The National Forest
Genetic Electrophoresis
Laboratory

NFGEL
USDA FOREST SERVICE
2375 Fruitridge Road
Camino, CA 95709

Phone 530-642-5067
Fax 530-642-5093
email: vhipkins@fs.fed.us

Final Report

Evaluation of Genetic Diversity in Tahoe yellow cress (*Rorippa subumbellata*)

Report prepared by Robert C. Saich and Valerie D. Hipkins
August 29, 2000

INTRODUCTION

Rorippa subumbellata (Roll.), Tahoe yellow cress, is endemic to sandy beaches on the shores of Lake Tahoe in California (El Dorado and Placer counties) and Nevada (Carson City Rural Area, Washoe and Douglas counties). The plants are found where the beach is wide enough to offer a back beach area, out of wave action and behind the highest debris deposit line (Ferreira, 1987). The distribution around the lake edge is patchy, with most occurrences found on the west and south shores in California, where the greatest expanse of beaches occur (CSLC, 1998). *R. subumbellata* is a rare plant restricted by both geography and habitat requirements, which periodically undergoes significant fluctuations in number of individuals and sites as a result of naturally and artificially induced phenomena. This species is a Federal candidate for listing under the Endangered Species Act of 1973.

According to a CSLC (California State Lands Commission) Biological Assessment (1998), the number of *R. subumbellata* occurrences in any particular year is strongly related to lake level fluctuations. During periods of low water, additional habitat for *R. subumbellata* is exposed and becomes available for colonization, such as occurred during the 1992-1993 season. When the lake elevation is high, much of the habitat for *R. subumbellata* is inundated, and therefore unavailable for plant colonization. While high lake levels may cause mortality in some *R. subumbellata* sites and pose an immediate threat to existing individuals, it may benefit the species in the long term by removing other plant species and opening new habitat when lake levels drop. Unfortunately under current conditions, dam operations alter the historical seasonal fluctuation of the lake, maintaining higher water elevations during the spring and summer, the growing season for *R. subumbellata*. Substrate disturbance, construction, other development, and recreation are the primary human caused disturbances which have been documented impacting *R. subumbellata* and its habitat.

R. subumbellata is a perennial plant which is capable of re-sprouting each season from dormant rootstalks, though it is unknown if rootstalks can survive being inundated for long periods of time. Little detailed information concerning the reproductive biology of *R. subumbellata* is available. Pollinators have not been identified or recorded. The dominant mechanism of site colonization, whether by seed, re-sprouting, or the deposition of vegetative plant material by water processes, has not been determined (CSLC 1998). The longevity and germinative capabilities of the seed of *R. subumbellata* are unknown. Seeds are small (< 1mm) and probably drop down, establishing in close proximity to the plant that shed them. Seed could be dispersed by the wind and wave action of the lake. The CSLC Biological Assessment explains that beaches at the mouths of streams are completely reformed during periods of high spring runoff, such as occurred in 1982, 1983, 1986, and 1997. During such beach forming events, aerial stems and rootstocks of *R. subumbellata* are removed. This material may be deposited around the lake, providing a mechanism for *R. subumbellata* to distribute propagules to other lakeshore locations. At this time there are not data to either support or refute this idea.

The purpose of this investigation is to determine the genetic characteristics of *R. subumbellata*. This study provides information on genetic variability among *R. subumbellata* sites, which will be used to assess long-term population viability under existing Lake Tahoe management scenarios. In accordance with the Scope of Work for Interagency Agreement FWS 14-48-0001-95813, the National Forest Genetic Electrophoresis Laboratory (NFGEL) evaluated

the genetic diversity in *R. subumbellata*. The starch gel electrophoresis method was used to assess isozyme markers within and among *R. subumbellata* populations.

METHODS

Sample collection: In 1999, water levels receded to sufficient levels to allow for the collection of 140 individuals of *R. subumbellata* from 11 populations (Table 1, Map). All individuals of the small populations (<30 individuals) were sampled. The largest population, composed of approximately 80 individuals located at Upper Truckee-East, was sampled by collecting individuals from a distance of at least three to five feet between plants and sampling throughout its patchy distribution. Collections occurred on August 15, 1999 and September 1, 1999. Between one to two stems per plant were removed per individual and placed in zip-lock bags. Bags were kept cool in ice chests and transported to NFGEL in Camino, CA.

Sample preparation: Samples were prepared using standard NFGEL procedures (Anonymous 1995). An approximately 1cm long section of stem per individual (40 mm^2), was clipped from a stem tip and submerged in 100 microliters of a Tris buffer pH. 7.5 (Gottlieb 1981). Tissue was frozen at –70C until electrophoresis. Two backup preparations of each individual were prepared and frozen. All sample tissue was green, healthy, and disease-free. There was ample tissue available for preparing samples for analysis. Tissue was prepared on 8/18/99 and 9/3/99.

Electrophoresis: Methods of electrophoresis followed the general methodology of Conkle et al. (1982) except that most enzyme stains are somewhat modified (Anonymous 1995). A lithium borate electrode buffer (pH 8.3) was used with a tris citrate gel buffer (pH 8.3) (Conkle et al. 1982) to resolve the enzymes fluorescent esterase (FEST), leucine aminopeptidase (LAP), phosphoglucose isomerase (PGI), aconitase (ACO), phosphoglucomutase (PGM), and malic enzyme (ME). A sodium borate electrode buffer (pH 8.0) was used with a tris citrate gel buffer (pH 8.8) (Conkle et al. 1982) to resolve uridine diphosphoglucose pyrophosphorylase (UGPP), glutamate-oxaloacetate transaminase (GOT), triosephosphate isomerase (TPI), glycerate-2-dehydrogenase (GLYDH), and catalase (CAT). A morpholine citrate electrode and gel buffer (pH 6.1) (Conkle et al. 1982) was used to resolve malate dehydrogenase (MDH), diaphorase (DIA), isocitrate dehydrogenase (IDH), phosphogluconate dehydrogenasee (6PGD), and shikimic acid dehydrogenase (SKD). All enzymes were resolved on 11% starch gels. Enzyme stain recipes follow Anonymous (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For quality control, 7% of the individuals were run and scored twice.

Data Analysis: The genus *Rorippa* has a base number of n=8 as determined from chromosome counts of five species. Four of the species are diploid, with two of these species containing tetraploid populations. The fifth species is reported to be hexaploid (Darlington and Wylie 1955). There is no record of a chromosome count for *R. subumbullata*. There are also no known isozyme studies concerning *R. subumbullata*, therefore, genetic interpretations were inferred directly from isozyme phenotypes under the assumption that *R. subumbullata* is diploid.

Resulting genetic data was analyzed using Popgene version 1.31 (Yeh et al. 1999) and Biosys-1 version 1.7 (Swofford and Selander 1989). A locus was considered polymorphic if an alternate allele occurred once. Hierarchical structure was given to single populations and

multiple populations. Statistics calculated included allele frequency, Shannon-Weaver Diversity Index (Shannon and Weaver 1949), unbiased genetic distances (Nei 1978), observed heterozygosity, expected heterozygosity (Nei 1978), F-statistics (Wier 1990), and effective allele number (Kimura and Crow 1964). A dendrogram was generated using Biosys-1 version 1.7 (Swofford and Selander 1989) using UPGMA and Nei's unbiased genetic similarity.

RESULTS

Eight of the eleven *Rorippa subumbullata* populations sampled were monomorphic for 23 enzyme loci (Table 2). Two of the populations have UGPP-1 locus variation, Taylor Creek and Upper Truckee-East, with Taylor Creek also exhibiting variation at the PGI-1 locus (Table 2). Tahoe Meadows showed variation at the DIA-1 locus only (Table 2). Among all the populations sampled, *R. subumbullata* has low enzyme variability (Table 3). The percentage of polymorphic loci at the species level was 13%, while at the population level was 1.6%. Within population diversity as measured by expected heterozygosity equaled 0.000 for all populations except Taylor Creek (0.0229, S.E=0.0790), Upper Truckee-East (.0050, S.E.=0.0241) and Tahoe Meadows (.0250, S.E.=0.1118) (Table 3). Tahoe Meadows was found to be the most diverse population via its unique variation in DIA-1. Fst (the proportion of the total variation measured found among populations within the taxon) equaled 0.2175. The dendrogram generated by Biosys shows that the most diverse population is Tahoe Meadows, followed by Taylor Creek and Upper Truckee-East (Figure 1).

DISCUSSION

Genetic Variation

Isozyme variation in *Rorippa subumbellata* was extremely limited. Eight of the eleven populations studied were completely monomorphic at the 23 loci assayed (Table 2). Therefore, no heterozygosity was observed, and all within population diversity values were equal to zero (Table 3). These data suggest that these eight populations consist of the same clone. The remaining three populations, Tahoe Meadows, Taylor Creek, and Upper Truckee–East, were slightly variable, and were also monomorphic for many of the same loci as the other eight populations (Table 2). The Upper Truckee–East population contained a small amount of variation at the UGPP-1 locus (two out of the 33 individuals were monomorphic for a second allele). Three of the eight individuals in the Tahoe Meadows population contained variation (they were monomorphic for a second allele) at the DIA-1 locus. The Taylor Creek population showed variation at two loci. Two out of the ten inviduals in the population were heterozygous at the PGI-1 locus, and also were monomorphic for an alternate allele at the UGPP-1 locus. The only heterozygosity observed in the study was in the Taylor Creek population at the PGI-1 locus. All other populations contained no heterozygosity.

R. subumbellata has much less isozyme variation than that observed in the average endemic taxon (Hamrick and Gott 1990). The average endemic species has 40% polymorphic loci, 1.80 alleles per locus, and 1.15 effective alleles per locus. Comparatively, *R. subumbellata* has 13.0% polymorphic loci, 1.13 alleles per locus, and 1.00 effective alleles per locus.

However, other species with similar characteristics as *R. subumbellata* (restricted range and/or moist habitat), have also been found to be nearly to completely monomorphic. These species include *Sisyrinchium sarmentosum* (Wilson et al. 2000), *Iris lacustris* (Simonich and Morgan 1994), *Howellia aquatilis* (Lesica et al. 1988), *Limnanthes macounii* (Kesseli and Jain 1984), and *Lespedeza leptostachya* (Cole and Biesboer 1992).

Of the total variation measured by isozymes, 21.75% (Fst = 0.2175) is found among populations, indicating that, as a whole, populations are moderately differentiated. This slightly high among-population variation value is the result of three of the populations containing unique variation (Table 2). The Tahoe Meadows population contains an alternate DIA-1 allele at moderate frequency that exists in no other population sampled. The Taylor Creek population contains a unique low frequency alternate allele at the PGI-1 locus. Taylor Creek and Upper Truckee-East both contain an allele at the UGPP-1 locus that exists in no other population. This shared allele occurs at a frequency of 20% in Taylor Creek and 6% in Upper Truckee-East. Among the other eight populations that contain exactly the same isozymes, the among-population variation is, of course, 0% (they are monomorphic for all the same isozymes measured). Even though there are some genetic differences among a few of the populations, gene flow appears to be quite low. The presence of alleles unique to populations (allele 2 in DIA-1; allele 2 in PGI-1; allele 2 in UGPP-1) indicates that gene flow is limited, otherwise more populations would share these alleles.

All the populations share above 99% genetic similarity (Table 4, Figure 1). The Tahoe Meadows population is the most dissimilar overall, followed by the Taylor Creek population. The Upper Truckee–East population, even though it does contain variation (a rare allele shared with Taylor Creek), is more similar to the monomorphic group of populations than to either Taylor Creek or Tahoe Meadows. Taylor Creek and Tahoe Meadows are the most genetically dissimilar pair of populations sampled (Table 4).

Gene Conservation

One goal of conservation biology is to preserve overall levels of genetic diversity. Isozyme electrophoresis is often used to estimate diversity in natural populations. Common measures of diversity include the percent of all loci that are polymorphic (P), the average number of alleles per locus (A), the effective number of alleles per locus (Ae), the observed frequency of heterozygotes (H_o), and the frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions (H_e). It is common to find low genetic diversity in populations of many rare or threatened species (Hamrick et al. 1991). However, individual endemic species vary widely in their genetic diversity (Gitzendanner and Soltis 2000). Although it is sometimes thought that low genetic diversity puts a species future at risk, a strong causal relationship between diversity and population viability has not been shown. Diversity is often reduced in rare species, likely because of bottlenecks associated with constrictions on population size (Falk and Holsinger 1991). It is important to point out that species that show low amounts of isozyme variation due to bottlenecks, may still contain ample variation at other gene loci, especially those loci involved in quantitative, adaptive traits. Also, if populations expand rapidly after experiencing a bottleneck, they can generate sufficient amounts of genetic variation to ensure future adaptive potential (Barrett and Kohn 1991).

There are several possibilities why the *R. subumbellata* populations are so invariant. First, low population sizes for several generations would reduce variation, regardless of whether

the populations were initially variable (a genetic bottleneck). Second, the species could be reproducing largely vegetatively. Although vegetatively reproducing taxa are often variable (Ellstrand and Roose 1987), low variation could characterize an entirely vegetatively reproducing species. Third, low variation can result from many generations of selfing, or mating within the same clone (geitonogamous pollination). Selfing would produce a preponderance of homozygous individuals, which is observed in the *Rorippa* populations. Because the reproductive biology of *R. subumbellata* is unknown, it is difficult to distinguish between these possible causes of the observed lack of variation. The present level and structure of diversity is probably a result of a combination of all the above. When water levels were lower, *Rorippa* probably formed much larger and more extensive populations. At that time, the entire south shore may have been one slightly subdivided population. It is even possible that the plants around the entire lake comprised a single population.

CONCLUSIONS

The observed lack of genetic variation (as measured with isozymes) suggests that few restrictions need be placed on programs to increase the *R. subumbellata* populations through transplants. Moving plants (seeds or rhizomes) among populations may still be undesirable because it might lead to loss of rare genetic variations now restricted to single populations. However, as long as gene flow is as limited as it appears, establishment of new populations at sites not now occupied by *R. subumbellata* need be restrained only by the pragmatic test that populations can be established only where the plants can grow.

This study suggests that preserving enzyme (and genetic) diversity in *R. subumbellata* requires preserving many populations because some, like Taylor Creek, Tahoe Meadows, and Upper Truckee-East, contain unique alleles. Also, large populations should be maintained at these areas, the only populations known to be variable. However, this study also suggests that management practices directed toward demographics, rather than genetics, can be effective in long-term preservation of *R. subumbellata* biodiversity. Demographic changes can be as good, or better indicators of the biological status of rare species than information about the level and structure of genetic variation (Schemske et al. 1994). This is especially true in a species such as *R. subumbellata* that has little genetic variability.

REFERENCES

Anonymous. 1995. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, USDA Forest Service, Camino, California.

Barrett, S. C. H., and J. R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. Pages 3-30 *In* Falk, D. A., and K. E. Holsinger (editors) Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, England.

CSLC (California State Lands Commission). 1998. Tahoe Yellow Cress Draft Biological Assessment (Administrative Draft), Sacramento CA 95825-8202.

Cole, C. T., and D. D. Biesboer. 1992. Monomorphism, reduced gene flow, and cleistogamy in rare and common species of *Lespedeza* (Fabaceae). American Journal of Botany 79:567-575.

Conkle, M. T., P. D. Hodgskiss, L. B. Nunnally, and S. C. Hunter. 1982. Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual. Gen. Tech. Rep. PSW-64. Berkeley, CA: Pacific Southwest Forest and Range Experiment Station, Forest Service, U.S. Department of Agriculture. 18pp.

Crawford, D. J. 1990. Plant Molecular Systematics: Macromolecular Approaches. John Wiley & Sons, New York, New York.

Darlington, C. D., and A. P. Wylie. 1955. Chromosome Atlas of Flowering Plants. University Press, Aberdeen, Great Britain. 519 pp.

Ellstrand, N. C., and M. L. Roose. 1987. Patterns of genotypic diversity in clonal plant species. American Journal of Botany 74:123-131.

Falk, D. A., and K. E. Holsinger (editors). 1991. Genetics and Conservation of Rare Plants. Oxford University Press, Oxford, England.

Ferreira, J. E. 1987. The population status and phenological characteristics of *Rorippa subumbellata* (Roll.) at Lake Tahoe, California and Nevada. M.A. Thesis, California State University, Sacramento, CA.

Gitzendanner, M. A., and P. S. Soltis. 2000. Patterns of genetic variation in rare and widespread plant congeners. American Journal of Botany 87:783-792.

Gottlieb, L. D. 1981. Gene number in species of *Asteraceae* that have different chromosome numbers. Proceedings of the National Academy of Science, USA 78:3726-3729.

Hamrick, J. L., and M. J. W. Godt. 1990. Allozyme diversity in plant species. Pages 43-63 *In* Brown, A. H. D., M. T. Clegg, A. L. Kahler, and B. S. Weir (editors) Plant Population Genetics, Breeding, and Genetic Resources. Sinauer Associates, Inc., Sunderland, Maryland.

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

Kesseli, R. V., and S. K. Jain. 1984. New variation and biosystematic patterns detected by allozyme and morphological comparisons in *Limnanthes* sect. Reflexae (Limnanthaceae). Plant Syst. Evol. 147:133-165.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725-738.

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

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

Schemske, D. W., B. C. Husband, M. H. Ruckelshaus, C. Goodwillie, I. M. Parker, and J. G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584-606.

Shannon, C. E., and W. Weaver. 1949. The Mathematical Theory of Communication. Univ. of Illinois Press, Urbana, Illinois.

Simonich, M. T., and M. D. Morgan. 1994. Allozymic uniformity in *Iris lacustris* (dwarf lake iris) in Wisconsin. Canadian Journal of Botany 72:1720-1722.

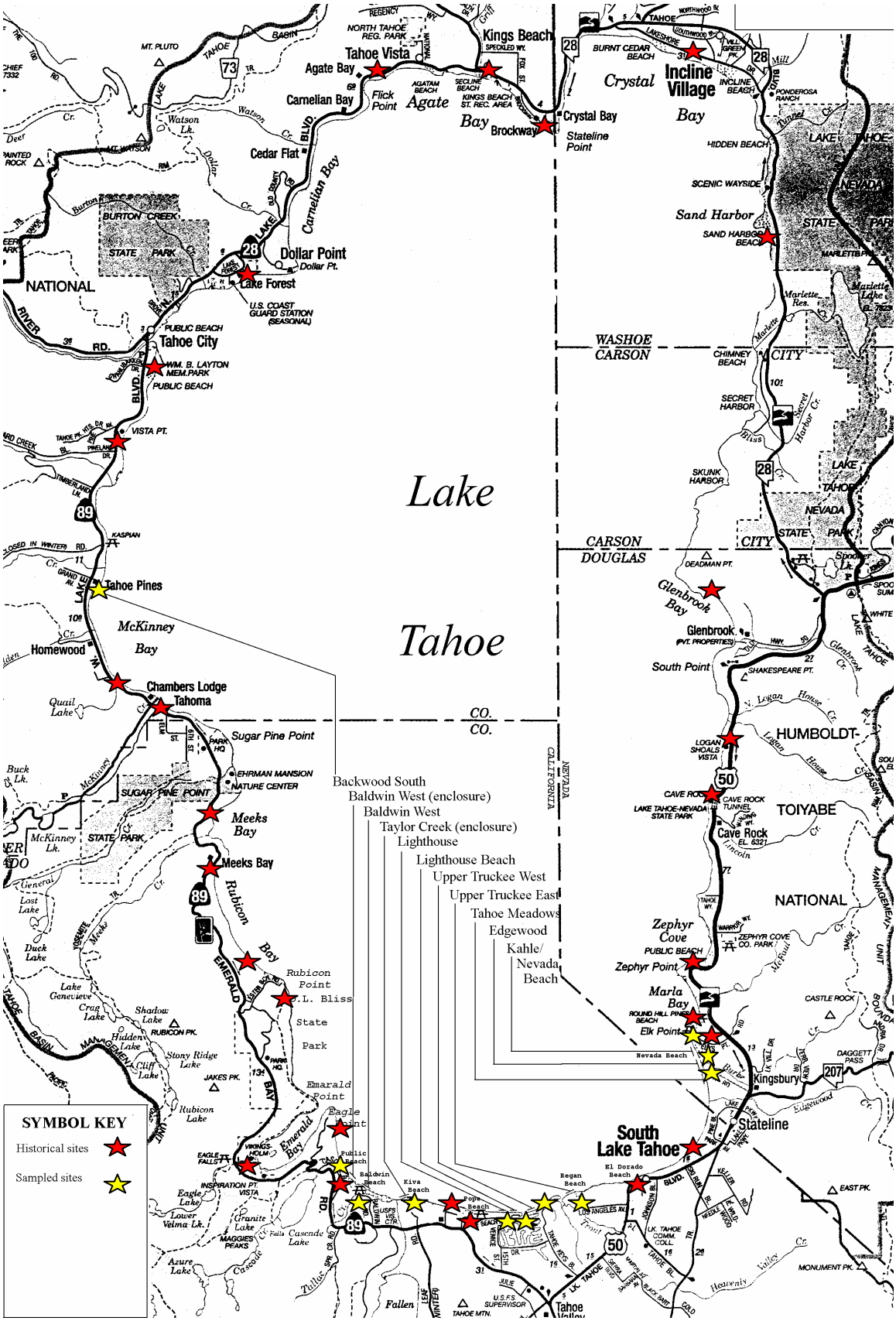
Swofford, D. L., and R. B. Selander. 1989. BIOSYS-1, a computer program for the analysis of allelic variation in population genetics and biochemical systematics, release 1.7 edition. Illinois Natural History Survey, Champaign, Ill.

Weir, B. S. 1990. Genetic data analysis. Sunderland, Massachusetts, Sinauer.

Wendel, J. F., and N. F. Weeden. 1989. Visualization and interpretation of plant isozymes. Pages 5-45 *In* Soltis, D. E., and P. S. Soltis (editors) Isozymes in Plant Biology. Dioscorides Press, Portland, Oregon.

Wilson, B. L., D. L. Doede, and V. D. Hipkins. 2000. Isozyme variation in *Sisyrinchium sarmentosum* (Iridaceae). Northwest Science 74:(in press).

Yeh, F. C., R.-C. Yang, and T. Boyle. 1997. Popgene Version 1.20. University of Alberta, Edmonton, Alberta.


Lake
Tahoe
SYMBOL KEY
Historical sites
Sampled sites
Backwood South
Baldwin West (enclosure)
Baldwin West
Taylor Creek (enclosure)
Lighthouse
Lighthouse Beach
Upper Truckee West
Upper Truckee East
Tahoe Meadows
Edgewood
Kahle/
Nevada
Beach
Tahoe Vista
Agate Bay
Kings Beach
Crystal Bay
Incline Village
Crystal Bay
Stateline Point
Cedar Flat
Dollar Point
Lake Forest
Tahoe City
Sand Harbor
Tahoe Pines
Homewood
McKinney Bay
Chambers Lodge
Tahoma
Sugar Pine Point
Meeks Bay
Rubicon Point
Emerald Bay
Glenbrook
South Point
Zephyr Cove
Zephyr Point
Marla Bay
Elk Point
Cave Rock
Kingsbury
Stateline
South Lake Tahoe
Tahoe Valley
WASHOE
CARSON
CARSON
DOUGLAS
CO.
CO.
CALIFORNIA
NEVADA
HUMBOLDT-
TOIYABE
NATIONAL

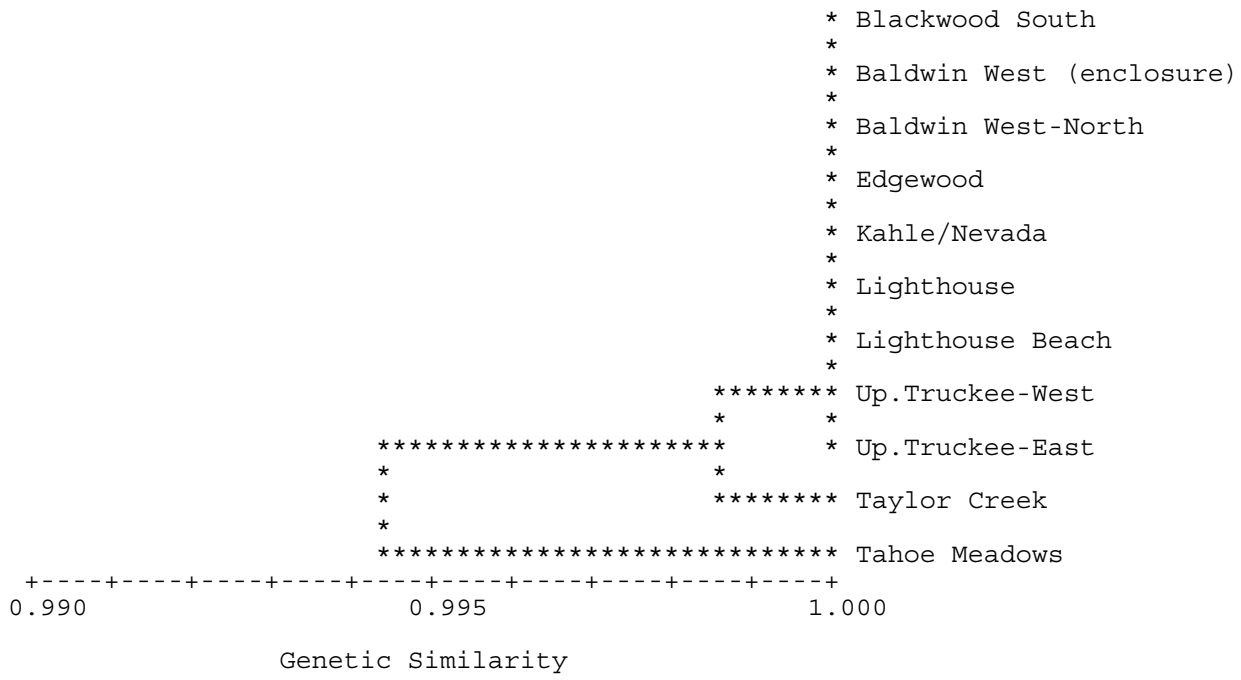


Figure 1. Dendrogram based on genetic similarity. Tahoe Meadows (0.99437) and Taylor Creek (0.99855) populations are shown as the most genetically dissimilar.

Table 1. Population name and number of *R. subumbellata* individuals sampled per occurrence.

Population Name	**# Individuals Sampled**	**Collection Date**
Upper Truckee East	33	8/15/99
Upper Truckee West	2	8/15/99
Edgewood	18	8/15/99
Taylor Creek (enclosure)	10	8/15/99
Baldwin West – N of parking lot	4	8/15/99
Baldwin West (enclosure)	13	8/15/99
Tahoe Meadows	8	9/01/99
Lighthouse Beach	7	9/01/99
Lighthouse	11	9/01/99
Kahle/Nevada	7	9/01/99
Blackwood South	27	9/01/99
TOTAL	**140**	

Table 2. Allele frequencies for 23 isozyme loci in *Rorippa subumbellata.* Migration distances are actual distances (mm) from the origin at which the enzyme band produced by this allele was observed, under the electrophoretic conditions used in this study. Alleles were numbered in the order in which they were observed, not in order of migration speed or frequency. Missing data is indicated with an asterisk.

Locus	Allele	Migration	Entire Study	Blackwood South	Baldwin West (enclosure)	Baldwin West-North	Edgewood	Kahle/ Nevada	Lighthouse	Lighthouse Beach	Tahoe Meadows	Taylor Creek	Upper Truckee-East	Upper Truckee-West
PGI-1	1	36	0.9929	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9000	1.0000	1.0000
PGI-1	2	40	0.0071									0.1000		
PGI-2	1	27	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
ME7	1	25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
LAP-1	1	42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
PGM-1	1	43	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
PGM-2	1	31	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-1	1	51	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-2	1	46	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-3	1	39	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
FEST-4	1	34	1.0000	1.0000	1.0000	1.0000	1.0000	*	1.0000	*	*	1.0000	1.0000	1.0000
ACO-1	1	41	1.0000	1.0000	1.0000	*	1.0000	*	1.0000	1.0000	*	1.0000	1.0000	*
UGPP-1	1	47	0.9714	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8000	0.9394	1.0000
UGPP-1	2	40	0.0286									0.2000	0.0606	
GOT-1	1	48/45/42	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TPI-1	1	55	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
TPI-2	1	43	1.0000	*	1.0000	1.0000	1.0000	*	1.0000	1.0000	*	1.0000	1.0000	1.0000
GLYDH	1	7	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
CAT-1	1	18	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-1	1	24	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
DIA-1	1	26	0.9786	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6250	1.0000	1.0000	1.0000
DIA-1	2	23	0.0214								0.3750			
IDH-1	1	21/27	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6PGD-1	1	28/25	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
6PGD-2	1	13	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SKD-1	1	32	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Table 3. Summary of genetic variability in *Rorippa subumbellata* populations. N = mean number of individuals sampled per locus, per population; P = % of all loci that are polymorphic; A = average number of alleles per locus; Ae = effective number of alleles per locus; H_o = observed frequency of heterozygotes; H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions. S-W = Shannon-Weaver diversity index.

	N (S.E.)	P	A (S.D.)	Ae (S.D.)	H_o (S.E.)	H_e (S.E.)	S-W (S.D.)
Species level							
Entire Study	247	13.04%	1.1304 (0.3444)	1.0048 (0.0149)	0.0003 (0.0015)	0.0046 (0.0142)	0.0112 (0.0339)
Population level							
Entire study - Mean	11.2 (2.7)	1.58%	1.0164 (0.0300)	1.0068 (0.0144)	0.0004 (0.0004)	0.0048 (0.0029)	0.0067 (0.0127)
Blackwood South	22.2 (1.8)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Baldwin West (enclosure)	13.2 (0.6)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Baldwin West-North	2.9 (0.1)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Edgewood	16.3 (0.8)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Kahle/Nevada	5.9 (0.5)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Lighthouse	8.9 (0.7)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Lighthouse Beach	6.1 (0.4)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)
Tahoe Meadows	6.5 (0.6)	4.35%	1.0500 (0.2236)	1.0441 (0.1973)	0.0000 (0.0000)	0.0250 (0.1118)	0.0331 (0.1479)
Taylor Creek (enclosure)	9.0 (0.4)	8.70%	1.0870 (0.2881)	1.0250 (0.0996)	0.0043 (0.0209)	0.0229 (0.0790)	0.0304 (0.1105)
Upper Truckee- East	30.9 (1.4)	4.35%	1.0435 (0.2085)	1.0056 (0.0268)	0.0000 (0.0000)	0.0050 (0.0241)	0.0099 (0.0477)
Upper Truckee-West	1.9 (0.1)	0.00%	1.0000 (0.0000)	1.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)	0.0000 (0.0000)

Table 4. Genetic identities (above the diagonal) and distances (below the diagonal) (Nei's (1978) unbiased estimates) among populations of *Rorippa subumbellata*.

Population	Blackwood South	Baldwin West (enclosure)	Baldwin West-North	Edgewood	Kahle/ Nevada	Lighthouse	Lighthouse Beach	Tahoe Meadows	Taylor Creek	Upper Truckee East	Upper Truckee West
Blackwood South	****	1.000	1.000	1.000	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Baldwin West (enclosure)	0.000	****	1.000	1.0000	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Baldwin West-North	0.000	0.000	****	1.000	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Edgewood	0.000	0.000	0.000	****	1.000	1.000	1.000	0.994	0.998	1.000	1.000
Kahle/Nevada	0.000	0.000	0.000	0.000	****	1.000	1.000	0.994	0.998	1.000	1.000
Lighthouse	0.000	0.000	0.000	0.000	0.000	****	1.000	0.994	0.998	1.000	1.000
Lighthouse Beach	0.000	0.000	0.000	0.000	0.000	0.000	****	0.994	0.998	1.000	1.000
Tahoe Meadows	0.006	0.006	0.006	0.006	0.006	0.006	0.006	****	0.992	0.994	0.994
Taylor Creek (enclosure)	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.009	****	0.999	0.998
Upper Truckee- East	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.001	****	1.000
Upper Truckee-West	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.002	0.000	****