C. palmatus* habitat vary from alkali or saline vernal pools of several hundred square meters to dozens of interconnected and isolated vernal pools and swales within an alkali sink.

A previous genetic study of *C. palmatus*, based on six polymorphic enzyme loci, found that approximately 98% of genetic variation was found within populations whereas just 2% of genetic variation was found among populations. One of the five populations contained a disproportionate amount of the within-population genetic variability (Fleishman et al 2001). Fine-scale genetic structure of less than four meters was detected in this latter population. Because dearth of isozyme polymorphism may have influenced those conclusions, we aimed to investigate genetic structure using many nuclear DNA loci. Another *Cordylanthus* species, *C. mollis* sp. *hispidus* occurs sympatrically with the most genetically variable population of *C. palmatus*. Therefore, we wished to determine whether hybridization between the two species might explain increased enzyme allelic diversity in this population.

The ultimate goal of our research was to inform conservation strategies for *C. palmatus* by increasing our understanding of microevolutionary processes. To achieve this understanding, we coupled data on population size over time and potential gene flow (movement of seed and pollen) with assessments of population-level genetic diversity and structure obtained from nuclear DNA markers.

METHODS

Study system

We examined genetic variation and estimated abundance of all five known populations of

C. palmatus. Climate throughout the species' range is similar. Virtually all rainfall occurs during the winter, and summers are hot and dry. *C. palmatus* grows on seasonally-flooded, saline-alkali soils in lowland plains and basins. It grows primarily along the edges of channels and drainages, with a few individuals scattered in seasonally-wet depressions, alkali scalds, and grassy areas. *C. palmatus* co-occurs with other species tolerant of high salt concentrations, such as iodine bush (*Allenrolfea occidentalis*), alkali heath (*Frankenia salina*), glasswort (*Salicornia subterminalis*), seepweed (*Suaeda moquinii*), and salt grass (*Distichlis spicata*).

The Mendota population (Fresno County) is the southernmost population of *C. palmatus*. The population was established in 1972 by Lawrence Heckard, who planted 10 individuals derived from seed from a nearby roadside population that since has been extirpated (correspondence from L. Heckard to R. Huddleston). In 1978, the population was incorporated into the newly-established Alkali Sink Ecological Reserve, which currently covers 382 ha.

The Livermore population is located in the southern part of a ca. 7,000 ha hydrologic basin, the Springtown Alkali Sink (Alameda County). About 300 ha of the sink, of which 109 ha are owned by the city of Livermore as a preserve, contain a mosaic of alkali sink shrubs and grasses, annual grasslands dominated by non-native grasses, natural and channelized stream drainages, and permanent and seasonal streams and vernal pools. *C. palmatus* has been known from the sink since 1982. The upper portions of the basin contain agricultural lands and annual grasslands.

The Woodland population occurs on the newly established Alkali Grasslands Preserve in the city of Woodland (Yolo County). The 73 ha preserve is owned by the city of Woodland and a private citizen. The preserve is dominated by annual grassland, with areas of alkali grassland, seasonal pools and swales, a vernal lake, and man-made irrigation channels and levees. The most abundant plant taxa in the annual grassland are non-native species, especially *Lolium multiflorum*. The hydrology of the preserve has been highly altered by agricultural activities. In at least two different years, observations detected no mature plants or seedlings of *C. palmatus* (M.A. Showers, personal observations); and the population likely reestablished from seeds in the soil seed bank. Within the preserve, *C. palmatus* occurs in two discrete locations separated by ca. 1.6 km but connected by seasonal drainages.

The Colusa population is located within the 1,872 ha Colusa National Wildlife Refuge (NWR) (Colusa County). Colusa NWR lies within the Colusa Basin and was historically covered by alkali vernal pools and associated alkali meadows. Prior to its designation as a refuge, the land was used for livestock grazing and winter wheat and rice agriculture. Parts of the refuge subsequently have reformed into functional vernal pools and alkali meadows. We sampled *C. palmatus* from two adjacent vernal pools.

The Delevan National Wildlife Refuge (Colusa County) includes more than 1,820 ha of intensively managed wetlands and 486 ha of uplands. *C. palmatus* occurs primarily in alkali meadows and large vernal pools, with plants scattered along the roadsides.

Demographic assessments

In 1993, long-term monitoring techniques were developed to allow rapid assessment of the distribution and abundance of *C. palmatus* over time (CCB 1993). Initially validated on the Livermore population, these methods were sufficient to identify substantial changes in abundance relatively cheaply and quickly. In brief, transects that collectively represented local

gradients in land cover and habitat quality were established in four subareas of the site. Each transect was subdivided further into segments representing different vegetation types that are known to support *C. palmatus* or that appeared to be potential habitat for the species. Each transect segment was walked for a predetermined period of time. The number of *C. palmatus* encountered was estimated on a semi-logarithmic scale (none, tens to hundreds, thousands to tens of thousands, and so forth). The number of individuals in each subarea was estimated by summing the totals for each segment.

In 1992 and 1993, populations of *C. palmatus* were surveyed at the Delevan and Colusa National Wildlife Refuges and the Mendota Alkali Sink Ecological Reserve (CCB 1993, 1994). The Woodland population was surveyed in 1992 (CCB 1993). All populations were again visited during 2004. During each visit, biologists familiar with the species estimated population sizes within an order of magnitude using the rapid-assessment method described above. In addition, annual population censuses have been conducted by managers of the Delevan and Colusa NWRs since 1997 (Silviera, 2005) and by a local biologist at Woodland.

Genetic methods and analyses

Plant and Primer Selection, DNA Extraction, and PCR

Thirty plants were sampled from each of the five extant populations of *C. palmatus*. We collected two to three robust green branches per plant. The coordinates of each sampled plant were recorded using a Trimble Geoexplorer 3 GPS meter (Trimble, Sunnyvale, California). At the Springtown Alkali Sink, six samples were taken from a population of *C. mollis hispidus* that co-occurred with *C. palmatus* to develop species-specific DNA fragments that could be used to test for interspecific hybridization.

DNA was extracted using the proteinase K method detailed in Daehler et al (1999). Preliminary screening of primers identified 29 Inter-Simple-Sequence-Repeat (ISSR) primers (of 100 examined; University of British Columbia kit 800 ISSR primers) that amplified *C. palmatus* or *C. m. hispidus* DNA fragments. We did not include samples from the Livermore population of *C. palmatus* in the preliminary screening. Instead, we wished to determine species-specific bands for *C. palmatus* from allopatric populations to investigate potential interspecific hybridization. MgCl₂ and annealing temperature were simultaneously optimized on a Eppendorf Mastercycler Gradient thermalcycler; 17 and 12 primers had annealing temperatures of 50° and 54°, respectively (primers and temperatures available from the senior author on request).

We used a final set of 134 bands for our genetic analyses: 85 ISSR bands were polymorphic in *C. palmatus* (69 of which were absent in *C. mollis hispidus*), and 34 ISSR bands were specific to and almost all ubiquitous in *C. mollis hispidus*.

PCR conditions were 94° for 90 sec followed by 40 cycles of 94° for 15 sec, the optimized annealing temperature for 30 sec, and 72° for 2 min. Reaction volumes of 15 mL contained 10% by volume MgCl₂-free 10X reaction buffer A (Promega, Madison, Wisconsin), 0.6 units Taq polymerase (Promega, Madison, Wisconsin), 360 picounits primer (University of British Columbia), 3 mmol/L MgCl₂, 200 mmol/L each dATP, dCTP, dGTP, and dTTP (Promega, Madison, Wisconsin), and 30 ng genomic DNA. Most reactions were repeated twice to confirm consistency. Following electrophoresis on 1.5% agarose gels, DNA was stained with ethidium bromide and visualized under ultraviolet light. Gels were hand-scored for polymorphic DNA fragments.

Data Analyses

Genetic and Geographical Distance. Genetic distance between individuals was calculated using the Euclidean distance metric implemented with NTSTSpC (version 2.10 Exeter Software, Setauket, NY). Hierarchical patterns of genetic distance were visualized using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method applied to Sequential, Agglomerative, Hierarchical, and Nested (SAHN) cluster analysis. For *C. palmatus* only, we calculated geographic distance between individuals. The correlation between genetic and geographic distance among and within local populations was calculated and tested using a Mantel test with 1000 permutations (Mantel, 1967). We conducted Mantel tests using the “Matrix Comparison Plot” program of NTSYS.

Analysis of Molecular Variance (AMOVA). A matrix of squared Euclidean genetic distances was calculated between all individuals using the program Arlequin 3.0 (Excoffier et al. 1992; <http://lgb.unige.ch/arlequin/>). A total of 79 loci (out of 85 loci) were used in the analysis after removing loci with replicate patterns or with >5% missing values. The matrix was then partitioned into sub-matrices corresponding to subdivisions identified by the UPGMA cluster analysis. The sums of squares in the matrix and submatrices yield sums of squares for the various hypothetical divisions in the population. The sums of squares were placed into an analysis of variance framework allowing for hypothesis tests of between-group and within-group differences at two hierarchical levels. We ran two analyses. The first considered broad scale regional population structure. The second included subpopulation definition within the Colusa population in which each subpopulation was composed of plants found in one of two vernal pools that were separated by ca. 100 m. A matrix of pair wise differences among populations, calculated by Arlequin 3.0, was subjected to UPGMA and SAHN cluster analysis using NTSYS to generate a population-level dendrogram of genetic relatedness.

Genetic variation. We counted the number of loci, out of the 85 ISSR polymorphic loci specific to *C. palmatus*, that were variable within each population.

RESULTS

Demographic assessments

The Mendota Alkali Sink Ecological Reserve population increased from the 10 founding plants in 1972 to 30 individuals by 1982 (L. Heckart, personal observation) and to 800 plants by 1987, but no seedlings or adult plants were observed in the early 1990s (Cypher, 1998). Although the population has rebounded during the past decade, presumably from a seed bank in the soil, the population size remains within the hundreds. Similarly, the Woodland population has not exceeded several hundred individual plants (Fig. 1).

The abundances of the Colusa, Delevan, and Livermore populations have consistently ranged from several hundred to many thousands of individuals. Abundance of the Colusa population has been highly variable since the early 1990s with 3-fold to 7-fold annual fluctuations, whereas annual fluctuations in the abundance of the Delevan and Livermore populations have been comparatively modest (Fig. 1).

Genetics

We found no evidence of hybridization between *C. palmatus* and *C. mollis hispidus*. None of the 34 bands specific to *C. mollis hispidus* were found in the Livermore population of *C. palmatus*, nor in any other population of *C. palmatus*; none of the 69 bands specific to *C. palmatus* were found in *C. mollis hispidus*.

Multivariate cluster analysis of genetic similarity between individual *C. palmatus* suggested that the species has hierarchical genetic-geographic patterns (Fig. 2). The southernmost population, Mendota, was distinct from the four populations to the north. Individuals from the Delevan and Livermore populations each formed cohesive and distinct aggregations, whereas the Colusa and Woodland populations were less distinct from each other. Within populations, we found no correlation between genetic and geographic distance among individuals.

We found no genetic structure between the Woodland subpopulations. However, individuals in each vernal pool at Colusa grouped together (Fig. 3). In addition, six samples taken from a small roadside aggregation of plants (< 20 individuals) at the Delevan National Wildlife Refuge, separated by only 14 linear m from individuals growing in a vernal pool, were genetically distinct from other *C. palmatus* at Delevan (Fig. 2, small cluster labeled “Delevan” below the Livermore aggregation).

On the basis of the hierarchical genetic patterns revealed by multivariate cluster analysis, we performed two analyses of molecular variance (AMOVA). The first AMOVA did not consider subpopulation structure (Table 1a), whereas the second incorporated structure at the level of vernal pools at Colusa (Table 1b). In both analyses, 76-78% of genetic variation was found within populations and the remaining genetic variation was held among populations or subpopulations, resulting in similar F_{ST} s ($p < 0.001$) of ca. 0.22. In the second model, almost 10% of among-population variation was attributed to subpopulation structure at Colusa.

Cluster analysis of pairwise differences between populations showed regional division between northern and southern populations (Fig. 4). While the dendrogram based on individual genetic similarity suggested that Livermore was more closely related to northern populations, the population analysis placed it with, although genetically distant from, the southernmost Mendota population. The pattern of the northern population group was consistent with that of the individual-based dendrogram.

Populations of *C. palmatus* contained, on average, 59 (69%) of the species' 85 polymorphic loci (Table 2). The amount of genetic variation in the Mendota and Livermore populations were the same (56 loci) despite current differences in abundance between those populations. Similarly, despite recent population bottlenecks, the small Woodland population contained the greatest number of polymorphic loci (63), comparable to the large population at Delevan (61 loci).

DISCUSSION

In addition to maintaining populations of viable size, conservation efforts generally aim to sustain evolutionary potential by maintaining the genetic structure and variation that arose over time in response to gene flow, adaptation, and demographic stochasticity (Hufford and

Mazer, 2004). Conservation of evolutionary potential requires an assessment of genetic structure coupled with an evaluation of potential structuring mechanisms. We employed neutral genetic markers to assess gene flow and genetic variation in *Cordylanthus palmatus* and evaluated the various mechanisms by which gene flow might occur. However, we did not assess structure that may reflect environmental adaptation.

In plants, gene flow occurs either by movement of haploid gametes in pollen grains or by seed dispersal. Early plant population biologists based their understanding of population boundaries on vectors of pollen and mechanisms of seed dispersal. Since the mid 1990s, molecular DNA markers have made empirical delineation of population boundaries more straightforward (e.g. Ayres and Ryan 1997). We found that geographically defined populations of *C. palmatus* corresponded well to genetically defined populations, suggesting that populations have been isolated for many generations. There was no apparent genetic structure within the Mendota, Livermore, and Woodland populations. In contrast, two adjacent vernal pools at Colusa contained distinct genetic subpopulations. Although we did not detect structure within the Delevan population, our sample sizes from several vernal pools were insufficient to conclude definitively that no genetic differences exist among pools.

Genetic structure at the scale of individual vernal pools has been considered theoretically (e.g. Elam, 1998) but, to our knowledge, not verified. Early genetic analyses of vernal pool species examined species-wide genetic structure rather than populations within single vernal pools, and were based on isozyme variation (Crawford and Ornduff, 1989; Dole and Sun, 1992). A challenge in basing genetic inferences on isozymes, especially for rare species, is that the enzymes do not exhibit sufficient variation for robust analysis of population structure. For example, six of nine populations of *Limnanthes floccosa* ssp. *californica* were invariant at 28 isozyme loci, and variable populations were polymorphic at only a single locus (Dole and Sun, 1992).

Previous genetic analyses of *C. palmatus* based on isozyme alleles (Fleishman et al. 2001) revealed less than 10% of the genetic structure ($F_{ST} = 0.019$) we detected using nuclear DNA markers. In addition, previous work suggested that the large populations at Delevan and Colusa were virtually invariant, whereas we found that almost 60% of ISSR loci within these populations were variable. However, our estimate of total variation was affected by our use of polymorphic DNA markers. Both studies found that genetic variability was not correlated with population size. We believe that the isozyme analysis did not contain enough alleles to discriminate adequately among genotypes and therefore underestimated genetic divergence. It appears that relatively small populations of *C. palmatus*, even those that have experienced severe bottlenecks, can contain relatively high levels of genetic variation. Maintenance of genetically variable populations in the face of demographic stochasticity is likely attributable to a genetically variable soil seed bank.

The assumption that very small populations of plants lack genetic variation is cited as a reason to avoid taking measures to preserve these populations or salvage them when human activities present unavoidable impacts (Hamrick and Godt, 1989; but see Gitzendanner and Soltis, 2000). “Emergency salvage” is generally not recommended as a tool for plant preservation (Allen, 1994; Howald, 1996), but when it is used, genetic factors are considered. While we concur that this tool should be used only when other techniques such as habitat

protection fail, we also suggest that the use of emergency salvage as a tool for preservation of rare plants, even on a small scale, should not be ruled out on the basis of genetics alone

Potential Mechanisms of Genetic Structure

Self-compatible plants with local pollinators can form local neighborhoods of related plants if seed dispersal is also limited. This may explain why isozyme studies detected genetic structure across distances less than 4 m (Fleishman et al. 2001). Genetic analyses based on nuclear DNA taken from samples more than 10 m apart did not detect local genetic structure. Further, we found no correlation between genetic distance and geographic distance.

Our results suggest that hydrologic connectivity has considerable influence on genetic structure within populations and that dispersal of buoyant seeds has facilitated long-term gene flow in *C. palmatus*. For example, lack of genetic structure within the Livermore and Woodland populations may reflect historical or recent overland flow. By contrast, genetic differences between individuals in adjacent vernal pools at Colusa suggest that overland flow between the pools is infrequent.

Application of Results to Conservation

Our work suggests that conservation efforts for *C. palmatus* are most likely to be effective when historical patterns of hydrology are considered. Our results indicate that hydrologic barriers in populations with genetic substructure should be maintained to the greatest extent possible, whereas overland flow should be maintained where populations do not appear to have local genetic substructure.

If historical patterns of overland water flow cannot be maintained, sowing of seed collected from throughout a given population may mimic historical exchange of genes within that population. However, this strategy bears a risk of disrupting locally coadapted gene complexes or generating outbreeding depression (Hufford and Mazer, 2003). There is considerable evidence that local adaptation strongly influences genetic structure in plants (Linhart and Grant, 1996). In most cases, there appears to be no correlation between patterns of molecular genetic markers and morphological traits (McKay and Latta, 2002). Accordingly, genetic patterns driven by gene flow and revealed by selectively neutral molecular markers may not correspond with morphological patterns driven by long-term environmental adaptation.

Ideally, we would directly map across environmental gradients the distributions of genetic and morphological traits potentially associated with adaptive responses to environmental variation. In reality, it is extremely difficult to identify the environmental drivers of adaptation, map those drivers across the landscape, identify the plant traits most responsive to variation in drivers, evaluate the genetic basis of those traits, and map plant traits across the landscape. Although this approach was accomplished by Linhart (1988) for *Veronica peregrine*, a cleistogamous species that occupies vernal pools distributed across marked moisture gradients, documented cases are rare in the literature.

Because all populations of *C. palmatus* are genetically variable and distinct, we recommend that seed collection and sowing be conducted within rather than among populations. Our work suggests that within populations, sowing and collection of seeds should consider historical hydrology; populations that may be hydrologically isolated, such as those within vernal pools, should be maintained intact. Where human activities have impeded overland flows, site-

wide sowing of seed collected throughout the population may simulate historical patterns of seed movement.

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Fig. 1. Changes in abundance of five extant populations of *Cordylanthus palamtus*. The Mendota population was established in 1972.

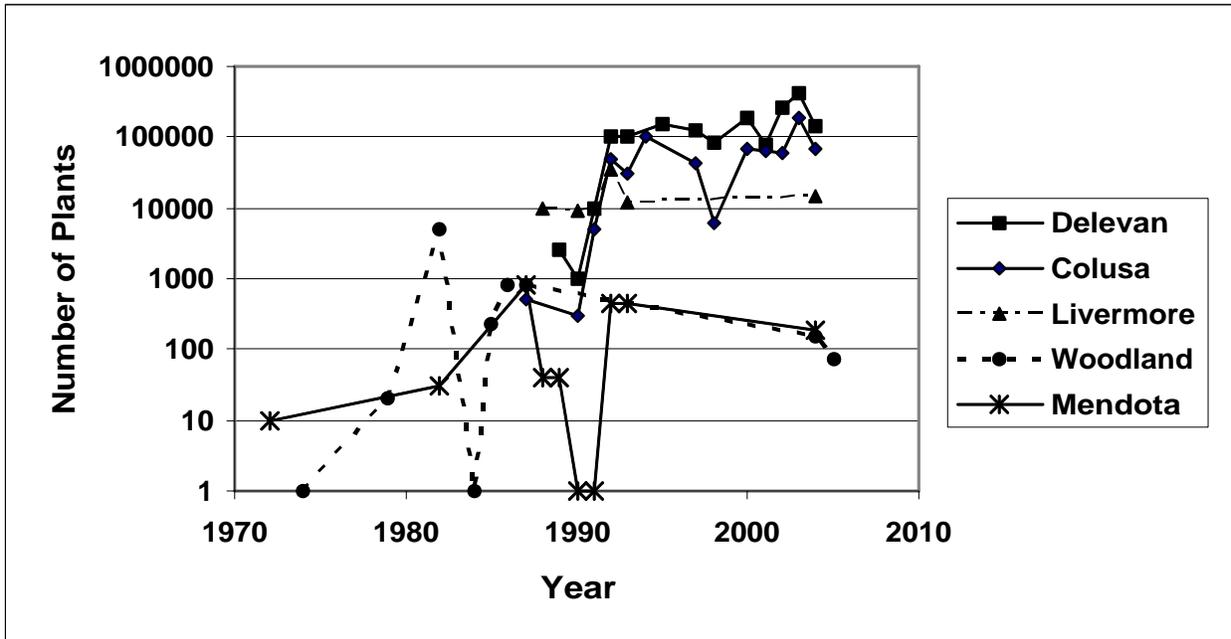


Fig. 2. Dendrogram of individuals of *Cordylanthus palmatus* clustered according to genetic similarity and location of populations within California, USA.

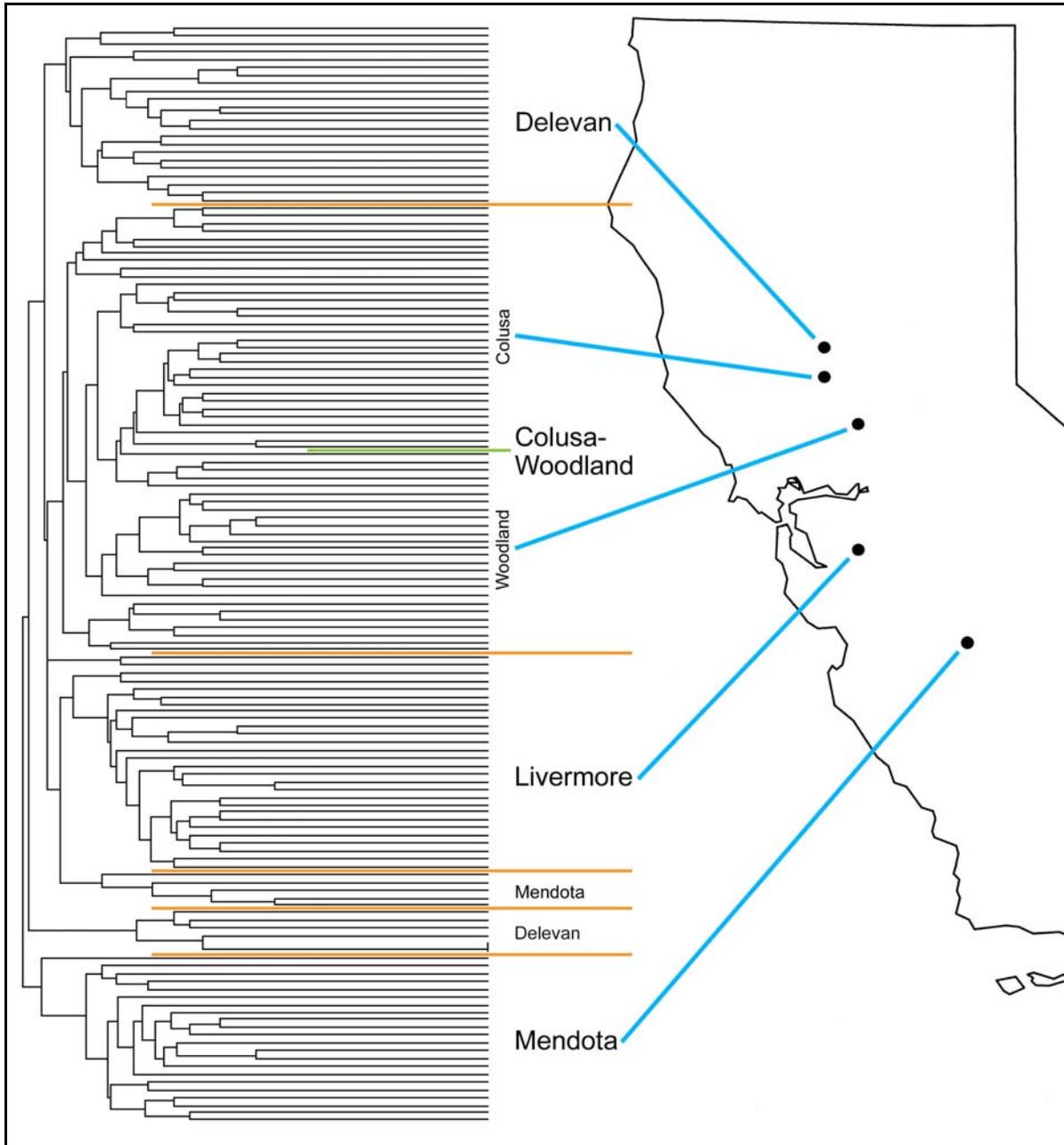


Fig. 3. Aerial photograph of the Colusa National Wildlife Refuge. Small circles indicate locations of samples of *Cordylanthus palmatus* from two vernal pools. Dendrogram illustrates genetic differences within the Colusa population (D = individual from the Delevan population, W = individual from the Woodland population).

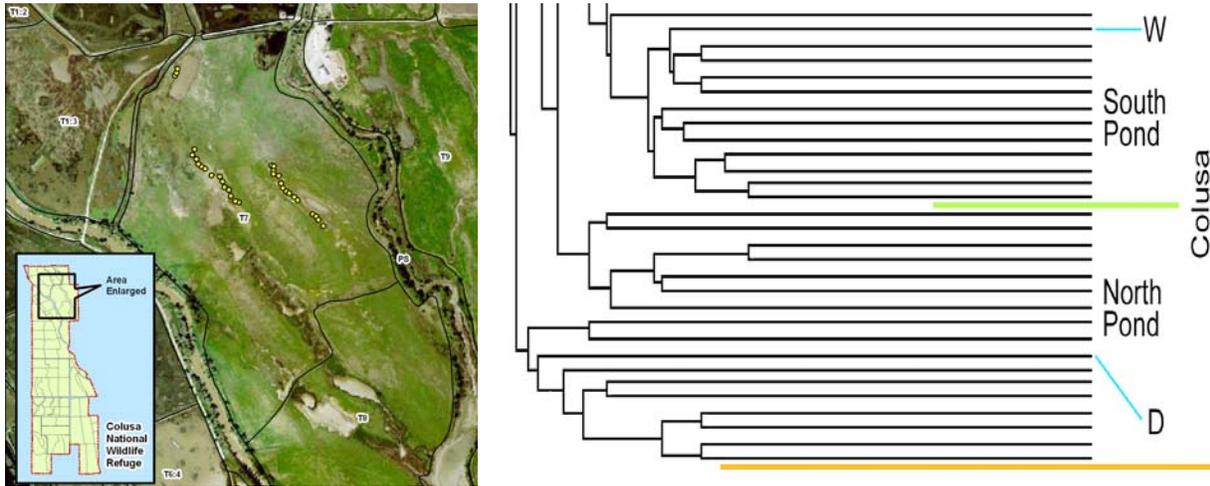


Fig. 4. Dendrogram of populations of *Cordylanthus palmatus* clustered according to genetic similarity.

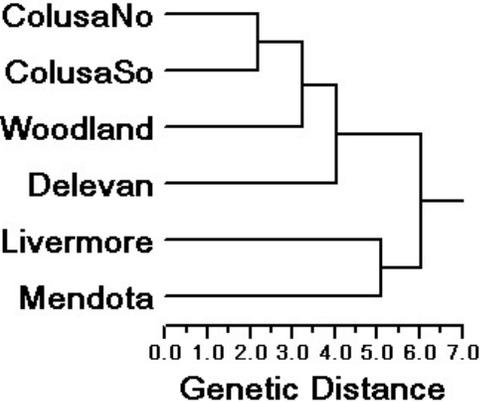


Table 1. Analyses of Molecular Variance (AMOVA) among and within populations of *Cordylanthus palmatus*. Table 1a: potential genetic structure within the Colusa population not considered in analysis. Table 1b: potential genetic structure within the Colusa population included in analysis.

Table 1a.

Source of variation	d.f.	Sum of squares	Variance	% of variation (P-value)
Among populations	4	315.74	2.38	22.0 (<0.001)
Within populations	143	1208.3	8.45	78.0 (<0.001)
F_{ST}	0.22 (<0.001)			

Table 1b.

Source of variation	d.f.	Sum of squares	Variance	% of variation (P-value)
Among populations	4	315.7	1.44	13.28 (0.067)
Among subpopulations	1	25.9	1.06	9.84 (<0.001)
Within populations	142	1182.0	8.33.	76.89 (<0.001)
F_{ST}	0.23 (<0.001)			

Table 2. Number of polymorphic loci (out of 85 species-wide polymorphic loci) that were variable within each population of *Cordylanthus palmatus*.

Population	Number of polymorphic loci
Mendota	56
Livermore	56
Woodland	63
Colusa	58
Delavan	61