

## Dissimilar patterns of genetic variation in two insular endemic plants sharing species characteristics, distribution, habitat, and ecological history

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### Abstract

*Camissonia guadalupensis* ssp. *clementina* (Onagraceae) and *Cryptantha traskiae* (Boraginaceae) share many species characteristics that affect levels and pattern of genetic variation. In addition, they have a similar distribution, are found in the same habitat, and share ecological history by virtue of co-occurring at nearly all of the locations at which they are found. All populations of the two taxa on San Clemente Island were surveyed for genotypes at 16 allozyme loci, revealing generally low levels of genetic variation. However, *Cam. g. clementina* has more genetic variation than *Cryp. traskiae* at the taxon level ( $P = 37.5$  versus 18.8,  $A = 1.69$  versus 1.31, and  $H_E = 0.088$  versus 0.003) and significantly more alleles and higher heterozygosity within populations ( $A = 1.09$  versus 1.03,  $A_E = 1.02$  versus 1.00;  $H_O = 0.013$  versus 0.001 and  $H_E = 0.017$  versus 0.003). Some populations of each taxon are monomorphic at all loci. Much greater differentiation among populations occurs in *Cam. g. clementina* ( $G_{ST} = 0.810$ ) than in *Cryp. traskiae* ( $G_{ST} = 0.042$ ). Locations of the more variable populations of each taxon do not coincide, and the pattern of genetic similarity of localities differs in the two taxa. Striking population genetic differences exist between these taxa despite shared species characteristics, distribution, habitat, and recent history.

### Introduction

Since 1966, when gel electrophoresis was first used by population geneticists (Harris 1966; Lewontin and Hubby 1966), thousands of studies have been published that report genetic data on natural populations of plants and animals. These have been summarized in many reviews (e.g. Brown 1979; Hamrick et al. 1979; Gottlieb 1981; Hamrick 1983; Loveless and Hamrick 1984; Hamrick and Godt 1989, 1996a). One of the features that still makes allozyme studies so attractive for initial surveys of genetic variation (aside from being a relatively fast and inexpensive source of genetic information) is the wealth of data for a broad range of plants and animals that provides a comparative context for any new study.

The role of various ecological and life-history characteristics in determining the level and distribution of genetic variation has been investigated in a series of reviews of the plant allozyme literature (Hamrick 1983; Loveless and Hamrick 1984; Hamrick and Godt 1989, 1996a). A number of characteristics have been identified as having a significant effect: taxonomic status, life form, geographic range, regional distribution, breeding system, seed dispersal, mode of reproduction, and successional status. However, these eight traits only explain 24% of the variation in genetic diversity at the species level, 28% at the population level, and 47% of the variation in population differentiation when species are categorized with respect to single traits (Hamrick and Godt 1989). Categorization of species for two-trait combinations

does not increase the proportion of variation explained by the variables (Hamrick and Godt 1996a). Moreover, flagrant exceptions to the general patterns revealed by these reviews have been reported (Young and Brown 1996; Young et al. 1999; Helenurm 2001). Thus, although these analyses have provided us with important insights regarding the importance of various species characteristics to the level and distribution of genetic variation, they clearly do not provide an adequate basis for predicting genetic variation in unstudied species.

The low predictive power of species characteristics may be partly clue to an imprecise relationship between concepts such as “outbreeding”, “animal-pollinated”, or “wind dispersed” and population genetic parameters such as outcrossing rate or gene flow. However, many additional factors are also likely to affect the levels and patterns of genetic variation reported for natural populations. These include ecological history (principally, fluctuations in the number and size of populations), design of the study (sample sizes and the number and spatial distribution of populations sampled), and technique (number and identity of loci surveyed; Hamrick and Godt 1996b). Species that share the entire eight-trait suite of characteristics mentioned above and are also similar in these additional factors may be expected to exhibit a high degree of similarity in amount and pattern of genetic variation. Whether this expectation is met is of practical concern in conservation biology because it would demonstrate whether known patterns of genetic variation can be extrapolated to unstudied but apparently similar species.

We report widely divergent amounts and patterns of genetic variation in *Cam. g. clementina* and *Cryp. traskiae*, two endemic plant taxa that, although phylogenetically unrelated, share all the principal traits demonstrated to affect genetic variation, and in addition share important aspects of their evolutionary and ecological histories. They occur in the same successional stage in the same habitat and occupy the same sites in virtually identical ranges. Moreover, genetic variation was surveyed in the two taxa by the same person, using similar sets of allozyme loci and similar buffer systems. We show that (1) the amount and distribution of genetic variation at the taxon level are highly dissimilar in *Cam. g. clementina* and *Cryp. traskiae*, and (2) the amount and distribution of

genetic variation among sites are not concordant in *Cam. g. clementina* and *Cryp. traskiae*. The study was initiated as part of an investigation of the conservation genetics of rare and endangered plant species of San Clemente Island, California.

## Methods

### *Study species*

*Cam. g. clementina* and *Cryp. traskiae* are insular endemic annual plants of California's Channel Islands that share many characteristics demonstrated to affect the amount and pattern of genetic variation. *Camissonia guadalupensis* (S. Watson) Raven ssp. *clementina* (Raven) Raven (Onagraceae; San Clemente Island evening primrose; termed herein as “*Cam. g. clementina*”) is known only from San Clemente Island, while *Cryp. traskiae* I.M. Johnston (Boraginaceae; Trask's Cryptantha, termed herein as “*Cryp. traskiae*”) is found primarily on San Clemente Island, although three populations are also known from the adjacent San Nicolas Island. Both taxa are limited to sand dunes and sandy coastal flats, and populations generally co-occur at suitable localities. They are found in the same successional stage and appear to have comparable population sizes (see below).

Although no quantitative estimates of their breeding systems have been reported, both *Cam. g. clementina* and *Cryp. traskiae* appear likely to be selfing taxa, based on Raven's (1969) description of *Cam. g. clementina* as autogamous and on observations of copious autogamous seed production in greenhouse populations of *Cryp. traskiae* (K. Helenurm, unpublished data). Seeds of both taxa are small and lack any special dispersal mechanism. Thus, these two taxa are very similar in their categorization by the eight characteristics known to affect genetic variation within and among populations, using descriptions from original papers or floras (following Hamrick and Godt 1989). They are similar in taxonomic status (dicots), life form (annual), geographic range (endemic), regional distribution (temperate), breeding system (selfing, based on observations of autogamy), seed dispersal (gravity, based on no special mechanism), mode of reproduction (sexual), and successional status (based on co-occurrence at nearly all localities at which the taxa are found).

Thus, they share features of taxonomy, distribution, life-history, and ecology.

#### Study sites

The distributions of *Cam. g. clementina* and *Cryp. traskiae* are limited by availability of suitable habitat on San Clemente Island, and populations of both taxa generally occur at all localities. Although populations range from the northern to the southern tip of the island, the majority of individuals of both taxa are found in the area of sand dunes in the north-central portion of San Clemente Island (Figure 1). Population sizes vary

over an order of magnitude in both taxa, with similar ranges (Table 1) and means (20,000 for *Cam. g. clementina*, 33,000 for *Cryp. traskiae*).

Leaf tissue was sampled from all populations of *Cam. g. clementina* and *Cryp. traskiae* (Figure 1, Table 1). Since the area of sand dunes contains a large, scattered population of both taxa, sampling was performed at three localities in this area.

#### Tissue collection and electrophoresis

Thirty to fifty individuals were haphazardly sampled from each population. Leaves were stored in plastic bags, and kept moist and cool until they

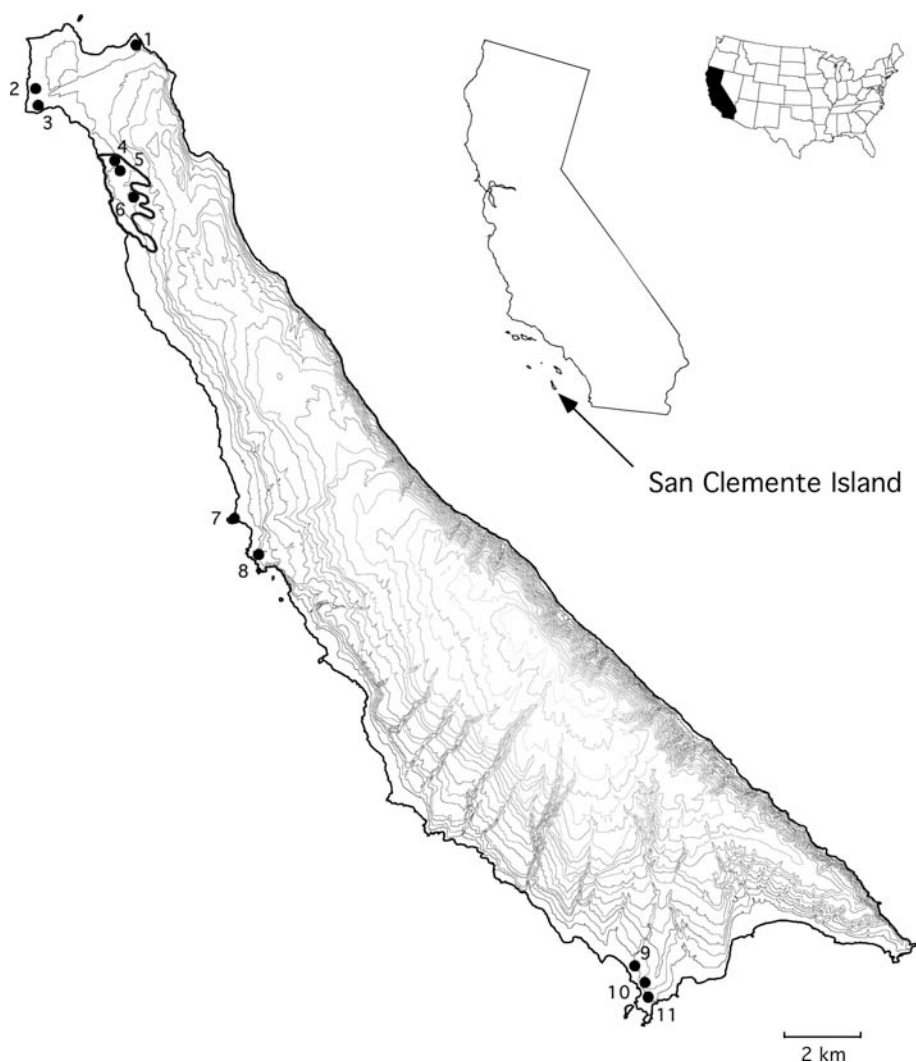


Figure 1. Locations of populations of *Camissonia guadalupensis* ssp. *clementina* and *Cryptantha traskiae* on San Clemente Island, California. The polygon enclosing populations 4-6 indicates the location of sand dunes.

Table 1. Population number, collection locations, approximate population sizes (1996), and sample sizes of *Camissonia guadalupensis* ssp. *clementina* and *Cryptantha traskiae* on San Clemente Island

Population		<i>Camissonia guadalupensis</i> ssp. <i>Clementina</i>		<i>Cryptantha traskiae</i>	
Number	Location	Population size	Sample size	Population size	Sample size
1	Northwest Shore	10,000	50	75,000	50
2	Whale Point	50,000	50	—	—
3	West Cove	10,000	50	1,500	30
4	North Dunes	50,000	50	50,000	50
5	Mid Dunes	30,000	50	35,000	50
6	South Dunes	25,000	50	15,000	50
7	Eel Point	3,000	50	50,000	50
8	Eel Cove	5,000	50	20,000	50
9	China Beach	2,500	50	—	—
10	China Cove	2,500	50	50,000	30
11	China Point	—	—	2,000	30

were transported to the laboratory, where they were stored at 5 °C.

Electrophoretic methods followed (Soltis et al. 1983). Leaf tissue was crushed in phosphate extraction buffer (Conkle et al. 1982) and stored at -80 °C until electrophoresis was conducted. Three buffer systems were used to resolve loci coding for 12 enzymes in *Cam. g. clementina*. Alcohol dehydrogenase (Adh), isocitrate dehydrogenase (Idh), malate dehydrogenase (Mdh), malic enzyme (Me), phosphoglucosomerase (Pgi), phosphoglucomutase (Pgm) and phosphogluconate dehydrogenase (Pgd) were resolved using a histidine-citrate, pH 6.2 buffer system (modified from Soltis et al. 1983) with 11.0% starch gels. Diaphorase (Dia), fluorescent esterase (Est), glutamate dehydrogenase (Gdh), and hexokinase (Hex) were resolved using a lithium hydroxide, pH 8.3 buffer system (Soltis et al. 1983) with 11.0% starch gels. Shikimic acid dehydrogenase (Skd) was resolved using a Tris-borate-EDTA, pH 8.6 buffer system (Soltis et al. 1983) with 11.0% starch gels. Two buffer systems were used to resolve loci coding for nine enzymes in *Cryp. traskiae*. Alcohol dehydrogenase (Adh), isocitrate dehydrogenase (Idh), malate dehydrogenase (Mdh), malic enzyme (Me), and phosphogluconate dehydrogenase (Pgd) were resolved using a histidine-citrate, pH 6.2 buffer system (modified from Soltis et al. 1983) with 11.0% starch gels. Diaphorase (Dia), fluorescent esterase (Est), phosphoglucosomerase (Pgi), phosphoglucomutase (Pgm) were resolved using a lithium hydroxide, pH 8.3 buffer system (Soltis

et al. 1983) with 11.0% starch gels. Staining recipes for all enzymes followed Soltis et al. (1983), except for diaphorase (Murphy et al. 1990). Loci were numbered sequentially with the most anodally migrating enzyme designated "1."

#### Data analysis

Data were analyzed using the computer program Genestrut (Constantine et al. 1994). Percentage of polymorphic loci ( $P$ ), mean number of alleles per locus ( $A$ ) and per polymorphic locus ( $A_P$ ), effective number of alleles ( $A_E$ ), observed heterozygosity ( $H_O$ ), and expected heterozygosity ( $H_E$ ) were calculated. Loci were considered polymorphic if more than one allele was detected. Levels of genetic variation were calculated for individual populations and for all individuals pooled across all populations on San Clemente Island. Mann-Whitney tests were performed to test for significant differences among population parameters for the two taxa. Correlations between the measures of genetic variability and estimates of population size were tested using Spearman's rank correlation analysis. Fixation indices ( $F$ ) were calculated to detect deviations from Hardy-Weinberg equilibrium. The partitioning of genetic diversity within and among all populations was analyzed using  $F$  statistics (Nei 1973). Nei's (1978) unbiased genetic identity ( $I$ ) was calculated for pairwise comparisons of populations. The relationship between genetic and geographic distance on San Clemente Island was tested using Mantel (1967) analysis

(Miller 1997). A cluster analysis was performed using UPGMA and Rogers' (1972) genetic distance. Gene flow was estimated using Wright's (1951) formula  $Nm = (1-F_{ST})/4F_{ST}$ , with  $F_{ST}$  considered equivalent to  $G_{ST}$  (Nei 1977). A second estimate was based on the frequency of private alleles (alleles found in a single population; Slatkin 1985).

## Results

### *Loci and alleles scored*

Enzyme electrophoresis resulted in clear and consistent staining for 12 enzymes encoded by 16 putative loci in *Cam. g. clementina*: Adh, Dia, Est-1, Est-2, Gdh, Hex, Idh, Mdh-1, Mdh-2, Mdh-3, Me, Pgd, Pgi, Pgm-1, Pgm-2, and Skd. All enzymes migrated anodally. Six loci were polymorphic: Est-1,

Est-2, and Skd (two alleles each), Pgi (three alleles), and Idh and Pgd (four alleles each). In total, 27 alleles were detected across all 16 loci.

Clear and consistent staining was obtained for nine enzymes encoded by 16 putative loci in *Cryp. traskiae*: Adh, Dia-1, Dia-2, Est-1, Est-2, Idh, Mdh-1, Mdh-2, Mdh-3, Me, Pgd-1, Pgd-2, Pgi-1, Pgi-2, Pgm-1 and Pgm-2. All enzymes migrated anodally. Three loci were polymorphic: Est-2 and Idh (two alleles each), and Pgi-2 (four alleles). In total, 21 alleles were detected across all 16 loci.

### *Measures of genetic variability*

Populations of *Cam. g. clementina* contained from zero (two populations) to three polymorphic loci with an average number of alleles per polymorphic locus of 2.12 and average expected heterozygosity of 0.017 (Table 2). No significant correlations were found between genetic diversity parameters and

Table 2. Genetic variability at 16 loci in 10 populations of *Camissonia guadalupensis* ssp. *clementina* and nine populations of *Cryptantha traskiae* on San Clemente Island

Population	$P$	$A$	$A_P$	$A_E$	$H_O$	$H_E$
<i>Camissonia guadalupensis</i> ssp. <i>clementina</i>						
1. Northwest Shore	12.5	1.12	2.00	1.06	0.039	0.057
2. Whale Point	12.5	1.12	2.00	1.03	0.015	0.032
3. West Cove	6.2	1.06	2.00	1.00	0.004	0.004
4. North Dunes	12.5	1.12	2.00	1.02	0.011	0.015
5. Mid Dunes	12.5	1.12	2.00	1.02	0.011	0.017
6. South Dunes	6.2	1.12	3.00	1.01	0.007	0.007
7. Eel Point	0.0	1.00	–	1.00	0.000	0.000
8. Eel Cove	18.8	1.19	2.00	1.01	0.013	0.012
9. China Beach	6.2	1.06	2.00	1.02	0.029	0.022
10. China Cove	0.0	1.00	–	1.00	0.000	0.000
Mean	8.8	1.09	2.12	1.02	0.013	0.017
SE	1.9	0.02	0.12	0.01	0.004	0.006
Subspecies	37.5	1.69	2.83	1.10	0.013	0.088
<i>Cryptantha traskiae</i>						
1. Northwest Shore	6.2	1.06	2.00	1.01	0.002	0.005
3. West Cove	6.2	1.06	2.00	1.01	0.000	0.008
4. North Dunes	0.0	1.00	–	1.00	0.000	0.000
5. Mid Dunes	6.2	1.06	2.00	1.00	0.001	0.001
6. South Dunes	0.0	1.00	–	1.00	0.000	0.000
7. Eel Point	12.5	1.12	2.00	1.01	0.002	0.014
8. Eel Cove	0.0	1.00	–	1.00	0.000	0.000
10. China Cove	0.0	1.00	–	1.00	0.000	0.000
11. China Point	0.0	1.00	–	1.00	0.000	0.000
Mean	3.5	1.03	2.00	1.00	0.001	0.003
SE	1.5	0.02	0.00	0.00	0.000	0.002
Species	18.8	1.31	2.67	1.00	0.001	0.003

estimated population sizes for populations of *Cam. g. clementina* ( $P$ : Spearman's  $\rho = 0.564$ ,  $P = 0.089$ ;  $A$ : Spearman's  $\rho = 0.595$ ,  $P = 0.070$ ;  $A_E$ : Spearman's  $\rho = 0.510$ ,  $P = 0.132$ ;  $H_O$ : Spearman's  $\rho = 0.215$ ,  $P = 0.550$ ;  $H_E$ : Spearman's  $\rho = 0.420$ ,  $P = 0.227$ ).

Populations of *Cryp. traskiae* contained from zero (five populations) to two polymorphic loci with an average number of alleles per polymorphic locus of 2.00 and average expected heterozygosity of 0.003 (Table 2). No significant correlations were found between genetic diversity parameters and estimated population sizes for populations of *Cryp. traskiae* ( $P$ : Spearman's  $\rho = 0.601$ ,  $P = 0.087$ ;  $A$ : Spearman's  $\rho = 0.601$ ,  $P = 0.087$ ;  $A_E$ : Spearman's  $\rho = 0.368$ ,  $P = 0.330$ ;  $H_O$ : Spearman's  $\rho = 0.296$ ,  $P = 0.439$ ;  $H_E$ : Spearman's  $\rho = -0.589$ ,  $P = 0.095$ ).

At the taxon level, *Cam. g. clementina* had more variation than *Cryp. traskiae* in all measures of genetic diversity:  $P$  (37.5 versus 18.8),  $A$  (1.69

versus 1.31),  $A_P$  (2.83 versus 2.67),  $A_E$  (1.10 versus 1.00),  $H_O$  (0.013 versus 0.001), and  $H_E$  (0.088 versus 0.003). At the population level, *Cam. g. clementina* had significantly greater levels of variation than *Cryp. traskiae* in  $A$  (1.09 versus 1.03; Mann–Whitney  $U = 69.5$ ,  $P = 0.035$ ),  $A_E$  (1.02 versus 1.00;  $U = 69.0$ ,  $P = 0.036$ ),  $H_O$  (0.013 versus 0.001;  $U = 78.0$ ,  $P = 0.005$ ), and  $H_E$  (0.017 versus 0.003;  $U = 71.0$ ,  $P = 0.029$ ). *Cam. g. clementina* also had greater mean  $P$ , but this is barely non-significant (8.8 versus 3.5;  $U = 67.5$ ,  $P = 0.053$ ).

#### Genetic identity measures

Genetic identity values for *Cam. g. clementina* ranged from 0.837 to 1.000 with a mean of 0.919 (Table 3). Genetic identity values were considerably higher for *Cryp. traskiae*, ranging from 0.999 to 1.000 with a mean of 1.000 (Table 4).

Table 3. Matrix of Nei's (1972) genetic identities (above diagonal) and geographic distances (in km below diagonal) among 10 populations of *Camissonia guadalupensis* ssp. *clementina* on San Clemente Island

Population	1	2	3	4	5	6	7	8	9	10
1	–	0.925	0.860	0.880	0.884	0.876	0.921	0.921	0.856	0.837
2	3.1	–	0.929	0.916	0.919	0.913	0.976	0.976	0.905	0.889
3	3.0	0.5	–	0.877	0.936	0.937	0.875	0.875	0.948	0.937
4	3.3	2.6	2.2	–	0.946	0.940	0.940	0.939	0.901	0.877
5	3.4	2.9	2.4	0.3	–	0.999	0.943	0.942	0.962	0.936
6	4.1	3.6	3.2	1.0	0.8	–	0.937	0.937	0.962	0.937
7	12.9	12.4	12.0	10.1	9.9	9.0	–	1.000	0.899	0.875
8	14.0	13.6	13.1	11.2	11.0	10.2	1.2	–	0.899	0.875
9	27.7	27.5	27.4	25.3	25.1	24.3	15.8	14.7	–	0.948
10	28.2	28.0	27.9	25.8	25.6	24.8	16.3	15.2	0.5	–

Table 4. Matrix of Nei's (1972) genetic identities (above diagonal) and geographic distances (in km below diagonal) among nine populations of *Cryptantha traskiae* on San Clemente Island

Population	1	3	4	5	6	7	8	10	11
1	–	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
3	3.0	–	1.000	1.000	1.000	0.999	1.000	1.000	1.000
4	3.3	2.2	–	1.000	1.000	0.999	1.000	1.000	1.000
5	3.4	2.4	0.3	–	1.000	0.999	1.000	1.000	1.000
6	4.1	3.2	1.0	0.8	–	0.999	1.000	1.000	1.000
7	12.9	12.0	10.1	9.9	9.0	–	0.999	0.999	0.999
8	14.0	13.1	11.2	11.0	10.2	1.2	–	1.000	1.000
10	28.2	27.9	25.8	25.6	24.8	16.3	15.2	–	1.000
11	28.4	28.1	26.0	25.8	25.0	16.5	15.4	0.2	–

The phenogram produced by UPGMA cluster analysis depicts a relatively close genetic relationship among all populations. No significant relationship exists between genetic and geographic distance for *Cam. g. clementina* ( $P = 0.8450$ ,  $N = 10$ ; Mantel 1967) or for *Cryp. traskiae* ( $P = 0.2940$ ,  $N = 9$ ; Mantel 1967).

#### Fixation indices

Mean fixation indices ( $F$ ) for populations of *Cam. g. clementina* ranged from  $-0.286$  to  $0.358$  with mean  $F = 0.108$  ( $N = 8$ ), indicating a deficiency of heterozygotes relative to Hardy–Weinberg expectations. Six of 14 fixation indices for individual loci were significant; four of these were positive.

Mean fixation indices for populations of *Cryp. traskiae* ranged from 0 to 1.000 with mean  $F = 0.495$  ( $N = 4$ ), indicating a deficiency of heterozygotes relative to Hardy–Weinberg expectations. Three of five fixation indices for individual loci were significant; all of these were positive.

The positive within-population inbreeding coefficients indicate that both inbreeding and outcrossing are occurring in natural populations of these taxa.

#### $F$ statistics and gene flow

Differentiation of populations of *Cam. g. clementina* was high and significantly different from 0 (mean  $F_{ST} = 0.810$ ,  $P < 0.01$ ; Table 5). Of the total gene diversity, 19.0% is found within populations and 81.0% among populations (mean  $H_S = 0.045$ , mean  $D_{ST} = 0.191$ , mean  $H_T = 0.235$ ,  $G_{ST} = 0.810$ ). The analysis was repeated after grouping populations by geographic

area into five groups (consisting of population 1, 2–3, 4–6, 7–8, and 9–11; Figure 1); 22.4% of the total gene diversity is found among populations within areas, and 58.7% among areas.

Differentiation of populations of *Cryp. traskiae* is low but significantly different from 0 (mean  $F_{ST} = 0.042$ ,  $P < 0.01$ ; Table 5). Of the total gene diversity, 95.8% is found within populations and 4.2% among populations (mean  $H_S = 0.017$ , mean  $D_{ST} = 0.001$ , mean  $H_T = 0.017$ ,  $G_{ST} = 0.042$ ). When populations are grouped according to geographic area (as above), 1.8% of the total gene diversity is found among populations within an area, and 2.4% among areas.

Gene flow among populations of *Cam. g. clementina* was  $Nm = 0.01$  using Slatkin's (1985) method based on seven private alleles with an average frequency of 0.546. Wright's (1951) method yielded an estimate of  $Nm = 0.06$ . Gene flow among populations of *Cryp. traskiae* was  $Nm = 1.93$  using Slatkin's (1985) method based on five private alleles with an average frequency of 0.047, and  $Nm = 5.70$  using Wright's (1951) method.

## Discussion

### *Dissimilar levels of genetic variation and differentiation*

Both *Cam. g. clementina* and *Cryp. traskiae* are low in levels of genetic variation at the taxon and population levels, as would be predicted based on narrow geographic range (Karron 1987; Hamrick and Godt 1989; Gitzendanner and Soltis 2000) and rarity (Cole 2003), and both fall within the range

Table 5. Summary of  $F$  statistics at polymorphic loci of *Camissonia guadalupensis* ssp. *clementina* and *Cryptantha traskiae*

<i>Camissonia guadalupensis</i> ssp. <i>clementina</i>				<i>Cryptantha traskiae</i>			
Locus	$F_{IS}$	$F_{IT}$	$F_{ST}$	Locus	$F_{IS}$	$F_{IT}$	$F_{ST}$
Est-1	-0.054	-0.005	0.046**	Est-2	1.000	1.000	0.039**
Est-2	1.000	1.000	1.000**	Idh	-0.008	-0.001	0.007
Idh-1	0.817	0.977	0.876**	Pgi-2	0.788	0.798	0.048**
Pgd-1	0.019	0.766	0.762**				
Pgi-1	-0.052	-0.021	0.030**				
Skd-1	-0.021	-0.002	0.018*				
Mean	0.231	0.854	0.810**		0.778	0.787	0.042**

Asterisks indicate  $F_{ST}$  values significantly different from zero ( $*P < 0.05$ ,  $**P < 0.01$ ).

reported for other species of San Clemente Island (Helenurm 2001; Dodd and Helenurm 2002; Helenurm 2003; Helenurm et al. 2005). However, the two taxa are substantially different for most parameters describing genetic variation. Overall, *Cam. g. clementina* has twice the proportion of polymorphic loci, and an order of magnitude more observed and expected heterozygosity of *Cryp. traskiae*. At the population level, *Cam. g. clementina* has approximately twice the proportion of polymorphic loci, an order of magnitude more observed heterozygosity, and five times the expected heterozygosity of *Cryp. traskiae*.

Even more striking is the difference between *Cam. g. clementina* and *Cryp. traskiae* in the degree of differentiation of populations. Most of the genetic variation in *Cam. g. clementina* is found among populations ( $G_{ST} = 0.810$ ) while only a small minority of the genetic variation in *Cryp. traskiae* is found among populations ( $G_{ST} = 0.042$ ). Genetic identity values (Tables 3 and 4), and the UPGMA phenogram (Figure 2) also illustrate this difference in partitioning of genetic variation. The greater differentiation among populations of *Cam. g. clementina* is consistent with indirect estimates of gene flow based on private

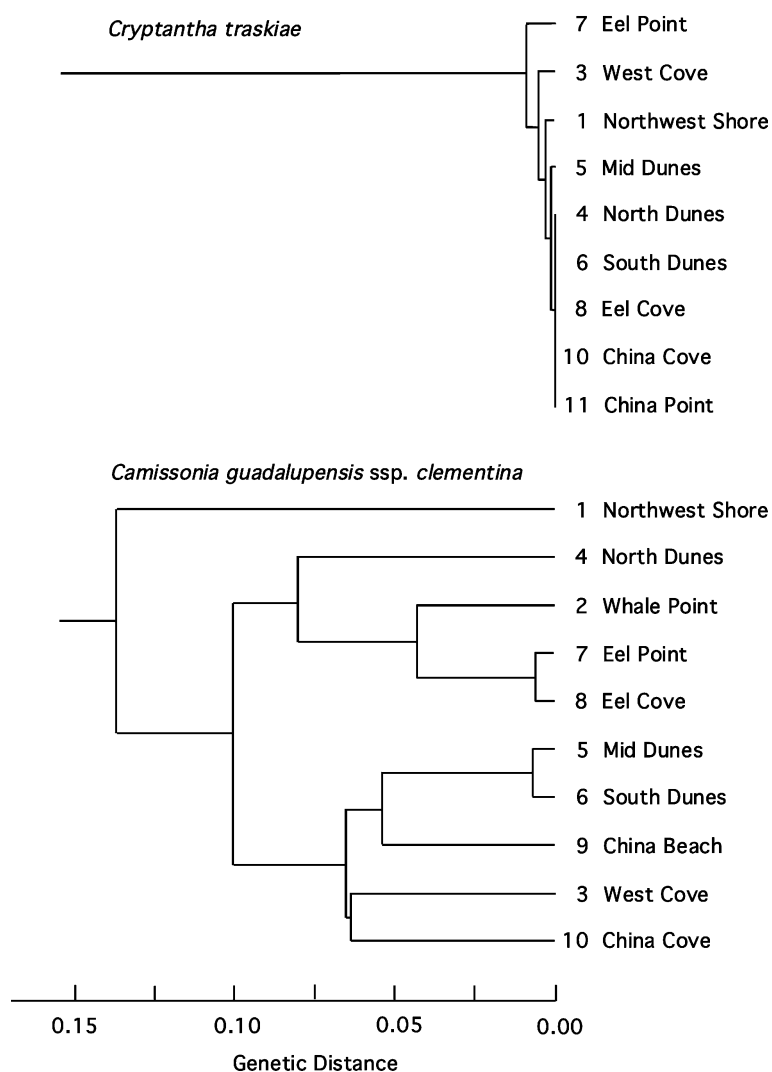


Figure 2. Cluster analysis of 10 populations of *Camissonia guadalupensis* ssp. *clementina* and nine populations of *Cryptantha traskiae* on San Clemente Island, California, using unweighted pair group method and Rogers' (1972) genetic distance values.



alleles (Slatkin 1985). Both Slatkin's (1985) and Wright's (1951) methods suggest that gene flow is two orders of magnitude lower in *Cam. g. clementina*; although these estimates may provide inaccurate absolute estimates of gene flow because of assumptions underlying the mathematical models or limitations of data collection (Slatkin 1994; Whitlock and McCauley 1999), their congruence is highly suggestive.

Grouping populations by geographic area showed that more of the genetic variation among populations of *Cam. g. clementina* is accounted for by differences among areas (58.7%) than by differences among population within areas (22.4%). Similarly, more of the genetic variation among populations of *Cryp. traskiae* is accounted for by areas (2.4%) than by population differences within areas (1.8%). Thus, genetic variation is apportioned similarly in the two taxa at this geographical scale, suggesting that seed dispersal, once it extends beyond a population, does not differ greatly between the taxa. The absence of correlations between genetic and geographic distance may be attributed to the small number of populations and, in *Cryp. traskiae*, to limited genetic differences among populations.

#### *Lack of concordance in patterns of variation and differentiation*

In addition to a lack of similarity between *Cam. g. clementina* and *Cryp. traskiae* in parameters summarizing genetic diversity and differentiation, there appears to be an utter lack of concordance in the pattern of variation and differentiation. First, the localities containing greater and lesser amounts of genetic variation are different in the two taxa (e.g. Eel Point and Eel Cove; Table 2). The sand dune area (Figure 1), which might be expected to function as a repository of genetic variation due to its large area of potential habitat, large total population size (reducing genetic drift which can cause loss of alleles) and central location (decreasing the maximum distance for gene flow to other populations), does not appear to be a center of genetic diversity for either taxon, but these populations tend to be intermediate in genetic diversity for *Cam. g. clementina* yet low for *Cryp. traskiae* (Table 2).

Second, the pattern of differentiation is very different for the two taxa (Figure 2). Localities

that are genetically similar for one taxon are not similar for the other (e.g. the adjacent Eel Point and Eel Cove), and the identity of the population most different from the others differs in the two taxa (Northwest Shore for *Cam. g. clementina* versus Eel Point for *Cryp. traskiae*).

#### *Similarity in species characteristics*

*Cam. g. clementina* and *Cryp. traskiae*, although phylogenetically unrelated, are very similar in their categorization by eight species characteristics known to affect genetic variation, and appear to share many similarities in their evolutionary and ecological history (see below). However, they are remarkably different in their amount and distribution of genetic variation. Important differences are likely to exist between these taxa, therefore, in specific features of their species characteristics, evolution, or ecology. Although it is possible to speculate and develop various hypotheses to explain the population genetic differences between the taxa, evaluation of these hypotheses will either require intensive research (to determine pollen and seed dispersal, for example) or will not be possible (if they depend on the history of population sizes or on extinct pollinators and past levels of gene flow, for example).

Although observations of autogamy in both taxa suggests a selfing breeding system, actual outcrossing rates in natural populations of the two taxa may be quite different. Both taxa are visited by several small, generalist pollinators, suggesting that at least some outcrossing may occur (K. Helenurm, personal observation). The fixation indices suggest that *Cryp. traskiae* may experience more inbreeding than *Cam. g. clementina*; however, these estimates are based on only one or two polymorphic loci per population. Moreover, they are inconsistent with the observed difference in distribution of genetic variation: higher outcrossing rates are generally associated with less, rather than more, differentiation among populations (Hamrick and Godt 1989).

Seed dispersal may be another possible difference between the two taxa. The allozyme data suggest a difference of more than an order of magnitude in gene flow among populations of the two taxa; this degree of difference is found by both of the indirect methods of estimation. Although both taxa have small, gravity-dispersed seeds

lacking any special dispersal mechanism, there may be significant differences in dispersal among populations. Unfortunately, *Cam. g. clementina* seeds (mean seed mass = 0.079 mg,  $N = 2500$ ), are lighter than *Cryp. traskiae* seeds (mean seed mass = 0.234 mg,  $N = 500$ ), suggesting greater dispersal among populations. This is inconsistent with our observation of greater differentiation among populations.

Other potentially significant differences among the taxa may also exist, including the longevity of seeds in the soil (greater longevity would retard loss of genetic variation due to drift), competitive ability relative to introduced competitors (see below), or palatability to goats and other herbivores. These factors may all affect population sizes, but none of them can be identified without intensive study; categorization of *Cam. g. clementina* and *Cryp. traskiae* using readily available information suggests great similarity between them.

#### *Evolutionary history*

There are several parallel features in the evolutionary history of *Camissonia* and *Cryptantha* in spite of these genera belonging not only to different families but to distant subclasses of dicots (Qiu et al. 1999; Soltis et al. 1999). Onagraceae (order Myrtales, placed in subclass Rosidae, subclade Eurosids II; Judd et al. 1999) and Boraginaceae (order Solanales, placed in subclass Asteridae, subclade Euasterids I) diverged approximately 100 mya (Sanderson and Doyle 2001). *Camissonia* consists of 62 species in western North America and one species in South America (Wagner and Raven 1993). Three other *Camissonia* taxa (native but not endemic) are found on San Clemente Island and five on other Channel Islands (Wallace 1985). The other insular endemic taxon, *C. guadalupensis* ssp. *guadalupensis*, is found only on Guadalupe Island, several hundred km further south (Wallace 1985).

*Cryptantha* consists of approximately 160 species, primarily found in western North America (Kelley and Wilken 1993) but with some species known from southwestern South America (Munz 1974). Three other *Cryptantha* taxa (native but not endemic) are found on San Clemente Island and five on other Channel Islands. The other insular endemic taxon, *C. foliosa*, is found only on Guadalupe Island (Wallace 1985). Thus, *Camissonia* and *Cryptantha*

share several aspects of evolutionary history, as reflected in the biogeography of the genera. They are both primarily western North American genera, both have at least one disjunct species in South America, and both have one insular endemic taxon on San Clemente Island, California, and another on Guadalupe Island, Mexico. In addition, several other taxa from each genus are found on San Clemente or other Channel Islands.

It is not known how long *Cam. g. clementina* and *Cryp. traskiae* have occurred on San Clemente Island, except they have presumably existed there long enough to evolve into insular endemics. It is possible that *Cryp. traskiae* evolved on San Nicolas Island and dispersed to San Clemente Island, but this is less likely. San Nicolas Island is considerably smaller (58 versus 145 km<sup>2</sup>) and geologically younger (D. Muhs, USGS), has fewer populations of *Cryp. traskiae* (only three have been reported), and has far fewer native species (139 versus 272) and endemic taxa (7% versus 13%; Junak et al. 1995). Nothing is known about the speciation process and subsequent dispersal of *Cam. g. clementina* or *Cryp. traskiae*, both of which may play a role in shaping the current levels and pattern of genetic differentiation. It is possible that the reduced genetic variation of *Cryp. traskiae* is due to its evolutionary history, either due to more recent speciation than *Cam. g. clementina* or perhaps to evolution from a lineage with reduced genetic variation. It is not currently possible to evaluate these hypotheses.

#### *Ecological history*

*Cam. g. clementina* and *Cryp. traskiae* presumably also share ecological history. Both taxa show strong habitat specificity, occurring only on sandy coastal flats and sand dunes. A limited number of localities with suitable habitat are found on San Clemente Island, and populations of both taxa occupy most of them. The majority of individuals of both taxa are found in the dunes area in the north-central portion of San Clemente Island, although both also have a large population at the northern end. Average estimated population sizes are similar (20,000 for *Cam. g. clementina*, 33,000 for *Cryp. traskiae*), suggesting that the demographic response of these taxa to their environment may be similar. Although population sizes are somewhat larger for *Cryp. traskiae* than for

*Cam. g. clementina*, populations of both taxa appear large enough currently to not be especially susceptible to the effects of genetic drift. Historical population sizes are unknown; it is possible that *Cryp. traskiae* populations sizes were smaller in the past, resulting in greater loss of alleles. Also unknown are the size of seed banks for the two taxa, and the longevity of seeds.

Thus, *Cam. g. clementina* and *Cryp. traskiae* not only have a virtually identical distribution and occur in the same habitat type, but they also occupy the same successional stage in the same sites and have similar population sizes. Although details of their ecology are unknown, it is likely that the number and size of populations of these two taxa have been affected by many of the same factors throughout their history. The main threat to most plant species of San Clemente Island since the late 1800s has been overgrazing by a variety of introduced animals (primarily goats since the 1930s) that were finally eliminated only in 1992 (Kellogg and Kellogg 1994; O'Malley 1994). More recently, native species have been threatened by introduced competitors; both *Cam. g. clementina* and *Cryp. traskiae*, in particular, are currently threatened by introduced *Mesembryanthemum* and *Carpobrotus* spp. (Vivrette 1973; Kellogg and Kellogg 1994).

#### *Experimental design and technique*

Sampling design was similar for both taxa: 30–50 individuals of *Cam. g. clementina* and *Cryp. traskiae* were sampled from each locality at which they were found, and both taxa were found at most localities. Thus, differences in genetic parameters for the two taxa cannot be due to sampling at different geographic scales, densities, or distances. Moreover, similar though not identical sets of enzymes (9 of 12 were surveyed in both taxa) and loci (13 of 19 were surveyed in both taxa) were used to assess genetic variation using similar buffer systems (only Pgi and Pgm were assayed using different buffer systems in the two taxa). All three loci that were polymorphic in *Cryp. traskiae* (Est-2, Idh, and Pgi-2) were also polymorphic in *Cam. g. clementina*. An additional two polymorphic loci in *Cam. g. clementina* (Est-1 and Pgd) were surveyed in *Cryp. traskiae* but were monomorphic. Only the remaining polymorphic locus in *Cam. g. clementina* (Skd) was not surveyed in

*Cryp. traskiae*. All other loci that were surveyed in just one of the two taxa were monomorphic, and therefore did not contribute greatly to the observed differences in level and distribution of genetic diversity between them.

#### *Conservation Implications*

On the basis of ecological information, all populations of *Cam. g. clementina* and *Cryp. traskiae* merit protection. These are annual taxa endemic to San Clemente Island (*Cam. g. clementina*) or to San Clemente and San Nicolas Islands (*Cryp. traskiae*) and, furthermore, are restricted to a small number of potential sites that have the appropriate substrate. The genetic data, however, suggest that management strategies may need to differ in these two taxa.

Genetic variation in *Cam. g. clementina* is primarily found among (81.0 %) rather than within populations. In contrast, most of the variation in *Cryp. traskiae* occurs within populations (95.8 %). Thus, regardless of whether the aim is to conserve variation in nature, to make *ex situ* collections or to acquire source material for reintroduction attempts, it is important that all populations of *Cam. g. clementina* be considered. This is less of a concern with *Cryp. traskiae*, in which populations appear to be virtually identical genetically.

The continued maintenance of genetic variation within natural populations may also require different management in *Cam. g. clementina*. Estimates of  $Nm$  are very low in this taxon ( $Nm = 0.01$  and  $0.06$ ), suggesting that gene flow has been too low to counteract the effects of genetic drift (Hartl and Clark 1989). Thus, it is especially important to try to maintain large population sizes in *Cam. g. clementina* to minimize the effects of drift in further eroding genetic variation. Estimates of gene flow in *Cryp. traskiae* are two orders of magnitude higher ( $Nm = 1.93$  and  $5.70$ ); even though allozyme variation is extremely low in this species, levels of gene flow among populations may be sufficiently high to counteract future loss of genetic variation due to drift. Effective management in *Cryp. traskiae* may thus require protection of intervening populations (in order to maintain gene flow among populations) in preference to maintaining large populations.

Prioritization of populations containing higher levels of allozyme variation may be premature, as

variation in both these taxa consists mainly of rare alleles found in different, and usually few, populations. While some populations in both taxa appear to be monomorphic, this may be an artifact of the low levels of genetic variation observed. Our study is susceptible to a form of sampling error because of the small number of polymorphic loci and rarity of variant alleles; had we observed additional polymorphic loci, different populations might have been identified as being more versus less variable. Moreover, it is arguable to what degree conclusions based on patterns of variation at allozyme loci can be extrapolated to traits of ecological importance. The primary strength of neutral molecular loci is to provide genetic markers with which to study evolutionary processes (such as the relative importance of gene flow and drift) that presumably affect all loci; however, they may fail to reveal genetic divergence of populations in ecologically important traits (e.g. Bekessy et al. 2003). The relationship between neutral molecular and quantitative genetic variation is unclear: there appears to be little correlation between molecular and quantitative measures of genetic variation within populations for specific traits (e.g. Reed and Frankham 2001), although fitness is positively correlated with neutral molecular estimates of heterozygosity (Reed and Frankham 2003) and differentiation in neutral marker loci appears to be correlated with differentiation in loci coding quantitative traits (e.g. Merila and Crnokrak 2001).

### Summary

*Cam. g. clementina* and *Cryp. traskiae* are insular endemics that share many species characteristics known to affect amount and distribution of genetic variation. The genera have parallel evolutionary histories, and the study taxa have a very similar distribution and share their habitat, with populations generally found at the same localities. It is likely that they have shared similar ecological constraints on population number and size throughout their history. In addition, they were studied using very similar sampling designs, buffer systems, and sets of loci.

Despite similarity in nearly all features except shared phylogeny, remarkable differences exist between *Cam. g. clementina* and *Cryp. traskiae* in the level and distribution of genetic variation.

Moreover, there is no concordance in their patterns of variation and differentiation among populations. Unfortunately, these population genetic differences are likely to be caused by factors requiring intensive study (such as quantitative estimates of seed viability, breeding system, and gene flow), or by factors that may remain unknown (such as past population sizes and details of the speciation process).

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### References

- Bekessy SA, Ennos RA, Burgman MA, Newton AC, Ades PK (2003) Neutral DNA markers fail to detect genetic divergence in an ecologically important trait. *Biol. Conserv.*, **110**, 267–275.
- Brown AHD (1979) Enzyme polymorphism in plant populations. *Theor. Popul. Biol.*, **15**, 1–42.
- Cole CT (2003) Genetic variation in rare and common plants. *Annu. Rev. Ecol. Syst.*, **34**, 213–237.
- Constantine CC, Hobbs RP, Lymbery AJ (1994) FORTRAN programs for analyzing population structure from multilocus genotypic data. *J. Hered.*, **85**, 336–337.
- Dodd SC, Helenurm K (2002) Genetic diversity in *Delphinium variegatum* (Ranunculaceae): a comparison of two insular endemic subspecies and their widespread mainland relative. *Am. J. Bot.*, **89**, 613–622.
- Gitzendanner MA, Soltis PS (2000) Patterns of genetic variation in rare and widespread plant congeners. *Am. J. Bot.*, **87**, 783–792.
- Gottlieb LD (1981) Electrophoretic evidence and plant populations. *Progress in Phytochemistry*, **7**, 1–46.
- Hamrick JL, Linhart YB, Mitton JB (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annu. Rev. Ecol. Syst.*, **10**, 173–200.
- Hamrick JL (1983) The distribution of genetic variation within and among natural plant populations. In: *Genetics and Conservation* (ed. CM Schonewald-Cox, SM Chambers, B MacBryde, WL Thomas), pp. 335–348. Benjamin/Cummings, Menlo Park, CA.
- Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding, and Genetic Resources* (ed. AHD Brown, MT Clegg, AT Kahler, BS Weir), pp. 43–63. Sinauer Associates, Sunderland, MA.

- Hamrick JL, Godt MJW (1996a) Effects of life history traits on genetic diversity in plant species. *Philos. T. Roy. Soc. Lond. B.*, **351**, 1291–1298.
- Hamrick JL, Godt MJW (1996b) Conservation genetics of endemic plant species. In: *Conservation genetics: Case histories from nature* (ed. JC Avise JL Hamrick), pp. 281–304. Chapman and Hall, New York.
- Harris H (1966) Enzyme polymorphism in man. *Proc. R. Soc. Lond. B.*, **164**, 298–310.
- Hartl DL, Clark AG (1989) *Principles of population genetics*, Sinauer Associates, Inc, Sunderland, MA.
- Helenurm K (2001) High levels of genetic polymorphism in the insular endemic herb *Jepsonia malvifolia*. *J. Hered.*, **92**, 427–432.
- Helenurm K (2003) Genetic diversity in the rare, insular endemic *Sibara filifolia* (Brassicaceae). *Madrono*, **50**, 181–186.
- Helenurm K, West R, Burckhalter SJ (2005) Allozyme variation in the endangered insular endemic *Castilleja grisea*. *Ann Bot.*, **95**, 1221–1227.
- Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) *Plant Systematics: A Phylogenetic Approach*, Sinauer Associates, Inc, Sunderland, Massachusetts.
- Junak S, Ayers T, Scott R, Wilken D, Young D (1995) *A Flora of Santa Cruz Island*, Santa Barbara Botanic Garden, Santa Barbara, CA
- Karron JD (1987) A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. *Evol. Ecol.*, **1**, 47–58.
- Kelley WA, Wilken DH (1993) Cryptantha. In: *The Jepson manual: Higher Plants of California* (ed. JC Hickman ), pp. 369–377. University of California Press, Berkeley, California.
- Lewontin RC, Hubby JH (1966) A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics*, **54**, 595–609.
- Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annu. Rev. Ecol. Syst.*, **15**, 65–95.
- Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res.*, **27**, 209–220.
- Merila J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *J. Evol. Biol.*, **14**, 892–903.
- Munz PA (1974) *A flora of Southern California*, University of California Press, Berkeley, California
- Murphy RW, Sites JW, Buth DB, Haufler CH (1990) Proteins I: Isozyme electrophoresis. In: *Molecular Systematics* (ed. DM Hillis, C Moritz), pp. 45–127. Sinauer Associates, Sunderland, MA.
- Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*, **70**, 3321–3323.
- Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. *Ann. Hum. Genet.*, **41**, 225–233.
- Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.
- O'Malley PG (1994) Animal husbandry on the three southernmost Channel Islands: A preliminary overview, 1820–1950. In: *The Fourth California Islands Symposium: Update on the Status of Resources* (ed. WL Halvorson GJ Maender ), pp. 157–164. Santa Barbara Museum of Natural History, Santa Barbara, California.
- Qiu Y-L, Lee J, Bernasconi-Quadroni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW (1999) The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. *Nature*, **402**, 404–407.
- Raven PH (1969) A revision of the genus *Camissonia* (Onagraceae). *Contributions from the United States National Herbarium*, **37**, 161–396.
- Reed DH, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.
- Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conserv. Biol.*, **17**, 230–237.
- Rogers JS (1972) Measures of genetic similarity and genetic distance. Studies in Genetics VII. *University of Texas Publication*, **7213**, 145–153.
- Sanderson MJ, Doyle JA (2001) Sources of error and confidence intervals in estimating the age of angiosperms from rbcL and 18S rDNA data. *Am. J. Bot.*, **88**, 1499–1516.
- Slatkin M (1985) Rare alleles as indicators of gene flow. *Evolution*, **39**, 53–65.
- Slatkin M (1994) Gene flow and population structure. In: *Ecological Genetics* (ed. LA Real), pp. 3–17. Princeton University Press, Princeton, New Jersey, USA.
- Soltis DE, Haufler CH, Darrow DC, Gastony GH (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern. J.*, **73**, 9–27.
- Soltis PS, Soltis DE, Chase MW (1999) Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. *Nature*, **402**, 402–404.
- Wagner WL, Raven PH (1993) Onagraceae. In: *The Jepson Manual: Higher Plants of California* (ed. Hickman JC), pp. 776–804. University of California Press, Berkeley, California.
- Wallace GD (1985) *Vascular Plants of the Channel Islands of Southern California and Guadalupe Island, Baja California, Mexico*, Contributions in Science No 365. Natural History Museum of Los Angeles County, Los Angeles, California.
- Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration:  $F_{ST} \neq 1/(4Nm + 1)$ . *Heredity*, **82**, 541–550.
- Wright S (1951) The genetic structure of populations. *Ann. Eugen.*, **15**, 323–354.
- Young AG, Brown AHD (1996) Comparative population genetic structure of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. *Conserv. Biol.*, **10**, 1220–1228.
- Young AG, Brown AHD, Zich FA (1999) Genetic structure of fragmented populations of the endangered daisy *Rutidosia leptorrhynchoides*. *Conserv. Biol.*, **13**, 256–265.