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# Allozyme diversity in the federally threatened golden paintbrush, *Castilleja levisecta* (Scrophulariaceae)

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**Abstract**

*Castilleja levisecta* (Scrophulariaceae), the golden paintbrush, is an insect-pollinated herbaceaous perennial found in the Pacific Northwest. Currently restricted to two island populations off British Columbia and nine populations (eight on islands) in Washington, *C. levisecta* is a rare species threatened with extinction. Allozymes were used to describe genetic diversity and structure in these eleven populations. Despite its threatened status and small geographic range, exceptionally high levels of genetic diversity are maintained within *C. levisecta*. All sixteen of the loci resolved were polymorphic within the species ($P_s = 100\%$), while the mean percentage of loci polymorphic within populations ($P_p$) was 65.7%. The mean number of alleles per polymorphic locus ($AP_s$) was 2.94 within the species and averaged 2.38 within populations ($AP_p$). Genetic diversity ($H_{es}$) was 0.285 for the species, whereas mean population genetic diversity ($H_{ep}$) was 0.213. Smaller populations had, on average, fewer observed alleles and less genetic diversity. A significant negative correlation ($r = -0.72$) was found between genetic identity and geographic distance, indicating reduced gene flow between distant populations. The most geographically isolated population was one of the larger populations, one of the most genetically diverse and the most genetically divergent. A wide range of pairwise population genetic identities ($I = 0.771 - 0.992$) was found, indicating considerable genetic divergence between some populations. Overall, 19% of the total genetic diversity was distributed among populations. Results of this survey indicate that genetic augmentation of existing populations is unnecessary. The high allelic diversity found for the species and within its populations holds promise for conservation and restoration efforts to save this rare and threatened plant species.

## Introduction

*Castilleja levisecta* Greenm. is a rare short-lived perennial herb in the figwort, or snapdragon family (Scrophulariaceae). Commonly known as ''golden paintbrush'', the species sports golden yellow leaf bracts that surround greenish flowers. *Castilleja levisecta* is endemic to the Pacific Northwest. It has been recorded on bluffs and in grasslands on islands of Puget Sound and the Georgia Basin, in the gravelly prairies of southern Puget Sound and in grasslands of the Willamette Valley in Oregon. Historically, *C. levisecta* was known from over 30 sites in British Columbia, Washington and Oregon (Sheehan and Sprague 1984; Caplow 2001). Currently the species persists on two islands off the coast of British Columbia and at nine sites (eight island populations) in Washington (Figure 1, Gamon 1995). It is extirpated from Oregon. Many extant populations are very limited in area (<0.4 ha) which elevates the risk of stochastic population extinctions (Gilpin

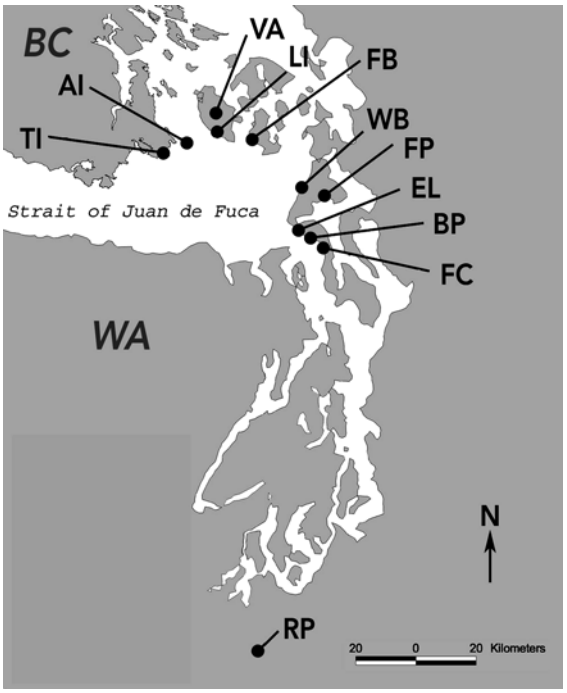


*Figure 1*. Locations of the eleven extant *Castilleja levisecta* populations. BC = British Columbia, WA = Washington, AI = Alpha Islet, TI = Trial Island, VA = Valley, FB = False Bay, LI = Long Island, WB = West Beach, FP = Forbes Point, EL = Ebey's Landing, BP = Bocker's Environmental Preserve, and FC = Fort Casey.

and Soule' 1986). Ten-year declines in population sizes have been documented at four of the remaining sites (Washington Natural Heritage Program records, 2002). In 1997 the US Fish & Wildlife Service listed *C. levisecta* as a ''threatened species'' under the federal Endangered Species Act (US Fish & Wildlife Service 1997). Washington lists the species as ''endangered'' and it is on British Columbia's ''Red List''.

*Castilleja levisecta* is a multi-stemmed herb that reproduces exclusively by seed (Caplow 2001). The species is protogynous, with pistils extending beyond the opening of the flower, and the stigma becoming receptive before anther dehiscence (Kaye and Lawrence 2003). Crossing experiments indicate that *C. levisecta* is nearly completely self-incompatible (Kaye and Lawrence 2003). *Bombus* species are the presumed pollinators (Wentworth 1994). No specialized seed dispersal mechanism has been reported for the species; most seeds probably germinate in the vicinity of their maternal parent (Caplow 2001). Like many members of the Scrophulariaceae, *C. levisecta* is hemiparasitic, obtaining some of its nutrients through haustoria that penetrate roots of other species (Heckard 1962). The range of host specificity of *C. levisecta* is unknown (Gamon 1995). Although the species can grow and flower without a host in the greenhouse (Wentworth 1994), plants without hosts grow more slowly (Kaye 2001).

Endangered plant species face a variety of threats. Frequently, however, species listed as endangered or threatened have experienced reductions in population sizes as well as population extinctions. As such species become increasingly rare, genetic diversity losses and inbreeding become of increasing concern (Ellstrand and Elam 1993). Diminished genetic diversity and inbreeding depression elevate extinction risks for these species (Gilpin and Soule' 1986).

Consideration of the long-term survival of endangered species requires strategies that maintain their genetic diversity (Barrett and Kohn 1991). To achieve this goal, an understanding of the species' genetic diversity and structure is necessary. Genetic diversity data permit informed choices of source populations for *ex situ* propagation. In addition, genetic augmentation may be considered if genetically depauperate populations are identified. Genetically diverse or unique populations may be given *in situ* conservation priority. Finally, an understanding of patterns of genetic diversity across the landscape should inform restoration and management decisions (Barrett and Kohn 1991). The objective of this study was to provide genetic data to enhance conservation and management plans for *C. levisecta*.

## Materials and methods

Leaf samples were collected from the 11 extant *C. levisecta* populations (Figure 1), kept on ice and shipped via overnight courier to the University of Georgia. Sample sizes were 48 per population except for Alpha Islet (39), Rocky Prairie (45), Long Island (31), Valley (47) and West Beach (45). Mean sample size/population was 45.0 (SD = 5.4). Prior to population collections, a series of trials was performed on 72 samples (total) from three sites to determine which of three extraction buffers (Mitton et al. 1979; Wendel and Parks 1982; Alvarez-Buylla

and Garay 1994) performed best, and to determine which enzyme/gel/electrode buffer combinations provided the best resolution. Although none of the extraction buffers was superior overall, the Mitton et al. (1979) buffer provided somewhat better resolution.

Leaf samples were crushed using a mortar and pestle to which clean ocean sand and the Mitton et al. (1979) extraction buffer were added. Enzyme extracts were adsorbed onto chromatography wicks that were placed in microtest plates and stored at −70 °C until needed for analysis. Standard starch gel electrophoretic techniques were used to obtain allele frequency data. The following 12 enzyme systems were successfully employed: aspartate amino acid transferase EC 2.6.1.1 (AAT), colorimetric esterase EC 3.1.1- (CE), diaphorase EC 1.6.4.3 (DIA), isocitrate dehydrogenase EC 1.1.1.42 (IDH), malate dehydrogenase EC 1.1.1.37 (MDH), menadione reductase EC 1.6.99.2 (MNR), phosphoglucoisomerase EC 5.3.1.9 (PGI), phosphoglucomutase EC 2.7.5.1 (PGM), 6-phosphogluconate dehydrogenase EC 1.1.1.44 (6-PGDH), shikimate dehydrogenase EC 1.1.1.25 (SKDH), triose phosphate isomerase EC 5.3.1.1 (TPI), and UTP-glucose-1-phosphate EC 2.7.7.9 (UGPP). Stain recipes were modified from Soltis et al. (1983), except for AAT and DIA (Cheliak and Pitel 1984) and UGPP (Manchenko 1994). The following loci were resolved on the buffer systems indicated (buffer numbers refer to recipes in Table 1 of Soltis et al. 1983): Buffer 4 resolved *Acon,* buffer 6 was used for *Ce-1* and *Ugpp-1*, buffer 11 was used for *Pgi-2*, *Pgm-2, 6-Pgdh*, *Skdh*, *Idh* and *Mdh*, and a modified buffer 8 (recipe available from the authors upon request) was used to resolve *Aat-1*, *Dia-1*, *Dia-2*, *Mnr-1*, *Tpi-1*, *Tpi-2* and *Tpi-3*. Enzyme banding patterns conformed to published expectations of loci number and enzyme subunit structure. Loci and alleles were numbered consecutively with the most anodal forms given the lowest number.

Measures of genetic diversity were estimated for each population (Hedrick 1985) and for the species (Hamrick and Godt 1989) using a statistical program (LYNSPROG) developed by M. D. Loveless (Department of Biology, College of Wooster, Wooster, OH, USA) and A. Schnabel (Department of Biology, University of Indiana, South Bend, IN, USA). Genetic parameters calculated included the percentage of polymorphic loci (*P*), the mean number of alleles per locus (*A*) and per polymorphic locus (*AP*), the effective number of alleles per locus ($A_e$), observed heterozygosity ($H_{obs}$) and Hardy–Weinberg expected heterozygosity ($H_e$). Genetic parameters subscripted with an "s" (e.g., $H_{es}$) indicate species

*Table 1.* Estimates of genetic diversity[a] within 11 *Castilleja levisecta* populations, and for the species

| Population | *P* | *AP* | *A* | $A_e$ | $H_{obs}$ (SD) | $H_e$ (SD) |
|---|---|---|---|---|---|---|
| Alpha Islet | 68.8 | 2.27 | 1.88 | 1.43 | 0.234 (0.054) | 0.232 (0.056) |
| Trial Island | 75.0 | 2.75 | 2.31 | 1.52 | 0.318 (0.057) | 0.283 (0.056) |
| Rocky Prairie | 75.0 | 2.67 | 2.25 | 1.51 | 0.288 (0.057) | 0.279 (0.053) |
| False Bay | 60.0 | 2.56 | 1.93 | 1.41 | 0.247 (0.052) | 0.231 (0.058) |
| Long Island | 66.7 | 2.30 | 1.87 | 1.34 | 0.219 (0.062) | 0.194 (0.055) |
| Valley | 66.7 | 2.60 | 2.07 | 1.48 | 0.256 (0.055) | 0.243 (0.058) |
| Ebey's Landing | 75.0 | 2.17 | 1.88 | 1.23 | 0.172 (0.046) | 0.146 (0.042) |
| Bocker's E.P. | 68.8 | 2.18 | 1.81 | 1.33 | 0.203 (0.052) | 0.193 (0.054) |
| Fort Casey | 46.7 | 2.29 | 1.60 | 1.22 | 0.151 (0.042) | 0.131 (0.046) |
| West Beach | 56.3 | 2.22 | 1.69 | 1.33 | 0.203 (0.048) | 0.174 (0.059) |
| Forbes Point | 64.3 | 2.22 | 1.79 | 1.44 | 0.258 (0.053) | 0.240 (0.059) |
| Mean Population | 65.7 | 2.38 | 1.91 | 1.39 | 0.232 | 0.213 |
| SD | 3.6 | 0.21 | 0.22 | 0.10 | 0.016 | 0.016 |
| Species Total | 100.0 | 2.94 | 2.94 | 1.56 | – | 0.285 |

[a] *P* is the percentage polymorphic loci, *AP* is the mean number of alleles per polymorphic locus, *A* is the mean number of alleles per locus, $A_e$ is the effective number of alleles, $H_{obs}$ is observed heterozygosity, $H_e$ is gene diversity, or expected heterozygosity and SD is the standard deviation.

values, whereas those subscripted with a "p" (e.g., $H_{ep}$) refer to population means. Allelic richness is reported as actual numbers observed, and considering sample size (i.e., with rarefaction techniques) using H-P Rare 1.0 (Kalinowski 2004a, b). For estimation of allelic richness with rarefaction, all samples were considered to consist of 96 genes, since our largest and most common sample size was 48 individuals.

Each polymorphic locus in every population was examined for conformity to Hardy–Weinberg expectations by calculating Wright's fixation index ($F$, Wright 1922) and examining the significance of these values with chi-square tests (Li and Horvitz 1953). Wright's $F_{IS}$ parameter was calculated to estimate overall deviations from random mating (Wright 1978).

The distribution of genetic diversity within and among populations was estimated using Nei's gene diversity statistics (Nei 1973, 1977). A $G_{ST}$ value (indicating the proportion of total genetic diversity found among populations) was calculated for each polymorphic locus. Significance of the $G_{ST}$ values was tested using chi-square tests (Workman and Niswander 1970). Overall population divergence was estimated by averaging $G_{ST}$ values across all polymorphic loci. Nei's (1972) genetic identity ($I$ ranges from 0.0 to 1.0) and distance measures ($D$ ranges from 0.0 to infinity) were also calculated for each population pair. The software program GENEPOP [originally designed by M. Raymond and F. Rousset (Universite de Montpellier II, Montpellier, France) and available at https://wbiomed.curtin.edu.au/genepop/index.html] was used to obtain pairwise population estimates of $F_{ST}$ (analogous to $G_{ST}$). GENEPOP was also used to test for isolation by distance by examining the relationship between $F_{ST}/(1-F_{ST})$ and ln distance between population pairs as discussed by Rousset (1997). The direct correlation between geographic distance between populations and genetic distance was examined using Mantel's test. An indirect estimate of historical levels of gene flow was calculated using the equation $Nm = [(1- G_{ST})/4G_{ST}]$ where $Nm$ is the number of migrants per generation (Wright 1931). A UPGMA (unweighted pair-group method based on arithmetic averages) phenogram based on genetic distances was generated using NTSYS (Rohlf 1992).

## Results

### *Genetic diversity*

All 16 loci resolved for *C. levisecta* were polymorphic ($P_s = 100\%$; Table 1). The number of alleles per locus ranged from two to six, with a mean of 2.94. Genetic diversity within the species was relatively high ($H_{es} = H_T = 0.285$).

Within populations, 10 of the 16 loci were polymorphic on average ($P_p = 65.7\%$, SD = 3.6; Table 1). The number of alleles per polymorphic locus within populations ranged from 2.17 (Ebey's Landing) to 2.75 (Trial Island), with a mean of 2.38. Mean expected heterozygosity within populations was 0.213 (SD = 0.016). Highest gene diversity ($H_{ep} = 0.283$) was found within the Trial Island population, followed by the Rocky Prairie population ($H_{ep} = 0.279$). Among populations, Fort Casey had the lowest genetic diversity as measured by the percentage polymorphic loci ($P = 46.7\%$), the mean number of alleles per locus ($A = 1.60$), the effective number of alleles per locus ($A_e = 1.22$) and expected heterozygosity ($H_{ep} = 0.131$).

Two loci (*Ugpp-1* and *Mdh-2)* could not be scored for all populations due to low activity. If these loci are excluded, Trial Island had the highest number of alleles (32), followed by the Rocky Prairie and Valley populations, each with 29 alleles (Table 2). The fewest alleles (23) were found in the West Beach population. The mean number of alleles per population was 26.5 (SD = 2.94), when *Ugpp-1* and *Mdh-2* were excluded from the analyses. Allelic richness based on rarefaction portrayed quite different results compared to the observed allelic richness (Table 2). Five populations (Rocky Prairie, Valley, Alpha Islet, West Beach and Long Island) were each estimated to have an allelic richness of 40 alleles. Fort Casey, with an estimated 22 alleles, had the lowest allelic richness. Six alleles were restricted to single populations. These alleles had a mean frequency of 0.175, and were found in Long Island (one allele), Valley (one), False Bay (one) and Rocky Prairie (three) populations.

Observed heterozygosity was equal to or higher than expected heterozygosity for every population (Table 1). Although 21 of 111 fixation indices tested were significantly different ($P < 0.05$) from zero, the overall $F_{IS}$ value ($= -0.071$) was relatively low. Of the 21 loci that differed significantly

*Table 2*. Population size estimates (1998–2002), allelic richness, genetic diversity and genetic identity

| Population | Mean size[a] | Allelic richness[b] | No. of Alleles[c] | $H_e$ | Mean genetic identity (SD) | Mean geographic distance (km) |
|---|---|---|---|---|---|---|
| Large populations (>1000) | | | | | | |
| Rocky Prairie | 5679 | 40 | 29 | 0.279 | 0.835 (0.029) | 159 |
| Ebey's Landing | 4353 | 29 | 26 | 0.146 | 0.928 (0.050) | 39 |
| Valley | 4021 | 40 | 29 | 0.243 | 0.934 (0.027) | 44 |
| Trial Island | 2150 | 33 | 32 | 0.283 | 0.890 (0.054) | 50 |
| Forbes Point | 1500 | 25 | 25 | 0.240 | 0.919 (0.042) | 41 |
| Mean | 3541 | 33.4 | 28.2 | 0.238 | 0.901 | 67 |
| SD | 1700 | 6.7 | 2.8 | 0.055 | 0.041 | 52 |
| Small Populations (<1000) | | | | | | |
| Alpha Islet | 877 | 40 | 25 | 0.232 | 0.868 (0.041) | 47 |
| West Beach | 381 | 40 | 23 | 0.174 | 0.906 (0.040) | 37 |
| False Bay | 269 | 31 | 26 | 0.231 | 0.942 (0.034) | 43 |
| Bocker's E.P. | 187 | 24 | 24 | 0.193 | 0.918 (0.051) | 39 |
| Fort Casey | 170 | 22 | 24 | 0.131 | 0.912 (0.056) | 41 |
| Long Island | 73 | 40 | 26 | 0.194 | 0.919 (0.030) | 40 |
| Mean | 326 | 32.8 | 24.7 | 0.193 | 0.911 | 41 |
| SD | 289 | 8.4 | 1.2 | 0.038 | 0.024 | 3 |

[a]Mean population size over 5 years; data was not available for all years for all populations.
[b]Rarefaction allelic richness for 14 loci (two loci that were not resolved for all populations were not included in the analysis) and 48 individuals per population.
[c]Observed number of alleles (two loci not resolved for all populations were excluded in the analysis).

from Hardy–Weinberg expectations, 13 had an excess of heterozygotes, and eight a deficit.

*Genetic divergence*

Pairwise genetic identities (Table 3) ranged from 0.771 (Rocky Prairie-Alpha Islet) to 0.992 (Ebey's Landing-Fort Casey). Mean genetic identity ($I$) was 0.907 (SD = 0.05). Allele frequencies were highly heterogeneous across populations ($P < 0.001$ for all loci except *Tpi-3*, which had minimal variation, Table 4). However, the percentage of total genetic variation found among populations ($G_{ST} = 0.189$) was moderate, and

*Table 3*. Nei's 1972 pairwise genetic identities (above diagonal) between *Castilleja levisecta* populations[1] and pairwise population $F_{ST}$ values (below diagonal)

| | ALPH | TRIAL | ROCK | FALSE | LONG | VALL | EBEY | BOCK | CASE | WEST | FORB |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ALPH | – | 0.853 | 0.771 | 0.910 | 0.910 | 0.899 | 0.873 | 0.875 | 0.855 | 0.849 | 0.889 |
| TRIAL | 0.280 | – | 0.821 | 0.965 | 0.904 | 0.945 | 0.863 | 0.850 | 0.841 | 0.886 | 0.969 |
| ROCK | 0.380 | 0.260 | – | 0.864 | 0.850 | 0.876 | 0.851 | 0.828 | 0.827 | 0.832 | 0.831 |
| FALS | 0.227 | 0.055 | 0.266 | – | 0.955 | 0.971 | 0.966 | 0.952 | 0.940 | 0.928 | 0.969 |
| LONG | 0.234 | 0.210 | 0.324 | 0.136 | – | 0.947 | 0.944 | 0.930 | 0.909 | 0.914 | 0.927 |
| VALL | 0.212 | 0.096 | 0.227 | 0.056 | 0.129 | – | 0.950 | 0.940 | 0.929 | 0.936 | 0.948 |
| EBEY | 0.260 | 0.223 | 0.352 | 0.098 | 0.199 | 0.142 | – | 0.973 | 0.992 | 0.954 | 0.918 |
| BOCK | 0.206 | 0.220 | 0.353 | 0.110 | 0.194 | 0.144 | 0.077 | – | 0.977 | 0.923 | 0.934 |
| CASE | 0.288 | 0.264 | 0.388 | 0.154 | 0.278 | 0.186 | 0.029 | 0.074 | – | 0.943 | 0.906 |
| WEST | 0.355 | 0.249 | 0.330 | 0.206 | 0.278 | 0.174 | 0.167 | 0.219 | 0.214 | – | 0.894 |
| FORB | 0.230 | 0.066 | 0.276 | 0.065 | 0.170 | 0.096 | 0.200 | 0.137 | 0.224 | 0.236 | – |

ALPHA = Alpha Islet, TRIAL = Trial Island, ROCK = Rocky Prairie, FALSE = False Bay, LONG = Long Island, VALL = Valley, EBEY = Ebey's Landing, BOCK = Bocker's E.P, CASE = Fort Casey, WEST = West Beach, FORB = Forbes Point.

indicated that about 81% of the variation was found within populations. If the less informative polymorphic loci (i.e., those with $H_T < 0.05$) are excluded from the analysis the $G_{ST}$ value increased slightly to 0.218. Estimates of gene flow based on these $G_{ST}$ values ranged from $Nm = 0.90$ to 1.07. Pairwise $F_{ST}$ values (Table 3) ranged from lows of 0.055 and 0.056 (for Trial Island/False Bay and Valley/False Bay, respectively) to highs of 0.388 and 0.380 (for Rocky Prairie with Alpha Islet and Fort Casey, respectively).

Estimated geographic distances between populations ranged from 2 to 176 km with a mean of 53 km (SD = 53; Table 2). The UPGMA phenogram (Figure 2) based on genetic distances indicated that the Rocky Prairie population was most distinct, followed by Alpha Islet. Ebey's Landing and Fort Casey were most similar and were clustered with Bocker's Environmental Preserve and West Beach. A second cluster linked the False Bay and Valley populations, followed by the Trial Island and Forbes Point populations, and then Long Island. A highly significant negative correlation ($r = -0.72$; $P < 0.0001$) was found between genetic identity and geographic distance. This was greatly influenced by the distant and diverse Rocky Prairie population. When Rocky Prairie was excluded from the analysis, the correlation between genetic and geographic distance fell to $r = -0.40$, but remained significant ($P < 0.006$). Similar results were found by analyzing the relationship between geographic distance and genetic divergence using Rousset's (1997) method of plotting $F_{ST}/(1 - F_{ST})$ vs ln distance between population pairs (Figure 3). A highly significant relationship ($P < 0.001$, $r = 0.60$) was found for all 11 populations. When Rocky Prairie was excluded the significance dropped to $P = 0.02$, with $r = 0.33$).

## Discussion

### *Genetic diversity*

*Castilleja levisecta* maintains unusually high genetic diversity compared to the mean genetic diversity found for species with similar geographic ranges (Table 5, Hamrick and Godt 1989). For example, the percentage of polymorphic loci and

*Table 4.* Number of alleles observed at each locus, and gene diversity statistics[a] (Nei 1973, 1977) for *Castilleja levisecta*

| Locus | Alleles | $H_T$ | $H_S$ | $G_{ST}$ |
|---|---|---|---|---|
| *Aat-1* | 4 | 0.425 | 0.260 | 0.389 |
| *Acon* | 5 | 0.599 | 0.474 | 0.208 |
| *Ce-1* | 6 | 0.692 | 0.547 | 0.210 |
| *Dia-1* | 3 | 0.379 | 0.302 | 0.204 |
| *Dia-2* | 2 | 0.257 | 0.235 | 0.088 |
| *Idh* | 2 | 0.286 | 0.258 | 0.097 |
| *Mdh-2* | 3 | 0.295 | 0.264 | 0.107 |
| *Mnr-1* | 3 | 0.006 | 0.006 | 0.046 |
| *Pgi-2* | 3 | 0.176 | 0.146 | 0.174 |
| *Pgm-2* | 2 | 0.068 | 0.043 | 0.367 |
| *6Pgdh-1* | 2 | 0.177 | 0.122 | 0.308 |
| *Skdh* | 3 | 0.385 | 0.343 | 0.109 |
| *Tpi-1* | 2 | 0.034 | 0.029 | 0.135 |
| *Tpi-2* | 2 | 0.210 | 0.173 | 0.175 |
| *Tpi-3* | 2 | 0.006 | 0.006 | 0.014 |
| *Ugpp-1* | 4 | 0.564 | 0.340 | 0.398 |
| Mean | 2.9 | 0.285 | 0.222 | 0.189 |
| SD | 1.2 | 0.212 | 0.161 | 0.120 |

[a] $H_T$ is total genetic variation, $H_S$ is the variation found within populations, $G_{ST}$ is the proportion of total variation found among populations and SD is the standard deviation. All $G_{ST}$ values are significantly different from zero ($P < 0.001$) except *Tpi-3*.

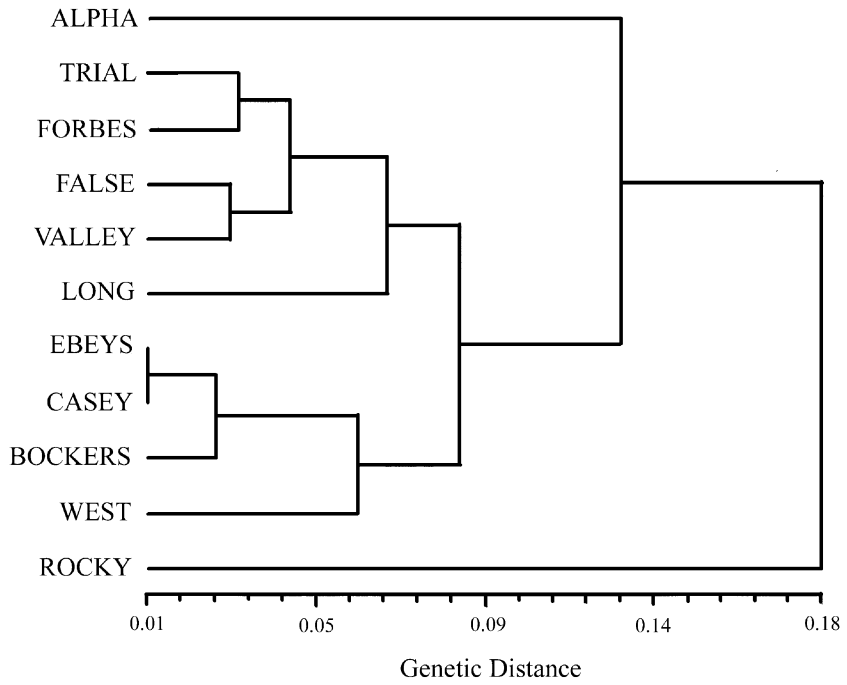


*Figure 2*. UPGMA phenogram of the eleven *Castilleja levisecta* populations based on Nei's (1972) genetic distance. Alpha = Alpha Islet, Trial = Trial Island, Forbes = Forbes Point, False = False Bay, Valley = Valley, Long = Long Island, Ebeys = Ebey's Landing, Casey = Fort Casey, Bockers = Bocker's Environmental Preserve, West = West Beach, Rocky = Rocky Prairie.

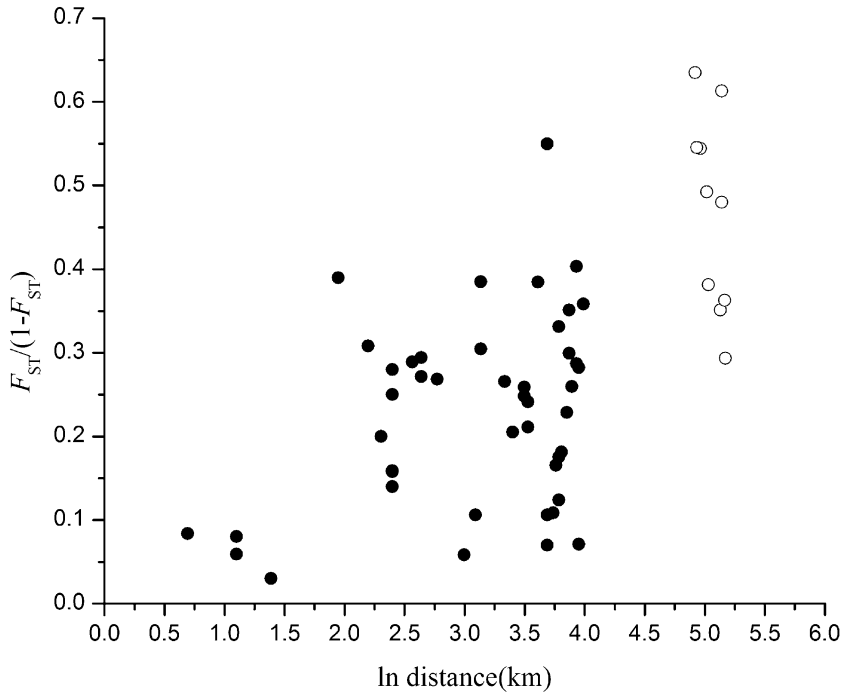


*Figure 3*. The relationship between pairwise population $F_{ST}$ /(1 - $F_{ST}$) values and ln distance (km). The linear regression (y = −0.005 + 0.081x) was highly significant ($P < 0.001$, $r = 0.60$), but greatly influenced by the distant Rocky Prairie population (open dots; see text).

gene diversity are more than twice the means found for endemics, both for the species and within populations. For *C. levisecta*, $P_s = 100\%$, $H_{es} = 0.285$, $P_p = 65.7\%$ and $H_{ep} = 0.213$. Means for 100 endemics (Hamrick and Godt 1989) are $P_s = 40.0\%$, $H_{es} = 0.096$, $P_p = 26.3\%$ and $H_{ep} = 0.063$. Although species with small geographic ranges tend to maintain less genetic diversity than geographically widespread species (Hamrick and Godt 1989) exceptions are not uncommon (Gitzendanner and Soltis 2000; Lopez-Pujol et al. 2002; Wang et al. 2004). A species very similar to *C. levisecta* in this respect is the federally endangered perennial aster *Liatris helleri* (Porter) Porter, or Heller's blazing star. *Liatris helleri* is restricted to seven granite-outcrop populations in the Blue Ridge Mountains of North Carolina. All populations are within a 30 km radius and most have declined in size (US Fish & Wildlife Service 1989). However, despite its small range and the few extant populations, *L. helleri* has high genetic diversity (e.g., $P_s = 88\%$, $H_{es} = 0.276$, $AP_s = 3.00$, Godt and Hamrick 1996).

Genetic diversity may be maintained in populations of threatened perennial plants for quite some time, particularly if population sizes remain large. Factors that may contribute to the maintenance of high variation in *C. levisecta* include a number of large populations (five number over 1000 plants), the persistence of multiple generations within populations, perenniality, and (potentially) a seed bank. *Castilleja levisecta* may also have acquired genetic diversity through introgression from one or two co-occurring *Castilleja* species (Gamon 1995; F. Caplow, pers. obs.). Our data, however, provide no evidence of recent hybridization.

The phylogenetic history of species (as well as their recent history) influences their genetic diversity. Some species originate having captured a small fraction of the diversity found within their presumed progenitor species (Loveless and Hamrick 1988; Pleasants and Wendel 1989). At the other extreme, some taxa originate as polyploids, having considerably more genetic diversity than their progenitors (Soltis et al. 1995; Soltis and Soltis 2000; Hardy and Vekemans 2001). Because speciation events are rarely documented (but see Gottlieb 1973; Soltis et al. 1995) the role of phylogeny in determining present-day genetic diversity is often difficult to ascertain. For rare species, genetic diversity comparisons with their more widespread congeners may provide the most useful insights into their genetic diversity (Karron 1987) because levels of genetic diversity between rare and widespread species are highly correlated (Gitzendanner and Soltis 2000). For example, low genetic diversity in a widespread species may provide an evolutionary explanation for low variation in its rare congener. Unfortunately, for most rare species congeneric genetic data is unavailable. To our knowledge, no other *Castilleja* species have been analyzed electrophoretically. Thus, our ability to interpret allozyme diversity in *C. levisecta* is somewhat limited by the lack of studies on more widespread *Castilleja*.

To provide an evolutionary context for *C. levisecta*'s genetic diversity we summarized results from 30 published studies within the Scrophulariaceae (Table 5). This summary encompasses eight genera and 27 species (and includes both annuals and perennials). Relative to the mean values for these 27 Scrophulariaceae species, *C. levisecta* maintains relatively high allozyme diversity (Table 5). Aside from *C. levisecta*, at least one other federally endangered member of the Scrophulariaceae (*Schwalbea americana* L.) has been genetically analyzed. In contrast to *C. levisecta*, *S. americana* (a hemiparasitic, fire-adapted perennial found nearly

*Table 5.* Comparisons of genetic diversity within *Castilleja levisecta*, endemic plants and plants in the Scrophulariaceae

| Species/group | $P_s$ | $A_s$ | $A_e$ | $H_{es}$ | $P_p$ | $A_p$ | $A_e$ | $H_{ep}$ |
|---|---|---|---|---|---|---|---|---|
| Endemic plants[a] | 40.0 | 1.80 | 1.15 | 0.096 | 26.3 | 1.39 | 1.09 | 0.063 |
| Scrophulariaceae[b] | 36.7 | 1.61 | 1.17 | 0.124 | 24.4 | 1.24 | 1.12 | 0.091 |
| *Castilleja levisecta* | 100.0 | 2.94 | 1.56 | 0.285 | 65.7 | 1.91 | 1.39 | 0.213 |

[a] $N = 52$ to 100; Hamrick and Godt (1989).
[b] Based on 30 literature reports; references available upon request.

exclusively in the southeastern US) has low genetic variation (e.g., $H_{es} = 0.006$) throughout its range (Godt and Hamrick 1998).

Population genetic theory predicts that larger populations will maintain higher allelic diversity (Hedrick 1985; Ellstrand and Elam 1993). For *C. levisecta*, highest observed allelic diversity was found for three of the larger populations, Rocky Prairie, Trial Island and Valley (Tables 1 and 2). These populations also had the highest genetic diversity ($H_e$). In 1980, Rocky Prairie was considered the ''best population'' within Washington in terms of overall site condition and population size (Washington Natural Heritage Program records, 2002).

Current population sizes (or short-term averages) may not always be reliable indicators of genetic diversity, however. Ebey's Landing, for example, numbers over 7000 plants, and is presently the largest *C. levisecta* population (Washington Natural Heritage data, 2002). However, it has the second lowest genetic diversity ($H_e = 0.146$), the second lowest effective number of alleles per locus ($A_e = 1.23$), and the fewest alleles per polymorphic loci ($AP = 2.17$). Low genetic diversity within this population may be related to founder effects or population bottlenecks. It is notable that this population was over an order of magnitude smaller in 1995 (300–400 plants, Washington Natural Heritage Program data, 2002).

Recently, the technique of rarefaction has been employed to predict the allelic richness of populations (El Mousadik and Petit 1996; Petit et al. 1998; Kalinowski 2004a). This technique, originally employed in ecology to estimate species richness (Hurlbert 1971), is designed to estimate allelic richness of populations when sample sizes differ. Larger samples should contain more alleles than smaller samples. Six of our samples were equivalent in size (48 individuals), while the other five consisted of 31(Long Island), 39 (Alpha Islet), 45 (Rocky Prairie and West Beach) and 47 (Valley) individuals. Based on rarefaction equations, Rocky Prairie, Valley, Alpha Islet, West Beach and Long Island populations had the highest, and equivalent, allelic richness (40 of 41 possible alleles). Fort Casey, which had one of the lower numbers of observed alleles, had the lowest allelic richness (22) based on rarefaction. Theoretical values of allelic richness differed considerably from observed values and changed the relative allelic richness of populations. West Beach, for example, had the lowest observed allelic richness but increased to the highest with rarefaction. These results were somewhat surprising given the overall similarity of sample sizes; we are inclined to give more credence to the observed values.

To examine the relationship between population size and genetic diversity, we arbitrarily grouped the 11 *C. levisecta* populations into two size classes (< or >1000 plants), based on population size estimates from 1998 to 2002. On average, the larger populations maintained more alleles (as observed) and had higher mean genetic diversity (Table 2), consistent with population genetic predictions. Differences between size classes diminished when rarefaction was employed to estimate allelic richness. Positive associations between population size and allozyme diversity have been documented for some plant species (e.g., Van Treuren et al. 1991; Godt et al. 1996; Paschke et al. 2002). However, since within-population genetic diversity is influenced by historical factors (e.g., founder effects, bottlenecks, extended time periods with low numbers of individuals and low gene flow rates), present-day population sizes may not be a reliable indication of genetic diversity. Long-term demographic records (and knowledge of gene flow rates) are necessary to infer causes of genetic diversity differences among populations.

Twenty-one significant deviations from Hardy–Weinberg were found among ten of the 16 polymorphic loci; these occurred among nine populations in *C. levisecta*. However, the mean $F_{IS}$ value (0.071) was negative and low, indicating that inbreeding is not a major factor in *C. levisecta* populations. These results are consistent with its self-incompatible breeding system (Kaye and Lawrence 2003). The loss of self-incompatibility (SI) alleles is of conservation concern for rare species, since fewer compatible mates are available as the number of SI alleles decrease in small populations (Byers and Meagher 1992). The loss of SI alleles may lead to the extinction of populations and the species (DeMauroqz 1993; Messmore and Knox 1997; Boscaiu and Guemes 2001). It is notable that seed germination rates (a measure of parental fitness) varied considerably (28% to >80%) among *C. levisecta* populations in one study (Kaye 2001), although germination rates did not appear to be correlated with population size.

*Genetic divergence*

A strong negative correlation ($r = -0.72$) was found between genetic identity and geographic distance, suggesting that geographically distant populations exchanged fewer genes (an isolation by distance effect). Although this correlation was strongly influenced by the distant Rocky Prairie population (mean interpopulation distance = 159 km), a significant correlation ($r = -0.40$) persisted when this population was eliminated from the analysis, suggesting that these populations have been at least partially isolated, despite their relatively local distribution. Isolation by distance was corroborated by the analysis suggested by Rousset (1997) which examines the correlation between pairwise values of $F_{ST}/(1-F_{ST})$ and ln distance. The strong association found between genetic identity and geographic distance is somewhat unusual for an outcrossing species, particularly one with a very small geographic range. High correlations between genetic distance and geographic distance have been found, however, for species with "island-like" distributions. Examples include the highly outcrossed *Liatris helleri* (Asteraceae), ($r = 0.55$, Godt & Hamrick 1996), the rock outcrop endemic *Tradescantia hirsuticaulis* Small (Commelinaceae, $r = 0.68$, Godt and Hamrick 1993), and the Australian rock outcrop tree *Eucalyptus caesia* Benth. (Myrtaceae, $r = 0.61$, Moran and Hopper 1983).

Geographically the *C. levisecta* populations may be grouped into three clusters, with Alpha Islet, Trial Island, Valley, False Bay and Long Island forming a northerly cluster, West Beach, Forbes Point, Ebey's Landing, Bocker's Environmental Preserve and Fort Casey forming a southern cluster, and the most isolated population, Rocky Prairie, comprising the third group (Figure 1). Of the total genetic diversity found 10.3% resides among these three clusters. Hence, about half (53%) of the total genetic divergence among populations can be attributed to differences among the three geographic clusters, while the remainder (47%) represents genetic divergence among populations within clusters. In the UPGMA phenogram (Figure 2) four of the five populations within the southern cluster (Ebey's Landing, Fort Casey, Bocker's Environmental Preserve and West Beach) grouped together. Of the northern grouping, Trial Island, False Bay, Valley and Long Island populations grouped together. Anomalies included grouping of Forbes Point population with the more northerly group, despite its geographic association with the southern group. An additional anomaly was the genetic divergence of the Alpha Islet population from nearby sites. Rocky Prairie was most distinct from the other populations, consistent with its geographical isolation.

Indirect estimates of gene flow [$Nm = 0.90$ to 1.07, based on $G_{ST}$ values of 0.189 (for all loci) and 0.218 (for loci with high genetic diversity)] suggest that gene flow within *C. levisecta* is relatively limited. However, it should be noted that indirect gene flow estimates are subject to error, since they are based on theoretical models whose assumptions are often not met by real populations (Whitlock and McCauley 1999). Furthermore, gene flow estimates based on genetic structure are historical estimates of gene flow, and they may not be indicative of current migration rates. However, for rare species, this is of more concern when gene flow estimates are high since they may reflect previous intermingling of populations of perennial plants and they should not be interpreted as indicating the present state of population isolation. On the other hand, low historical gene flow estimates are worrisome, because as rare plant populations decline or become extinct it is likely that gene flow will be reduced further and that populations will become increasingly isolated. Population genetic theory (Wright 1931) suggests that genetic drift may play a major role in populations with migration rates of $Nm < 1.0$. Pollen flow in *C. levisecta* may be affected by the reluctance of pollinators to fly between island populations, as well as the geographic isolation of the Rocky Prairie population. Because seeds have no specialized dispersal mechanisms, seed gene flow between populations on different islands seems unlikely.

Mean genetic identity among population pairs ($I = 0.91$) was lower than means ($I = 0.95$) reported for 22 species by Gottlieb (1977) and for 32 species by Crawford (1983). Furthermore, genetic identity values displayed a fairly large range among populations ($I = 0.77$ to 0.99), again indicating that gene flow between some populations has been limited. Twenty-two of the 55 pairwise identity values were $< 0.90$.

Although statistically significant allele frequency differences were found among populations for all loci, a moderate proportion (19–22%) of the total

genetic variation was distributed among populations. This is slightly less than mean $G_{ST}$ values found for 52 endemic plants (25%), but similar to means found for 60 animal-pollinated, mixed-mating species (22%) and for 124 animal-pollinated, outcrossing species (20%, Hamrick and Godt 1989).

Both ecological and genetic factors affect the persistence of endangered and threatened plant species. Decreasing numbers of plants and populations are predicted to lead to genetic diversity losses and increased inbreeding within populations, each of which can have detrimental effects on populations (Ellstrand and Elam 1993). Long-term population viability is likely to be affected by these genetic factors, even if habitat is maintained (Barrett and Kohn 1991). Although many *C. levisecta* populations have been extirpated, the persistence of several large populations has undoubtedly contributed to the maintenance of unusually high genetic diversity within this endemic species. Reduced gene flow may be expected, given that many populations have been extirpated, and most of the remaining ones occur on islands. Hence, maintenance of current population sizes is critically important to prevent the loss of genetic diversity within the species. Little evidence of inbreeding was detected in this study, consistent with the species' apparent self-incompatible breeding system (Kaye and Lawrence 2003).

The Federal Recovery Plan for *C. levisecta* (US Fish & Wildlife Service 2000) mandates the existence of at least 20 stable populations, 15 of which must reside on protected sites before the species is delisted. Populations are to be deemed "stable" if they have a 5-year average population size of 1000 plants (US Fish & Wildlife Service 2000). Population restorations (introductions into suitable sites and re-introductions into historic sites) are currently planned (Caplow 2001). These should include sufficient propagules to deter genetic drift in the re-established populations (Krauss et al. 2002). The geographic source of propagules should also be considered since a strong association was found between genetic distance and geographic distance, indicating an isolation by distance effect. Since six extant populations are considered small ($<$1000 individuals) augmentation is also under consideration (Caplow 2001). Given the relatively high genetic diversity within all populations and the genetic distance between them, population admixtures are not recommended for the proposed augmentation. Detailed restoration and management strategies based on *C. levisecta*'s genetic diversity and structure are given in Guerrant (2003).

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