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Forest Service

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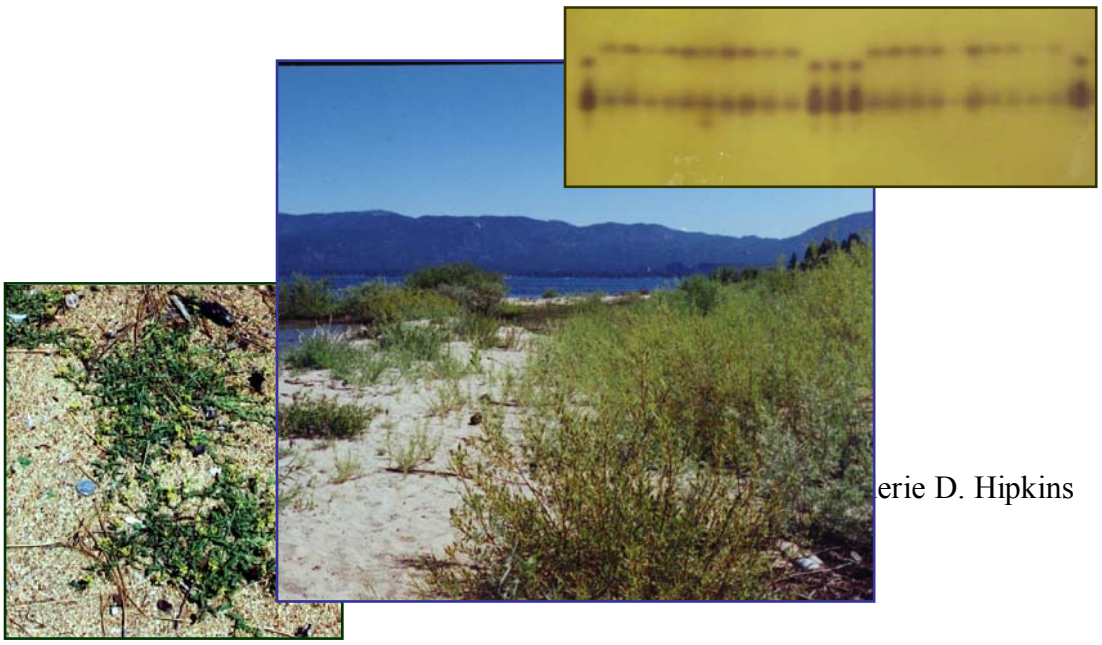
Final Report

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

(Service Agreement 14320-2-H401)



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ABSTRACT

Rorippa subumbellata, a small perennial Brassicaceae endemic to the shores of Lake Tahoe, was assessed for genetic variation using isozyme and DNA techniques. Samples from a total of twenty-five sites were collected over two years (2002-2003) and assayed for variation at 23 isozyme loci. Of the 553 total individuals genotyped, 540 (97.6%) had the same isozyme genotype. This genotype was the same as the common genotype found among 95.0% of the individuals sampled in the previous isozyme study (Service Agreement 14-48-0001-95813) conducted in 1999. The thirteen individuals found to contain variation in the 2002-03 collections were distributed among four populations: Eagle Creek (1 plant), Sugar Pine (9 plants), Tahoe Keys (2 plants), and Tallac Creek (1 plant). Neither random amplified polymorphic DNA (RAPD) nor sequences of the chloroplast genome resolved variation in individuals displaying isozyme variation. Of the ten populations sampled over more than one year, two showed evidence of some change in genetic structure between years based on isozyme analysis, including the apparent loss of one rare allele from the Upper Truckee East population. No variation was detected in the samples from the out-planted population at Emerald Bay Avalanche, but as the natural population at this site displayed only the common genotype, these plantings do not change the genetic structure of the population. Due to the lack of variation in most populations, the movement of seed among populations during restoration efforts will likely not affect the overall genetic structure of the species, although efforts to conserve the limited variation observed in the species are warranted.

INTRODUCTION

Rorippa subumbellata (Roll.), Tahoe yellow cress, is a small, perennial plant endemic to the sandy beaches of Lake Tahoe in California (El Dorado and Placer counties) and Nevada (Carson City Rural Area, Washoe and Douglas counties). Occurring only where beaches are wide enough to offer a back beach area protected from wave action (Ferreira 1987), populations are subject to annual variation in size and distribution (*i.e.* metapopulation dynamics), although population censuses have observed a net decline in the species over the past ten years (Pavlik *et al.* 2002). Based on its narrow habitat and declining population sizes, the California and Nevada state governments, local municipalities, and non-profit organizations have identified *R. subumbellata* as an endangered species, and the species is currently a candidate for federal protection under the U.S. Endangered Species Act of 1973, as amended (Pavlik *et al*. 2002).

The current conservation strategy (CS) for *R. subumbellata*, implemented by a coalition of federal, state, and local agencies and private organizations, is described in detail by Pavlik *et al.* (2002). Previous research established that metapopulation dynamics, or the local extinction and colonization of populations, are common in this species. Restoring these processes is considered important to the survival of this species. However, a complete understanding of the demographic processes contributing to these metapopulation dynamics is still lacking.

Metapopulation, migratory, and even reproductive processes can be interpreted from genetic data given sufficient variation in the species (Leberg 1996). In addition, conserving the genetic variation within and among populations is an important component of maintaining the long-term survival and evolutionary potential of a species (Sherwin and Moritz 2000). In the hope of applying genetic information to *R. subumbellata* conservation efforts, the National Forest Genetics Laboratory (NFGEL) was contracted in 1999 to assess 11 sites of *R.*

subumbellata for genetic variation at 23 isozyme loci (Saich and Hipkins, 2000). This previous study found very low levels of variation (eight of the eleven populations displayed no variation), and as a result could identify no patterns of genetic differentiation in the species.

As an extension of the previous genetic work performed by NFGEL, the current study was designed to screen 25 sites for variation at the same 23 isozyme loci. In order to determine if genetic variation is present but undetected by isozymes, additional analyses were completed using two DNA-based markers on a subset of samples. The isozyme analysis revealed low amounts of variation within and among populations, similar to those reported by Saich and Hipkins (2000). Neither of the DNA-based analyses detected variation in the samples screened. These results are consistent with those found in other narrowly-distributed endemic herbs (Hamrick and Godt 1990), and imply that maintaining patterns of genetic differentiation among populations of *Rorippa subumbellata* may be less critical than capturing genetic variation in seed collection and *ex situ* propagation activities.

METHODS

Study Area: Study sites were located along the southern half of Lake Tahoe, on the western, southern, and eastern shores (Figure 1). Samples were collected from 25 sites in 2002-2003 in conjunction with annual census performed by the U.S. Fish and Wildlife Service in cooperation with several federal, state, and local organizations. Several populations were located within protective enclosures, while other populations contained out-planted individuals established as part of restoration efforts (Table 1). In the latter case, collections from naturally occurring populations were collected and labeled separately from restored material (Native and Planted, respectively).

Sample Collection: Up to 30 samples per site of *R. subumbellata* were collected September 2002 and September 2003 for a total of 553 sampled individuals (Table 1). In addition, twenty samples from each of two populations of *R. curvisiliqua* were collected in September 2002. When fewer than 30 *R. subumbellata* plants were present, all plants were sampled. When more than 30 plants were present, samples were collected at sufficient intervals to insure individuals were sampled from throughout the range of the population. On one occasion, up to 60 samples were collected due to misunderstanding as to where the borders of the site were defined. One or two leaves were taken from each plant and placed in zip-lock bags. Bags were kept cool in ice chests during transport to NFGEL in Placerville, CA, and kept refrigerated until prepared for analysis.

Isozyme Analysis: Samples were prepared according to NFGEL Standard Operating Procedures (USDA Forest Service 2003) by submerging a 1cm long section of leaf (40 mm^2) in 100 uL of Tris buffer pH 7.5 (Gottlieb 1981). Samples were stored at –80°C until electophoresis.

Starch gel electrophoresis took place following NFGEL Standard Operating Procedures (USDA Forest Service 2003). A total of 23 loci were resolved in three buffer systems: a lithium borate electrode buffer-tris citrate gel buffer combination (LB), a sodium borate electrode buffer-tris citrate gel buffer system (SB), and a morpholine citrate electrode and gel buffer system (MC6). Eleven loci were resolved in system LB: aconitase (ACO1), leucine aminopeptidase (LAP1), malic enzyme (ME(7)1), phosphoglucose isomerase (PGI1 and PGI2), phosphoglucomutase (PGM1 and PGM2), and fluorescent esterase (FEST1, FEST2, FEST3, and FEST4). Six loci were resolved in system SB: aspartate aminotransferase (AAT1), catalase (CAT1), glycerate-2-dehydrogenase (GLYDH1),

triosephosphate isomerase (TPI1 and TPI2), and uridine diphosphoglucose pyrophosphorylase (UGPP1). Six loci were resolved in system MC6: diaphorase (DIA1), isocitrate dehydrogenase (IDH1), malate dehydrogenase (MDH1), phosphogluconate dehydrogenase (6PGD1 and 6PGD2), and shikimic acid dehydrogenase (SKD1). Two people independently scored each gel, and a third person resolved any disagreements in scores. As part of the NFGEL quality assurance (QA) program, duplicate preparations of 25 individuals (3%), and complete re-runs of 5 sets of 30 individuals (19%) were analyzed in order to confirm observed variation.

DNA Analysis: DNA was extracted from a total of 22 samples using a Qiagen DNEasy Mini Kit following the manufacturer's instructions. Six of the samples were chosen because they displayed the three genotypes observed at the locus UGPP1 during isoyzme analysis: TE-1, UTW-1, and ZS-3 were homozygous for the common allele; SP-1 and SP-9 were heterozygous at the locus, and TC-4 was homozygous for the rare allele. The remaining samples (LH-1, LH-10, LH-5, LH-6, R-21, R-22, RAT-1, RAT-18, SP-2, SP-3, TCW-30, TCW-31, UTE-1, UTE-20, UTW-10, and UTW-20) were selected at random in order to screen DNA markers for variation in the species.

The six samples chosen for their genotype at UGPP (herein the set of six samples) were assessed for variation using three DNA marker systems: random amplified polymorphic DNA (RAPDs), chloroplast DNA sequences in the *trn*L-*trn*F intergenic region (cpDNA), and three microsatellite loci (SSRs). The remaining samples were assessed for variation only using the cpDNA sequences. All amplification reactions took place on a MJ Research® PTC-100 thermalcycler.

The set of six samples were screened for variation using 10 RAPD primers obtained from Operon primer set B: primers 3 thru 12 (available through Qiagen DNA Oligos). Amplification reactions were carried out in a total volume of 25.0 uL, with 3.0 ng sample DNA, 1X reaction buffer (provided with enzyme), 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 20.0 pmol primer, and 1.0 U Taq DNA Polymerase (Qiagen). Amplification reactions involved 1-min. 30-sec. melting at 94 °C, 40 cycles of 1-min. 94 °C, 1-min. 40 °C, and 2-min. 72 °C, followed by a final extension of 10-min. at 72 °C. Products were separated on 1.4% agarose gels and visualized using ethidium bromide.

Bleeker and Hurka (2001) identified intra- and interspecific variation in the *trn*L-*trn*F intergenic region of the chloroplast genome in their study of three European *Rorippa* species. This variation was observed within and among populations at the intraspecific level. All 22 samples of *R. subumbellata* were screened for sequence variation at this locus using primer sequences from Taberlet *et al.* (1991). The intergenic region was first amplified using a standard polymerase chain reaction with a total volume of 25.0 uL, with 8.0 ng sample DNA, 1X reaction buffer (provided with enzyme), 200 uM each dNTP, 2.5 mM $MgCl_2$, 1 uM each primer, and 1.0 U Taq DNA Polymerase (Qiagen). Amplification reactions involved 2-min melting at 95°C, 35 cycles of 30-sec. 95 °C, 30-sec. 55 °C, and 2-min. 72 °C, followed by a final extension of 5-min. at 72 °C. PCR products were purified using Qiagen QiaQuick PCR Purification kits following the manufacturer's protocol. The concentration of the PCR product recovered was quantified using either fluorometery or agarose gel electrophoresis. The sequencing reaction took place in a total volume of 10.0 uL, with 100 ng DNA, 9.5 uM primer, and 4.0 uL of BigDye® Terminator v3.1 Cycle Sequencing Mix (Applied Biosystems, Inc.). The sequencing reaction involved 25 cycles of 10-sec. 96 °C, 5-sec. 50 °C, and 4-min. at 60 °C, with a temperature change rate of 1 °C/second between each step. Sequences were detected on an ABI-3100 capillary system.

Finally, the set of six samples was assessed for variation at three microsatellite loci (or simple sequence repeats, SSRs) developed by Suwabe *et al.* (2002) for *Brassica rapa*: BRMS-020, BRMS-025, BRMS-044. Although most microsatellite primers cannot readily transfer across genera, these three primers have been shown to transfer to *Arabidopsis*, which is classified in a different tribe than *Brassica* (Heywood *et al*. 1993). As a result, these markers had a greater probability of transferring to *Rorippa* than other microsatellite markers currently available. The amplification reaction recipe and conditions followed those described by Suwabe *et al.* (2002). Primers were fluorescently labeled, and products were detected on the ABI-3100 capillary system.

Data Analysis: A variety of species- and population-level parameters were estimated from the isozyme data. For each population, the percent polymorphic loci (*P*), mean alleles per locus (*A*), mean alleles per polymorphic locus (A_p), observed heterozygosity (H_o), and heterozygosity expected under Hardy-Weinberg equilibrium (H_e) were calculated. These six parameters were also estimated at the species level for the 2002 and 2003 collections.

In order to identify the genetic structure of the entire species, data from the 2002-2003 collections and the 1999 study were combined to create population phenograms, using the two *R. curvisiliqua* populations as outgroups. Nei's (1978) unbiased estimate of genetic distance was estimated for the isozyme data for all possible pairs of populations. The resulting distance matrix was then used to create two population phenograms using cluster analysis (UPGMA) and Neighbor-Joining (NJ) methods. All phenograms were executed using the software PHYLIP (Felsenstein 1993).

Finally, annual changes in genetic structure are not unexpected in species displaying metapopulation dynamics, and such variability in *R. subumbellata* may provide insight to the dynamics of this species. In order to test for annual differences in genetic structure in *R. subumbellata* populations, data from the 2002-2003 collections were combined with the data from the 1999 study, providing multi-year data for ten sites (Table 1). Temporal differences in genetic structure were identified from the isozyme data based on the genetic identity and genetic distance (Nei's (1978) unbiased estimate) among years for each site.

RESULTS

Isozyme Analysis: Isozyme analysis was marginal or failed for samples collected at 13 of the sites in 2002, and as a result, samples were recollected from those sites in 2003. Data for each year a site was sampled was analyzed independently. The resulting data set includes six sites each sampled twice (resulting in 12 "populations") and nineteen sites sampled once (19 populations), for a total of 31 populations in this analysis. Although isozyme analysis of the two samples collected at the D.L. Bliss site in 2002 failed, the site was not revisited in 2003, and has not been included in this report.

Twenty-seven of the 31 populations were monomorphic at the 23 isozyme loci analyzed (Appendix 1). All variation observed in the remaining four populations occurred at low frequency. Two populations, Sugar Pine 2002 and Tallac Creek 2002, contained variation at a locus previously found to be variable, UGPP1. The other two populations displayed novel variation: Eagle Creek 2003 at the locus FEST1, and Tahoe Keys 2003 at PGM2 (Table 2, Appendix 2). For the 2002-2003 collections, this variation results in a species-level estimate of percent polymorphic loci of 13.04%. The expected heterozygosity for all populations was 0.0000 except Sugar Pine 2002: 0.0159 (S.E. = 0.0154), Tallac Creek 2002: 0.0103 (S.E. = 0.0100); Eagle Creek 2003: 0.0062 (S.E. = 0.0061); and Tahoe Keys

2003: 0.0049 (S.E. = 0.0048). *R. curvisiliqua* contains greater levels of variation than does *R. subumbellata* (He=0.032 vs He=0.002, respectively; Table 3).

The population phenograms produced using UPGMA and NJ methods display a similar topology, each rooted by the two populations of *R. curvisiliqua* and illustrating the similarity in most populations. The UPGMA phenogram distinguishes only the Taylor Creek 1999 and Tahoe Meadows 1999 populations from the other *R. subumbellata* sites (available upon request). The Neighbor-Joining method depicts not only these sites as unique, but also reflects the variation observed in Sugar Pine 2002, Tallac Creek 2002, Upper Truckee East 1999, and Tahoe Keys 2003 (Figure 2).

Genetic distances detect temporal variation in genetic structure between the 1999 and 2003 collections of Tahoe Meadows, as variable between 1999 and 2003 (genetic distance = 0.005), although the single variable locus (DIA1) was not resolved in the 2003 collection. A change in allele frequency was detected in the samples collected at Upper Truckee East, with the rare allele at UGPP1 occurring at low frequency in 1999, but missing from the 2002 collections. All remaining pairs of collections produced an index of genetic identity of 1.000.

***DNA Analyses*:** Of the ten RAPD primers screened in the set of six samples, none produced consistent variation among individuals. Attempts to replicate potential variation failed to produce repeatable banding patterns. Despite their great potential due to the anonymous nature of primer binding, banding patterns produced by RAPD analyses are not always consistent or appropriate for genetic diversity studies (Jones *et al.* 1998), and the results of the *Rorippa* screening indicate that RAPDs are not appropriate for this species. As a result, none of the banding patterns were analyzed further. All 22 samples screened for variation at the *trn*L-*trn*F spacer of the chloroplast genome produced identical sequences (sequences available upon request.) Finally, none of the microsatellite primers produced peaks in the six samples screened in this test, indicating that these primers may not be easily transferred across species within this genus.

DISCUSSION

Genetic structure of *Rorippa subumbellata*

Low levels of variation were detected in only a handful of *R. subumbellata* populations, which is consistent with the findings of Saich and Hipkins (2000). Of the 31 populations (25 sites) sampled in this study, four contained variation at a single locus. Two populations, Sugar Pine 2002 and Tallac Creek 2002, contained the same alternate allele at the locus UGPP1 that Saich and Hipkins (2000) reported in previous collections. The other two populations displayed novel variation: Eagle Creek 2003 contained a single alternate allele at the locus FEST1, and Tahoe Keys 2003 contained an alternate allele at the locus PGM2 (Table 2, Appendix 1, Appendix 2). No variation was observed at two loci previously reported as variable: PGI1 and DIA1. The remaining 27 populations were monomorphic for the common allele at all loci. Other studies of extreme endemics (plants restricted to narrow habitats) have reported consistently low levels of variation, including *Pedicularis furbishiae* (Waller *et al.* 1987) and *Iris lacustris* (Simonich and Morgan 1994). However, *R. subumbellata* displays levels of variation that are consistently lower than the mean of 40% polymorphic loci reported in rare and endemic herbs by Hamrick and Gott (1990).

Given that all populations sampled contain the common genotype (*i.e.* individuals homozygous for the common allele at all loci) in high frequencies, efforts to supplement or reestablish populations with *ex situ* plants that contain only the common genotype should maintain the current genetic structure observed among populations. However, care should be

taken to preserve those populations containing genetic variation (see Gene Conservation below).

Saich and Hipkins (2000) discussed the possible causes of the low levels of variation in *R. subumbellata*, including genetic bottlenecks, clonal reproduction, and a mating system displaying high rates of selfing. The genetic findings of this study, together with the life history of the species described by Pavlik *et al.* (2002), reveals additional factors that may contribute to the observed genetic structure. The recurring extinction and colonization of populations in a metapopulation may lead to genetic bottlenecks and random genetic drift, and eventually to a decrease in heterozygosity in the species (Thrall *et al*. 2000). If metapopulation dynamics were historically important to the survival of this species, frequent turnover of populations may have maintained the low levels of variation currently observed.

Although metapopulation dynamics may play an important role in the structure of this species, these studies indicate that gene flow among established populations is rare. The presence of rare alleles in only one or a couple of populations is consistent over years (Pavlik *et al.* 2002, Saich and Hipkins 2000), and no evidence has been found that these rare alleles have moved (presumably via seed flow) into neighboring populations. In addition, the population differentiation reported by Saich and Hipkins (2000) is the consequence of rare alleles being restricted to no more than 2 populations, another indication that gene flow is rare in this species.

Temporal variation

Temporal differences in genetic variation were detected in two of the ten sites (20%) sampled over more than one year (1999-2003). These differences were indicated by genetic distances greater than 0.000, and by a difference in allele frequencies between collection years. Differences at one site, Tahoe Meadows, are due not to a potential change in allele frequency in the population over a four year period, but rather due to the fact that the single locus displaying variation in 1999, DIA1, was unresolved in the 2003 collections. Alternatively, the absence of the rare allele UGPP1-2 in the Upper Truckee East samples collected in 2002, indicates that the allele has likely been lost through random genetic drift since the 1999 collections (see Gene Conservation, below), although the allele may still be present at low frequencies due to the large size of the population. These patterns of temporal differentiation are consistent with the genetic consequences expected from the local extinction and colonization dynamics that define a metapopulation.

Gene conservation

Although the high frequency of the common alleles in *R. subumbellata* indicates that outplanting efforts using the common genotype should not change the genetic structure of the metapopulation, the conservation of the species as a whole will not be complete if the rare alleles are lost through genetic drift. One goal of conservation biology is to conserve patterns and levels of genetic diversity in species. Theoretically, maintaining levels of genetic variation is important in order to maximize the adaptive potential of the species of concern (Lande and Barrowclough 1987).

The genetic variation observed in *R. subumbellata* occurs in low levels and in a limited number of populations (Table 1, 2, 3). As a result, care should be taken to conserve these rare alleles and preserve their functional role in each population. Two rare alleles present in the 1999 samples, DIA1-2 and PGI1-2, were not observed in the present study. In one case, the locus DIA1 was not resolved in the 2003 collection from the Tahoe Meadows population (where it was previously observed), so the allele may still be present but not

detected. In another case, *R. subumbellata* was not recollected from the Taylor Creek Enclosure, where the PGI1-2, as well as the UGPP1-2 allele, were observed in 1999. These alleles were not observed in any of the neighboring sites, however (Taylor Creek East, Taylor Creek Middle, and Taylor Creek West; Appendix 1). In the last case, the allele UGPP1-2 was not observed in the 2002 collections at Upper Truckee East. In this case, the rare allele has likely been lost through genetic drift, since we would have expected to observe the allele based on the frequency of the allele in the 1999 collections.

If a rare allele is present at low frequencies in a large population of *R. subumbellata*, how many copies would we expect to observe in a sample of 30 individuals? This can be estimated as the product of the allele frequency of the rare allele (conservatively, the lowest population-level frequency observed for the allele) and the number of alleles sampled from the population (since *R. subumbellata* behaves as diploid, sampling 30 individuals resolves 60 alleles from the population). From this information, we expect to observe the following number of rare alleles for each variable locus (the allele frequency used in each estimate is given in parentheses): 22.5 occurrences of DIA1-2 (0.375), 4.02 of FEST1-2 (0.067), 6.0 of PGI1-2 (0.100), 3.12 of PGM2-3 (0.052), and 3.66 of UGPP1-2 (0.061). Allele frequencies reported by Saich and Hipkins (2000) were used to estimate the values for DIA1, PGI1, and UGPP1. These estimates indicate that, based on a sample of 30 individuals, the rare allele should have been observed if present in a population. Given these probabilities, we conclude that those populations containing more individuals than those sampled (4H, Blackwood North, Blackwood South, Emerald Avalanche, Emerald Point, Lighthouse, Rubicon, Sugar Pine, Tahoe Keys, Taylor Creek West, Upper Truckee East, and Upper Truckee West) likely do not contain rare alleles not reported herein. The remaining populations were sampled exhaustively, so allele frequencies at each site are conclusive.

Future directions

Isozyme variation can be considered a proxy for the total genetic variation that may be contained in a species. Based on the observed isozyme variation and the lack of variation at DNA markers in individuals known to display protein variation, this battery of isozyme loci currently provides the best tool to monitor the genetic structure of populations of *R. subumbellata*. In contrast, other DNA marker systems may prove more variable than isozymes, but are often either expensive to develop and screen (*e.g.*, microsatellites) or require a larger amount of tissue than may be available from a single *R. subumbellata* rosette without significantly damaging its chances for survival (*e.g.* AFLPs).

Rather than expanded searches for variable markers in *R. subumbellata*, the addition of populations from the northern half of Lake Tahoe may provide additional information about the genetic structure of this species. Pavlik *et al.* (2002) identified several sites along the northern shores of the lake that were once known to contain *R. subumbellata* populations (Figure 1), although recent surveys have determined populations at some of the sites to be extinct.

Finally, one goal of conservation biology is to conserve genes under the theory that species (or populations) displaying higher levels of variation have a greater chance of adapting to changing environments (Lande and Barrowclough 1987). To this end, care should be taken to conserve the genetic variation known to exist in *R. subumbellata*. With the goal of preserving the limited genetic variation observed in this species, efforts to tag individual plants containing rare alleles and track their survival over years, systematically collect seed from these individuals, or even vegetatively propagate these plants would be reasonable

additions to the conservation strategy. Sites containing known genetic variants should receive special attention for future seed collections (Table 2, Appendix 2).

SUMMARY AND CONCLUSIONS

Analysis of 693 individuals from 28 sites revealed low levels of variation in *Rorippa subumbellata* over three collection years (1999, 2002, and 2003). DNA analyses resolved no variation in individuals known to contain different isozyme genotypes. Genetic differences among populations and between years are due to the presence of rare alleles in five loci: DIA1, FEST1, PGI1, PGM2, and UGPP1 (Table 2). Populations found to contain variation are: Eagle Creek (2003), Sugar Pine (2002), Tahoe Keys (2003), Tahoe Meadows (1999), Tallac Creek (2002), Taylor Creek (1999), and Upper Truckee East (1999). Temporal changes in genetic variation observed at Upper Truckee East is likely due to the loss of a rare allele at one locus (UGPP1) between 1999 and 2002, although the allele may be present in low frequencies due to the large size of this population. The high frequency of the common alleles in every population sampled indicates that restoration activities using plants that are homozygous for the common genotype will not significantly change the genetic structure of the metapopulation. However, care should be taken to conserve the limited genetic variation observed in order to preserve the evolutionary potential of the species.

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Table 1. Name, abbreviation, and collection date of *Rorippa* study sites. Previous Name is the label used by Saich and Hipkins (2000), and is provided for reference. The number of individuals analyzed, if different from the number collected, is indicated in parentheses. Names or Dates in **bold** indicate collections that displayed isozyme variation. 1999 collections are part of the previous isozyme study conducted under Service Agreement 14-48-0001-95813; 2002 and 2003 collections are part of the current study, Service Agreement 14320-2-H401.

Population	Abbrev.	Previous Name	Date Collected	# Collected
Rorippa subumbellata				
4H	4H		9-4-2002	21 (0)
			9-9-2003	30
Baldwin	B	Baldwin West – N of lot	8-15-1999	4
			9-3-2002	3(2)
			9-2-2003	3
Blackwood North	BN		9-2-2003	24[a]
Blackwood South	BS	Blackwood South	9-1-1999	27
			9-4-2002	28 (5)
			9-2-2003	30
Cascade West	CW		9-3-2002	4 (0)
			9-2-2003	8
Eagle Creek	EC		9-4-2002	4 (0)
			9-3-2003	15
Edgewood	E		8-15-1999	18
Emerald Bay Avalanche, Native	EAN		9-4-2002	21 (1)
			9-3-2003	60
Emerald Bay Avalanche, Planted	EAP		9-3-2003	15
Emerald Point	EP		9-4-2002	11 (7)
			9-3-2003	30
Kahle/Nevada	K		9-1-1999	7
Lighthouse	L	Lighthouse	9-1-1999	11
		Lighthouse Beach	9-1-1999	7
			9-4-2002	31 (10)
			9-2-2003	35
Meeks Bay	MB		9-4-2002	12 (5)
			9-2-2003	7
Pope Beach	P		9-4-2002	7 (0)
			9-2-2003	9 (4)
Regan/Al Tahoe	RAT		9-3-2002	18
Rubicon	R		9-4-2002	30
Sugar Pine	SP		**9-4-2002**	30
Tahoe Keys	TK		9-4-2002	31 (0)
			9-2-2003	30
Tahoe Meadows	TM	Tahoe Meadows	**9-1-1999**	8
			9-4-2002	20 (0)
			9-9-2003	12
Tallac Creek	TC		**9-3-2002**	11
Tallac Enclosure	TE	Baldwin West (enclosure)	8-15-1999	13
			9-3-2002	10

[a]More than 24 plants present.

Table 1. Continued.

Population	Abbrev.	Previous Name	Date Collected	# Collected
Taylor Creek Enclosure	TAY		**8-15-1999**	10
Taylor Creek East	TCE		9-3-2002	12
Taylor Creek Mouth	TCM		9-3-2002	10
Taylor Creek West	TCW		9-3-2002	31
Upper Truckee East	UTE	Upper Truckee East	**8-15-1999**	33
			9-3-2002	30
Upper Truckee West	UTW	Upper Truckee West	8-15-1999	2
			9-3-2002	30
Zephyr Spit	ZS		9-4-2002	8
Rorippa curvisiliqua				
Tallac Creek	ROCUT		9-3-2002	20
Taylor Creek Enclosure	ROCUE		9-3-2002	20

Table 2. Genotype scores at the five variable isozyme loci for the 20 *R. subumbellata* individuals showing genetic variation in both studies (20 out of 693 total plants sampled; 2.9%). Allele numbers (1, 2, or 3) are defined in Appendix 1. All individuals are homozygous for the common allele ('11') at the other 18 loci (not listed).

Site	**Year**	**# of Plants**	**Locus**				
			UGPP1	PGM2	FEST1	DIA1	PGI1
Sugar Pine	2002	9	12	11	11	11	11
Tallac Creek	2002	1	22	11	11	11	11
Upper Truckee East	1999	2	22	11	11	11	11
Taylor Creek Enclosure	1999	2	22	11	11	11	12
Tahoe Meadows	1999	3	11	11	11	22	11
Eagle Creek	2003	1	11	11	22	11	11
Tahoe Keys	2003	1	11	13	11	11	11
		1	11	33	11	11	11

Table 3. Summary of genetic variability in *Rorippa* species. N = mean number of individuals per locus per population; P = % polymorphic loci; A = mean number alleles per locus; A_p = mean alleles per polymorphic locus; H_o = oberserved frequency of heterozygotes; H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium. Standard errors given in parentheses.

	N	*P*	*A*	*A*p	*H*o	*H*e
Species level						
R. subumbellata						
All populations (1999, 2002, 2003)	693	13.04	1.1304 (0.3444[a])	2.0000	0.0008 (0.0034[a])	0.0015 (0.0045[a])
R. curvisiliqua						
All populations (2002)	28	30.43	1.3478(0.1168)	2.1429	0.0457 (0.0414)	0.0324 (0.0221)
Population level						
(*R.subumbellata)*						
2002 mean	250	4.35	1.0588 (0.2425[a])	2.0000	0.0021 (0.0088[a])	0.0025 (0.0105[a])
2003 mean	303	8.70	1.0952 (0.3008[a])	2.0000	0.0002 (0.0008[a])	0.0009 (0.0029[a])
4H 2003	28.714	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
B 2002	1.929	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
B 2003	3.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BN 2003	21.750	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BS 2002	5.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
BS 2003	28.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
CW 2003	7.882	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EC 2003	14.150	5.00	1.0500 (0.0487)	2.0000	0.0000 (0.0000)	0.0062 (0.0061)
EAN 2002	1.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EAN 2003	52.588	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EAP 2003	14.500	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EP 2002	6.539	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
EP 2003	17.947	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
L 2002	10.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
L 2003	33.810	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
MB 2002	4.692	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
MB 2003	6.824	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
P 2003	3.895	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
RAT 2002	16.500	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
R 2002	27.188	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
SP 2002	26.875	6.25	1.0625 (0.0605)	2.0000	0.0188 (0.0182)	0.0159 (0.0154)
TK 2003	25.700	5.00	1.0500 (0.0487)	2.0000	0.0017 (0.0017)	0.0049 (0.0048)
TM 2003	10.350	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TC 2002	10.063	2.000	1.0625 (0.0605)	2.0000	0.0000 (0.0000)	0.0103 (0.0100)
TE 2002	10.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCE 2002	10.133	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCM 2002	9.438	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
TCW 2002	23.625	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
UTE 2002	28.235	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
UTW 2002	28.353	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)
ZS 2002	8.000	0.00	1.0000 (0.0000)		0.0000 (0.0000)	0.0000 (0.0000)

[a]Standard deviations

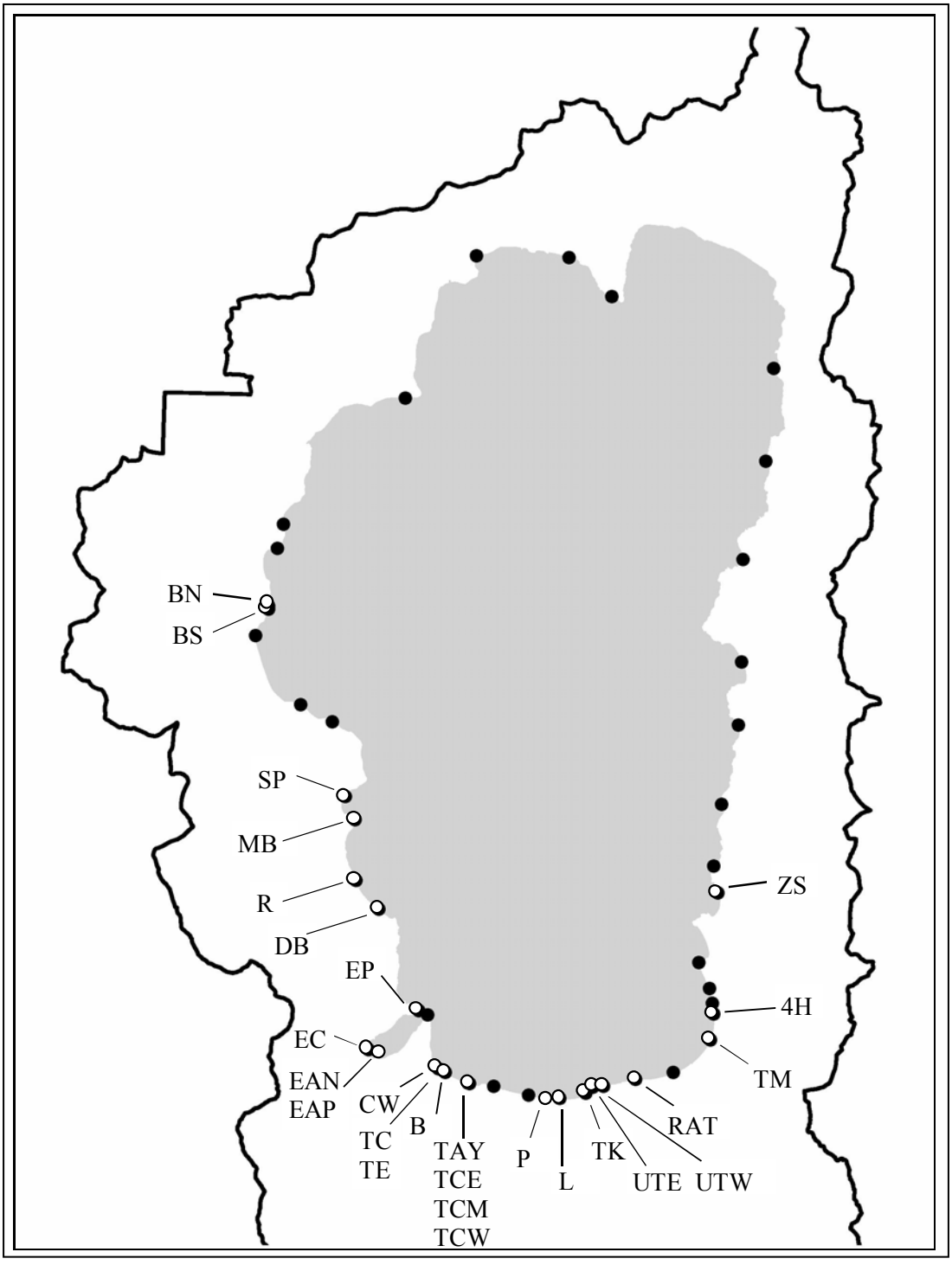


Figure 1. Locations of *R. subumbellata* populations sampled in this study. Open circles represent those populations sampled, with abbreviations identifying each site. Solid gray circles represent historic locations of populations. From Pavlik *et al.* 2002.

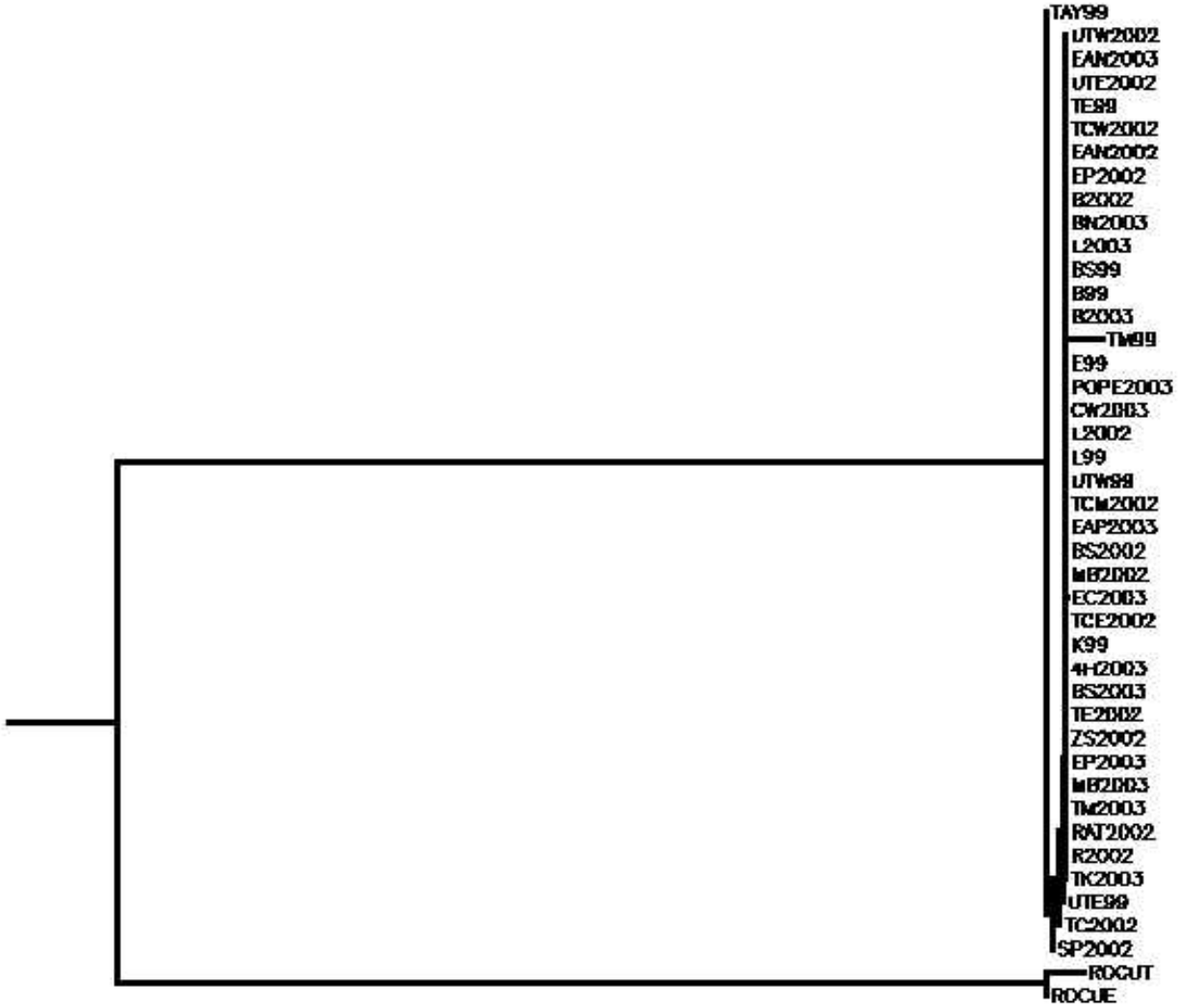


Figure 2. Population phenogram for 41 populations of *Rorippa subumbellata* and 2 populations of *R. curvisiliqua*. Phenogram is built from Nei's (1978) unbiased genetic distance using Neighbor Joining methods. Population abbreviations found in Table 1.

Appendix 1. Allele frequencies at 23 isozyme loci for *Rorippa subumbellata* and *R. curvisiliqua*. Alleles were numbered in the order they were observed, not in order of migration speed or frequency. Migration is the distance (mm) the allele migrated from the origin. * indicates missing data.

Locus	AAT1	ACO1			CAT1	DIA1		FEST1	
Allele	1	1	2	3	1	1	2	1	2
Migration	48/45/42	41	45	43	18	26	23	51	54
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000
Baldwin 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Baldwin 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Blackwood North 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Blackwood South 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Blackwood South 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Cascade West 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Eagle Creek 2003	1.000	*	*	*	1.000	1.000	0.000	0.933	0.067
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Emerald Bay Avalanche, Native 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	*	*	*	1.000	1.000	0.000	1.000	0.000
Emerald Point 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Emerald Point 2003	1.000	1.000	0.000	0.000	*	*	*	1.000	0.000
Lighthouse 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Lighthouse 2003	1.000	1.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000
Meeks Bay 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
Meeks Bay 2003	1.000	*	*	*	1.000	*	*	1.000	0.000
Pope Beach 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Regan/Al Tahoe 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Rubicon 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Sugar Pine 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Tahoe Keys 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Tahoe Meadows 2003	1.000	1.000	0.000	0.000	1.000	*	*	1.000	0.000
Tallac Creek 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Tallac Enclosure 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek East 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek Mouth 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Taylor Creek West 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Upper Truckee East 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Upper Truckee West 2002	1.000	1.000	0.000	0.000	1.000	*	*	*	*
Zephyr Spit 2002	1.000	1.000	0.000	0.000	*	*	*	*	*
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.500	0.500	0.000	1.000	*	*	*	*
Taylor Creek Enclosure 2002	1.000	0.475	0.475	0.050	1.000	*	*	*	*

Appenix 1 (cont'd)

Locus	FEST2	FEST3	FEST4	GLYDH1	IDH1		LAP1		MDH1
Allele	1	1	1	1	1	2	1	2	1
Migration	46	39	34	7	17/27	17/21	42	45	24
Rorippa subumbellata									
4H 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Baldwin 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Baldwin 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Blackwood North 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Blackwood South 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Blackwood South 2003	*	*	*	1.000	*	*	1.000	0.000	1.000
Cascade West 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Eagle Creek 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Native 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Native 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Emerald Bay Avalanche, Planted 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Emerald Point 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Emerald Point 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Lighthouse 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Lighthouse 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Meeks Bay 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Meeks Bay 2003	*	1.000	*	1.000	*	*	1.000	0.000	1.000
Pope Beach 2003	1.000	1.000	*	1.000	*	*	1.000	0.000	1.000
Regan/Al Tahoe 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Rubicon 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Sugar Pine 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Tahoe Keys 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Tahoe Meadows 2003	1.000	1.000	1.000	1.000	*	*	1.000	0.000	1.000
Tallac Creek 2002	*	*	*	1.000	1.000	0.000	1.000	0.000	1.000
Tallac Enclosure 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek East 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek Mouth 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Taylor Creek West 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Upper Truckee East 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Upper Truckee West 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
Zephyr Spit 2002	*	*	*	1.000	*	*	1.000	0.000	1.000
R. curvisiliqua									
Tallac Enclosure 2002	*	*	*	1.000	0.000	1.000	0.000	1.000	1.000
Taylor Creek Enclosure 2002	*	*	*	1.000	0.000	1.000	0.050	0.950	1.000

Appenidx 1 (cont'd)

Locus	ME(7)1	6PGD1		6PGD2	PGI1		PGI2		
Allele	1	1	2	1	1	2	1	2	3
Migration	25	28/25	32/27	13	36	40	27	30	28
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Baldwin 2002	1.000	1.000	0.000	*	1.000	0.000	1.000	0.000	0.000
Baldwin 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood North 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood South 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Blackwood South 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Cascade West 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Eagle Creek 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Point 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Emerald Point 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Lighthouse 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Lighthouse 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Meeks Bay 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Meeks Bay 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Pope Beach 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Regan/Al Tahoe 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Rubicon 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Sugar Pine 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tahoe Keys 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tahoe Meadows 2003	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tallac Creek 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Tallac Enclosure 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek East 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek Mouth 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Taylor Creek West 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Upper Truckee East 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Upper Truckee West 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
Zephyr Spit 2002	1.000	1.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.000	1.000	*	1.000	0.000	0.000	1.000	0.000
Taylor Creek Enclosure 2002	1.000	0.000	1.000	*	1.000	0.000	0.000	0.975	0.025

Appendix 1 (cont'd)

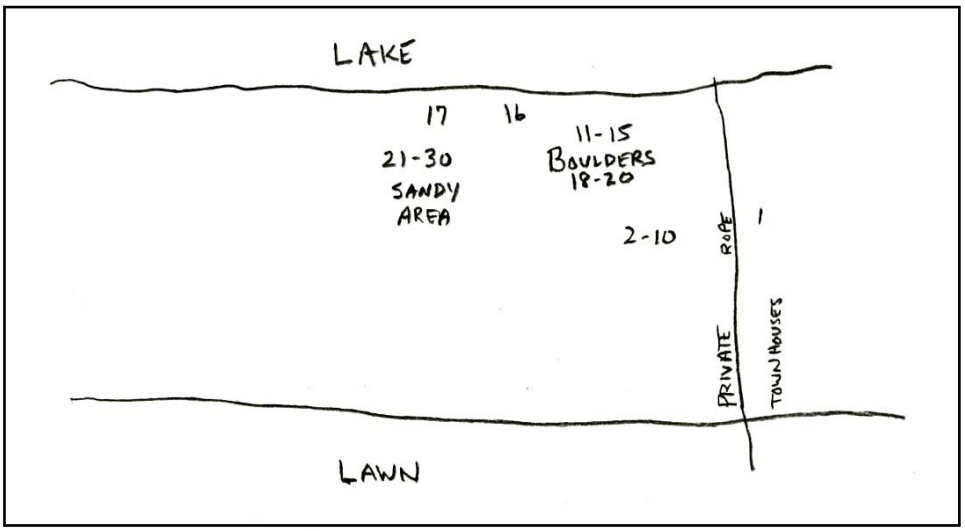
Locus	PGM1	PGM2			SKD1		TPI1		TPI2
Allele	1	1	2	3	1	2	1	2	1
Migration	43	31	33	24.5	32	37	55	51	43
Rorippa subumbellata									
4H 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Baldwin 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Baldwin 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Blackwood North 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Blackwood South 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Blackwood South 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Cascade West 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Eagle Creek 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Bay Avalanche, Native 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Emerald Bay Avalanche, Native 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Bay Avalanche, Planted 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Emerald Point 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Emerald Point 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Lighthouse 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Lighthouse 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Meeks Bay 2002	1.000	1.000	0.000	0.000	1.000	0.000	*	*	*
Meeks Bay 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Pope Beach 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Regan/Al Tahoe 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Rubicon 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Sugar Pine 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tahoe Keys 2003	1.000	0.948	0.000	0.052	1.000	0.000	1.000	0.000	*
Tahoe Meadows 2003	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tallac Creek 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Tallac Enclosure 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Taylor Creek East 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
Taylor Creek Mouth 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Taylor Creek West 2002	*	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Upper Truckee East 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Upper Truckee West 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
Zephyr Spit 2002	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	*
R. curvisiliqua									
Tallac Enclosure 2002	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	*
Taylor Creek Enclosure 2002	1.000	0.025	0.975	0.000	0.950	0.050	0.975	0.025	*

Appendix 1 (cont'd)

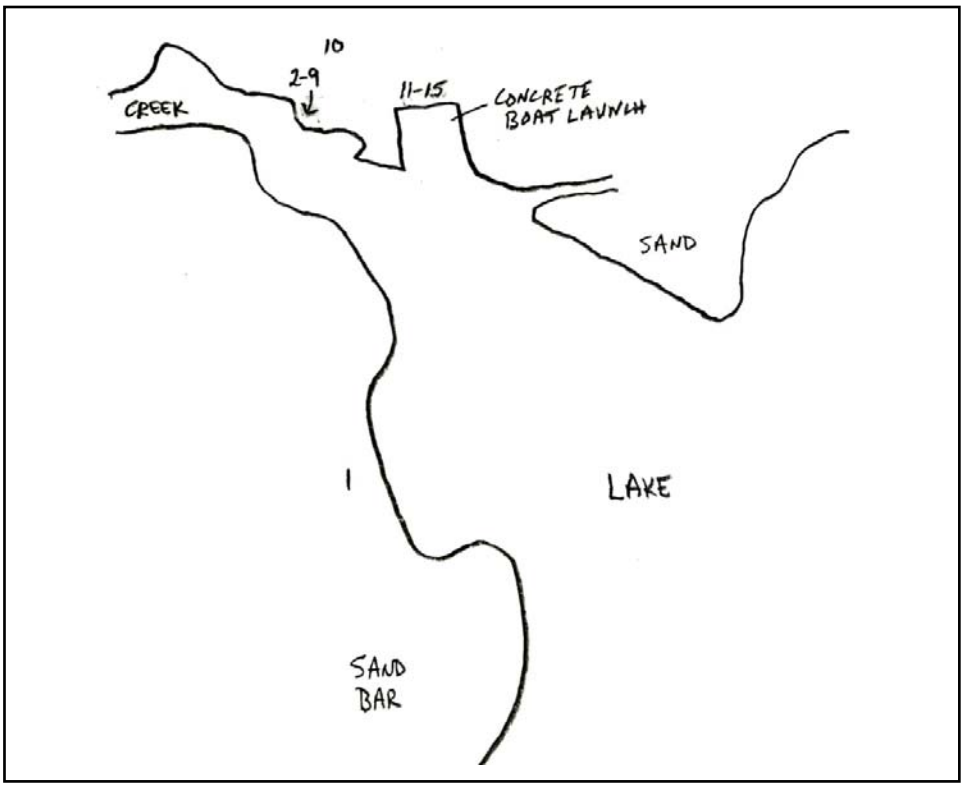
Locus	UGPP1		
Allele	1	2	3
Migration	47	40	52
Rorippa subumbellata			
4H 2003	1.000	0.000	0.000
Baldwin 2002	1.000	0.000	0.000
Baldwin 2003	1.000	0.000	0.000
Blackwood North 2003	1.000	0.000	0.000
Blackwood South 2002	1.000	0.000	0.000
Blackwood South 2003	1.000	0.000	0.000
Cascade West 2003	1.000	0.000	0.000
Eagle Creek 2003	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2002	1.000	0.000	0.000
Emerald Bay Avalanche, Native 2003	1.000	0.000	0.000
Emerald Bay Avalanche, Planted 2003	1.000	0.000	0.000
Emerald Point 2002	1.000	0.000	0.000
Emerald Point 2003	1.000	0.000	0.000
Lighthouse 2002	1.000	0.000	0.000
Lighthouse 2003	1.000	0.000	0.000
Meeks Bay 2002	1.000	0.000	0.000
Meeks Bay 2003	1.000	0.000	0.000
Pope Beach 2003	1.000	0.000	0.000
Regan/Al Tahoe 2002	1.000	0.000	0.000
Rubicon 2002	1.000	0.000	0.000
Sugar Pine 2002	0.850	0.150	0.000
Tahoe Keys 2003	1.000	0.000	0.000
Tahoe Meadows 2003	1.000	0.000	0.000
Tallac Creek 2002	0.909	0.091	0.000
Tallac Enclosure 2002	1.000	0.000	0.000
Taylor Creek East 2002	1.000	0.000	0.000
Taylor Creek Mouth 2002	1.000	0.000	0.000
Taylor Creek West 2002	1.000	0.000	0.000
Upper Truckee East 2002	1.000	0.000	0.000
Upper Truckee West 2002	1.000	0.000	0.000
Zephyr Spit 2002	1.000	0.000	0.000
R. curvisiliqua			
Tallac Enclosure 2002	0.000	0.000	1.000
Taylor Creek Enclosure 2002	0.050	0.000	0.950

Appendix 2. Location maps of 2002-2003 collection sites showing genetic variation. Maps are not to scale. Individual plant collections are indicated with number.

Site = Tahoe Keys (2003 collection). Genetically variable plants = #11 and #18. (Site mapped by J. DeWoody).

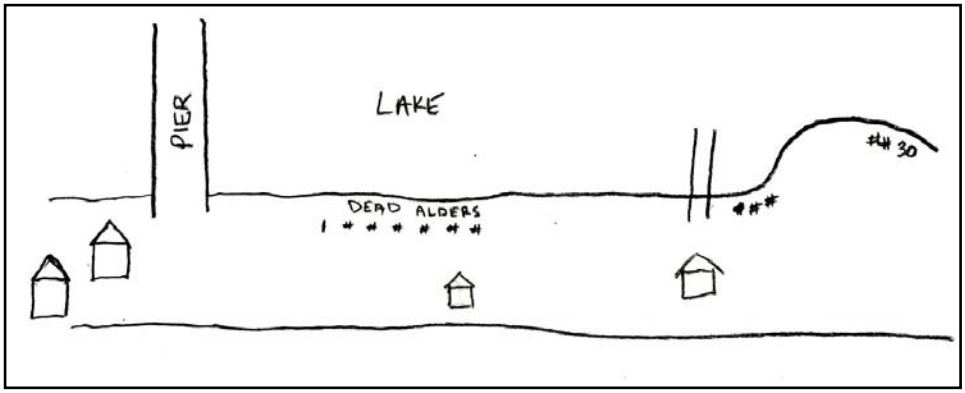


Site = Eagle Creek (2003 collection). Genetically variable plant = #3. (Site mapped by J. DeWoody).

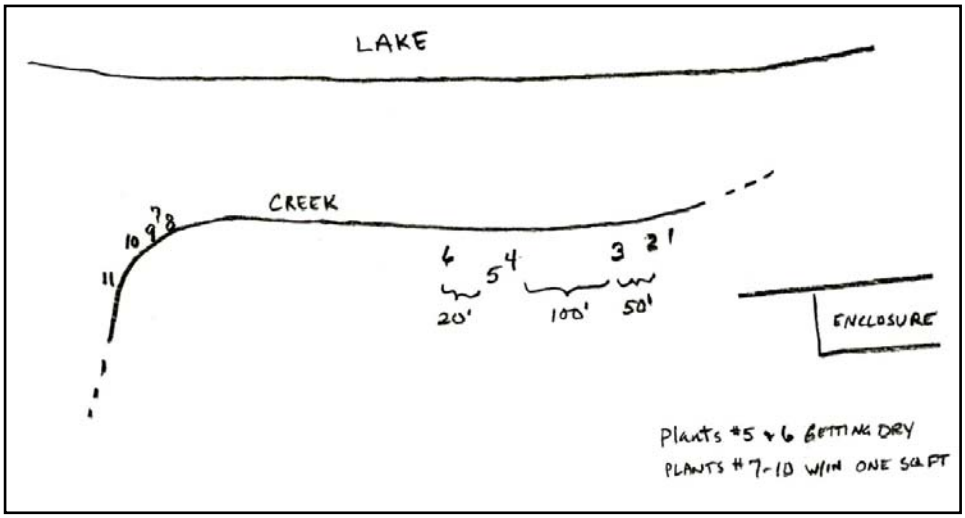


Appendix 2, continued.

Site = Sugar Pine (2002 collection). Genetically variable plants = #1 thru #9. (Site mapped by J. Fraiser). Individual plants were collected in order from #1 (northern most sample) to #30 (southern most sample).



Site = Tallac Creek (2002 collection). Genetically variable plant = #4. (Site mapped by V. Hipkins).



Appendix 3. Summary of Expenditures.

Item	Cost (dollars)
Rorippa subumbellata	
Plant material collection (24 sites, 546 plants)	
NFGEL staff (1 GS-9, 2 GS-5), 10 hrs	$924
Supplies and mileage	$120
Plant material laboratory preparation	
546 individuals/site	$1,818
Genetic Analysis	
546 individuals	$11,591
Analysis and Reporting	$310
Overhead (18%)	$2,658
Subtotal	$17,421
Rorippa curvisiliqua	
Plant material collection (2 sites, 20 plants per site)	
NFGEL staff labor	$128
Supplies and mileage	0
Plant material laboratory preparation	
40 individuals	$133
Genetic Analysis	
40 individuals	$849
Overhead (18%)	$200
Subtotal	$1,310
Total	$18,731