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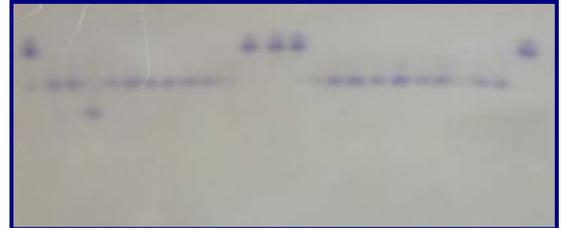
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Service

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Genetic Electrophoresis
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Project Report

Genetic Monitoring of *Rorippa subumbellata* (Tahoe Yellow Cress):



Analyzing Northeast Populations and Monitoring South Shore Populations.
(An SNPLMA Project; In Service Agreements 0519-05-607, 0519-06-601)



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NFGEL Project #194
Submitted: January 31, 2006



MANAGEMENT SUMMARY

This report details the results of the third study of the genetic structure of *Rorippa subumbellata* on the shores of Lake Tahoe. The following conclusions are based on a total of 430 plants sampled from 21 populations and assayed for 22 isozyme loci.

- 1) What is the genetic structure of *R. subumbellata* population on the northeastern shore of Lake Tahoe?

Ten sites were inspected for *R. subumbellata* on the east shore of Lake Tahoe in Nevada. Seven of the sites contained *R. subumbellata* plants, and a total of 152 plants were sampled for genetic analyses. Five plants in three populations contained genetic variation: Round Hill, Sand Harbor Enclosure, and Hidden Beach Enclosure. The pattern of genetic variation observed over all populations on the northeast shore is similar to that observed in populations on the south and west shores.

- 2) Do populations contain the same variation as was observed in the 1999/2002/2003 studies?

Seven populations that contained alternate alleles in at least one previous study were resampled in 2005. Five of these seven populations contained variation. One of the populations lacking variation (Eagle Creek) was sampled completely, and has likely lost the alternate allele through genetic drift. The other population (Upper Truckee East) is the largest population, and may contain variation in the individuals not sampled in this study.

Two of the five alternate alleles observed in previous studies were observed in the 2005 collections. Two of the three alleles not observed in this study have likely been lost to drift since those populations in which they were first reported were sampled completely (Eagle Creek and Tahoe Keys). The third allele may be present but unsampled in the relatively large population where it was first reported (Taylor Creek Enclosure 2). Four of the populations sampled in 2005 contained different alternate alleles than reported at these sites in earlier studies. This finding is likely due to regeneration of plants from dormant rootstock, the recruitment of plants from a seed bank, or the migration of seed among populations.

- 3) Are seed collection and outplanting efforts capturing any genetic variation observed in native populations?

Ex situ propagation efforts are capturing the genetic variation observed in native populations. Seven populations containing outplanted individuals were sampled, and three of those populations contained alternate alleles (Hidden Beach Enclosure, Sand Harbor Enclosure, and Taylor Creek Enclosure 2). A greater proportion of all sampled outplanted populations contain variation (43%) than sampled natural populations (23%), but the location of the outplanted sites results in an even distribution of genetic variation geographically around Lake Tahoe. Based on outplanting records, the sources of the genetic variation observed in outplantings on the northeast shore are populations located on the south shore. Thus, outplanting efforts are successfully managing the limited genetic variation observed in this species.

4) What is the mating system of *R. subumbellata*?

This objective was not addressed in 2005 due to a lack of coordination with the contractor overseeing the *ex situ* propagation and the lack of extra *R. subumbellata* plants from the 2005 outplantings. NFGEL is able and willing to perform this work in 2006, if desired.

INTRODUCTION

Rorippa subumbellata (Brassicaceae), or Tahoe yellow cress, is endemic to the sandy shores of Lake Tahoe in California and Nevada. Although *R. subumbellata* may be locally abundant at some sites, it is generally threatened by human impact, such as the intense use of public beaches, development within the shorezone, and raking on private lands. As a result, *R. subumbellata* is currently listed as endangered by the California and Nevada state governments, fully protected by the Tahoe Regional Planning Agency, and is under consideration for protection by the U.S. Endangered Species Act of 1973, as amended (Pavlik et al. 2002).

Due to the rare nature of this plant, several federal, state, and local agencies formed a coalition to develop and implement a conservation strategy (CS) for *R. subumbellata* (Pavlik et al. 2002). The fourth goal of the CS is to conduct research designed to optimize restoration activities and incorporate new findings into the management of *R. subumbellata* (Pavlik et al. 2002). As part of this goal, two studies were completed to investigate the genetic structure of populations located on the west and south shores of Lake Tahoe (Saich and Hipkins, 2000; DeWoody and Hipkins, 2004), but samples were not collected any further north or east than Zephyr Spit on the east shore in Nevada. These studies revealed extremely low amounts of genetic variation in *R. subumbellata*, and emphasized the possibility for that variation to be lost through genetic drift as populations decline (Saich and Hipkins 2000; DeWoody and Hipkins 2004). These previous studies also identified temporal differences in the amount of variation observed in populations of *R. subumbellata* (DeWoody and Hipkins 2004).

R. subumbellata is capable of both vegetative and sexual reproduction, and seeds are thought to float some distance on the water, providing a mechanism for colonization of appropriate habitat. Evidence of local extirpations followed by recolonization has been found through the annual surveys of historic and extant sites of *R. subumbellata* populations (Pavlik et al. 2002). These metapopulation dynamics (recurrent extirpation and colonization) may account for the variance in the levels of genetic variation observed across collections. In addition, the mating system of *R. subumbellata* may also affect the year-to-year genetic structure of populations, especially following episodes of abundant recruitment from seed.

The following study reports the genetic structure of *R. subumbellata* populations located on the east shore of Lake Tahoe, as well as the persistence of variation in previously sampled sites. Specifically, the project was designed to address four questions: 1) What is the genetic structure of *R. subumbellata* populations on the northeastern shore of Lake Tahoe? 2) Do populations contain the

same variation as was observed in the 1999/2002/2003 studies? 3) Are seed collection and outplanting efforts capturing any genetic variation observed in native populations? 4) What is the mating system of *R. subumbellata*?

METHODS

A total of 430 samples were collected from 21 populations of *R. subumbellata* (Table 1). As in previous years, confusion in site names arose from the lack of standardized naming conventions for all locations of *R. subumbellata*. Table 1 describes the names given by NFGEL in this and previous collections, and follows conventions used during collection planning conferences with J. Fraser and J. Howard. Appendix C is provided to reconcile site names with those used by BMP Ecosciences (provided by A. Stanton, personal communication).

For large populations, up to thirty plants were sampled, and when less than thirty plants were present, the population was sampled completely. Data from the 2003 and 2004 studies were used to identify ten sites on the east shore of Lake Tahoe from which to sample: Hidden Beach, Sand Harbor (natural population on the northwest end of beach, not the natural plants recently recruited near the outplantings), Secret Harbor, Skunk Harbor, Glenbrook Bay, Cave Rock Harbor, Skyland, Zephyr Cove (called Zephyr Spit in 2002 and by BMP Ecosciences; see Appendix C), Marla Bay, and Round Hill. All ten sites were inspected for natural populations of *R. subumbellata*. Populations were located and samples were collected from seven of those sites (Table 1).

In order to assess temporal variance in genetic variation, seven sites containing genetic variation in either 1999 or 2002/2003 were re-sampled in 2005: Eagle Creek, Sugar Pine, Tahoe Keys, Tahoe Meadows, Tallac Creek, Taylor Creek Enclosure 2 (the permanent enclosure and the same location sampled in 1999), and Upper Truckee East. Finally, to assess if outplanting efforts are capturing the genetic variation observed in natural populations, up to thirty samples were collected from the outplanted or enclosed populations located at seven sites: Hidden Beach Enclosure, Sand Harbor Enclosure, Zephyr Cove Outplanted, Taylor Creek Enclosure 1 Natural (plants located within and outside the seasonal enclosure), Taylor Creek Outplanted (within the seasonal enclosure), Upper Truckee East Outplanted, and Emerald Bay Avalanche Outplanted.

Pollinator exclusion experiments were not conducted during 2005 due to a lack of coordination with the contractor overseeing the *ex situ* propagation, and the lack of extra *R. subumbellata* plants from the 2005 outplantings. NFGEL is able and willing to perform this work in 2006, if desired.

Samples were collected, stored, and prepared for isozyme analyses following DeWoody and Hipkins (2004). Starch gel electrophoresis took place following NFGEL Standard Operating Procedures (USDA Forest Service 2003). A total of 22 isozyme loci were assayed in three buffer systems. Eleven loci were resolved in a lithium borate electrode buffer-tris citrate gel buffer combination (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). Five loci were resolved in a sodium borate electrode buffer-tris citrate gel buffer combination (system SB): aspartate aminotransferase (AAT1), catalase (CAT1), glycerate-2-dehydrogenase (GLYDH1), triosephosphate isomerase (TPI2), and uridine diphosphoglucose pyrophosphorylase (UGPP1). Six loci were resolved in a morpholine citrate electrode and gel buffer (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. All samples with putative variation were re-analyzed to confirm alternate genotypes. Allele frequencies for loci displaying variation were calculated using the software Biosys-1 version 1.7 (Swofford and Selander 1989).

RESULTS

No *R. subumbellata* were observed at Secret Harbor, Skunk Harbor, or Cave Rock Harbor. More than thirty samples were collected from two sites that had large populations. Thirty-one samples were collected at Glenbrook Bay in order to include two plants that were located approximately 200 m from the larger population, and forty samples from Tahoe Meadows, which had a large, nearly continuous population along the beach depression. All electrophoretic patterns matched those observed in previous studies except GLYDH1, which produced a novel three-banded pattern without variation. Twelve of the 430 plants assayed at 22 isozyme loci displayed genetic variation (Table 2). This variation was observed in two loci: DIA1 and UGPP1. Three other alternate alleles described by DeWoody and Hipkins (2004) were not observed in these collections. One of the seven natural sites sampled on the east shore in Nevada contained variation (Round Hill; Tables 1 and 2, Appendix A). Variation was also observed at five of the seven sites that contained alternate alleles in previous studies (Tables 1 and 2, Appendix A). Two of the seven outplanted or enclosure sites contained genetic variation (Hidden Beach Enclosure and Sand Harbor Enclosure; Table 1).

DISCUSSION

As in previous studies, extremely low levels of genetic variation were observed in the 2005 collections of *R. subumbellata* (Table 2). Alternate alleles were observed at only two loci (DIA1 and UGPP1), compared with three loci in 1999 (DIA1, UGPP1, and PGI1; Saich and Hipkins 2000) and three loci in 2002/2003 (UGPP1, FEST1, PGM2; DeWoody and Hipkins 2004). The status of each population that showed variation in previous years and the implications of the lack of variation in 2005 will be discussed below.

Populations sampled from the east shore in Nevada have similar probabilities of containing genetic variation as those populations sampled on the west and south shores (Saich and Hipkins, 2000; DeWoody and Hipkins 2004). In all studies, a total of 26 sites have been sampled on the west and south shores, and variation has been observed at least once in seven of those sites, meaning that 27% of the populations sampled contained variation. A total of 12 sites have been sampled from the east shore (including outplantings and the 4H and Zephyr Spit sites from DeWoody and Hipkins 2004), and three of those contained alternate alleles, meaning that 25% of the populations contained variation. Thus, when considered over all sites and outplantings, genetic variation is evenly distributed across the metapopulation of *R. subumbellata* on Lake Tahoe.

As described by DeWoody and Hipkins (2004), temporal variation was observed in the location and types of variation in *R. subumbellata* populations. Two populations that contained alternate alleles in previous studies contained only the common genotype in the 2005 collections (Eagle Creek and Upper Truckee East). The Eagle Creek population was sampled completely in 2005, so the genetic variation observed at this site in previous studies has likely been lost through genetic drift. Upper Truckee East supports the largest *R. subumbellata* population but was only represented by 30 samples in the current study. Given the low frequency of the alternate allele, it is possible the allele is still present in the population but was not captured in this collection.

Similarly, three of the five alternate alleles described in previous studies were not observed in these collections. Two of these three alleles occurred in populations that were sampled completely in this study (FEST1-2 in Eagle Creek and PGM2-3 in Tahoe Keys), and were likely lost through genetic drift. The third allele, PGI1-2, was observed in the natural population collected at Taylor Creek Enclosure in 1999 (labeled this year as Taylor Creek Enclosure 2), and may still be present but was not sampled in that larger population.

In addition, the particular alternate allele(s) observed at a site is not consistent over collections (Table 3). Three mechanisms may explain this observation. First, new observations of alternate alleles may arise through the random mutation of existing alleles. Given that it is possible for

alternate alleles to be first observed in the homozygous state (Tables 2 and 3), which would require two identical mutation events, and that the newly observed alleles are shared by different populations and across years, this explanation is unlikely. Rather, these alleles likely share a single origin. Second, the alternate alleles may have always been present in the population but were not sampled in previous studies. This is especially likely for larger populations (e.g. Sugar Pine in 2002) where not every plant was sampled. Additionally, the alleles may have been present in the seed bank or in dormant roots (although this physiological feature has not been confirmed in *R. subumbellata*), which would explain the lack of variation in some years, even when every living plant was sampled. Third, the alleles may have migrated into these populations as seed or vegetative propagules. Further study into the persistence of the seed bank and mechanism for dispersal of seed is necessary to determine which explanation is most likely.

Outplanted sites have a greater probability of containing variation than natural populations. Eight of the 35 natural populations sampled in total contained alternate alleles in at least one year, meaning the populations have a 23% probability of containing variation. Three of the seven sites that contain outplanted individuals contained alternate alleles, meaning that outplanted populations have a 43% chance of containing variation. Although this rate may seem inflated compared to the natural populations, given the location of the outplanted sites, the variation contained in them has served to mimic the natural geographic distribution of variation. For instance, two of the three populations containing variation on the east shore happen to be outplanted populations that contain variation (Sand Harbor Enclosure and Hidden Beach Enclosure). Lacking these outplanted sites, only one population on the east shore would contain an alternate allele (Round Hill), meaning 10% of those populations would contain variation. In this case, these outplantings have served to augment not only the size of the populations on the east shore, but also increase the variation contained in those populations. It is important to note that the seed sources for the outplantings, and thus the source of the variation observed on the east shore, are populations located on the south shore of Lake Tahoe (Appendix D). In this manner, *ex situ* collections and outplantings are capturing and conserving two of the alternate alleles observed in *R. subumbellata* (DIA1-2 and UGPP1-2). Other alternate alleles previously observed in this species were not observed in 2005 (FEST1-2, PGI1-2 and PGM1-2). Additionally, the third outplanted population that contained alternate alleles (Taylor Creek Enclosure 2) was sampled as a natural population in 1999 (the outplanted individuals were introduced in 2003 and 2004), at which time it contained variation (Saich and Hipkins, 2000). Based on the records from the outplanting efforts (Appendix D), the plants containing alternate alleles may be either outplanted individuals or remnants from the natural population. Since 1999, alternate alleles may have been lost

DeWoody and Hipkins, 2005
at two loci (PGI1 and UGPP1) but gained at a third (DIA1) at Taylor Creek Enclosure 2. This serves as further evidence of temporal variation in genetic structure in *R. subumbellata*. Genetic data from this study can be used as a baseline to monitor future outplanting efforts.

Together, these findings demonstrate the lack of genetic structure among populations of *R. subumbellata*, support the current design of *ex situ* collections and outplanting efforts, and confirm the potential for migration among populations. The populations on the east shore of Lake Tahoe contained no novel alleles, and most contained the common genotype. Three outplanted populations contain a total of two alternate alleles, and the location of these populations on the east shore mimics the natural pattern of genetic variation observed on the south and west shores. Migration is the most likely explanation for the temporal variation in the alternate allele observed at a site, which is consistent with the metapopulation dynamics observed through annual surveys. These findings further confirm the potential for *R. subumbellata* to migrate among habitat patches, and support the importance of unoccupied potential habitat for the long-term persistence of this species.

REFERENCES

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- Pavlik, B. *et al.* 2002. Conservation strategy for Tahoe yellow cress (*Rorippa subumbellata*). Tahoe Regional Planning Agency. Lake Tahoe, Nevada.
- Saich, R.C. and V.D. Hipkins. 2000. Evaluation of genetic diversity in Tahoe yellow cress (*Rorippa subumbellata*). National Forest Genetic Electrophoresis Laboratory. USDA Forest Service, Camino, California.
- 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.
- USDA Forest Service. 2003. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. Placerville, California.

Table 1. Summary of *Rorippa subumbellata* populations analyzed by NFGEL. The number of samples analyzed each year (1999, 2002, 2003, and 2005) is listed for each population. The abbreviations are used to depict population sites in Figure 1. Numbers in **bold** indicate that variation was observed at that site.

Population Name	Abbrev.	1999	2002	2003	2005
4H	4H			30	
Baldwin	B	4	2	3	
Blackwood North	BN			24	
Blackwood South	BS	27	5	30	
Cascade West	CW			8	
Eagle Creek	EC			15	24
Edgewood	E	18			
Emerald Bay Avalanche, Natural	EAN		1	60	
Emerald Bay Avalanche, Outplanted	EAP			15	30
Emerald Point	EP		7	30	
Glenbrook Bay	GL				31
Hidden Beach, Natural	HB				1
Hidden Beach, Enclosure	HBE				10
Kahle/Nevada	K	7			
Lighthouse	L	18	10	35	
Marla Bay	MAB				2
Meeks Bay	MB		5	7	
Pope Beach	P			4	
Regan/Al Tahoe	RAT		18		
Round Hill	RH				14
Rubicon	R		30		
Sand Harbor Enclosure	SAE				30
Sand Harbor Natural	SAH				6
Skyland	SK				11
Sugar Pine	SP		30		22
Tahoe Keys	TK			30	26
Tahoe Meadows	TM	8		12	40
Tallac Creek	TC		11		3
Tallac Enclosure	TE	13	10		
Taylor Creek East	TCE		12		
Taylor Creek Enclosure	TAY	10			
Taylor Creek Enclosure 1 Natural	TAY1				13
Taylor Creek Enclosure 2	TAY2				30
Taylor Creek Mouth	TCM		10		
Taylor Creek Outplanted	TCO				30
Taylor Creek West	TCW		31		
Upper Truckee East	UTE	33	30		30
Upper Truckee East Outplanted	UO				30
Upper Truckee West	UTW	2	30		
Zephyr Cove	ZC				17
Zephyr Cove Outplanted	ZCO				30
Zephyr Spit	ZS		8		

Table 2. Location and description of variation observed in the 2005 collections. “Number of Plants” indicates the number of samples having the indicated genotype. The common allele at each locus is designated as “1” and the alternate allele as “2.”

Population	Number of Plants	Locus	
		DIA1	UGPP1
Hidden Beach Enclosure	1	11	12
Round Hill	2	12	11
Sand Harbor Enclosure	1	11	12
	1	11	22
Sugar Pine	1	12	11
Tahoe Keys	2	11	22
Tahoe Meadows	1	12	12
Tallac Creek	1	11	22
Taylor Creek Enclosure 2	2	12	11

Table 3. Temporal changes in observed genetic variation in *R. subumbellata*. # of Plants refers to the number of samples having the indicated genotype. The common allele at each locus is designated as “1” and the alternate allele as “2.” See text for instances where genetic variation was lost from a population.

Population	Year	# of Plants	Locus				
			DIA1	UGPP1	FEST1	PGI1	PGM2
Sugar Pine	2002	9	11	12	11	11	11
Sugar Pine	2005	1	12	11	11	11	11
Tahoe Keys	2003	1	11	11	11	11	13
Tahoe Keys	2003	1	11	11	11	11	33
Tahoe Keys	2005	2	11	22	11	11	11
Taylor Creek Enclosure	1999	2	11	22	11	12	11
Taylor Creek Enclosure	2005	2	12	11	11	11	11
Tahoe Meadows	1999	3	22	11	11	11	11
Tahoe Meadows	2005	1	12	12	11	11	11

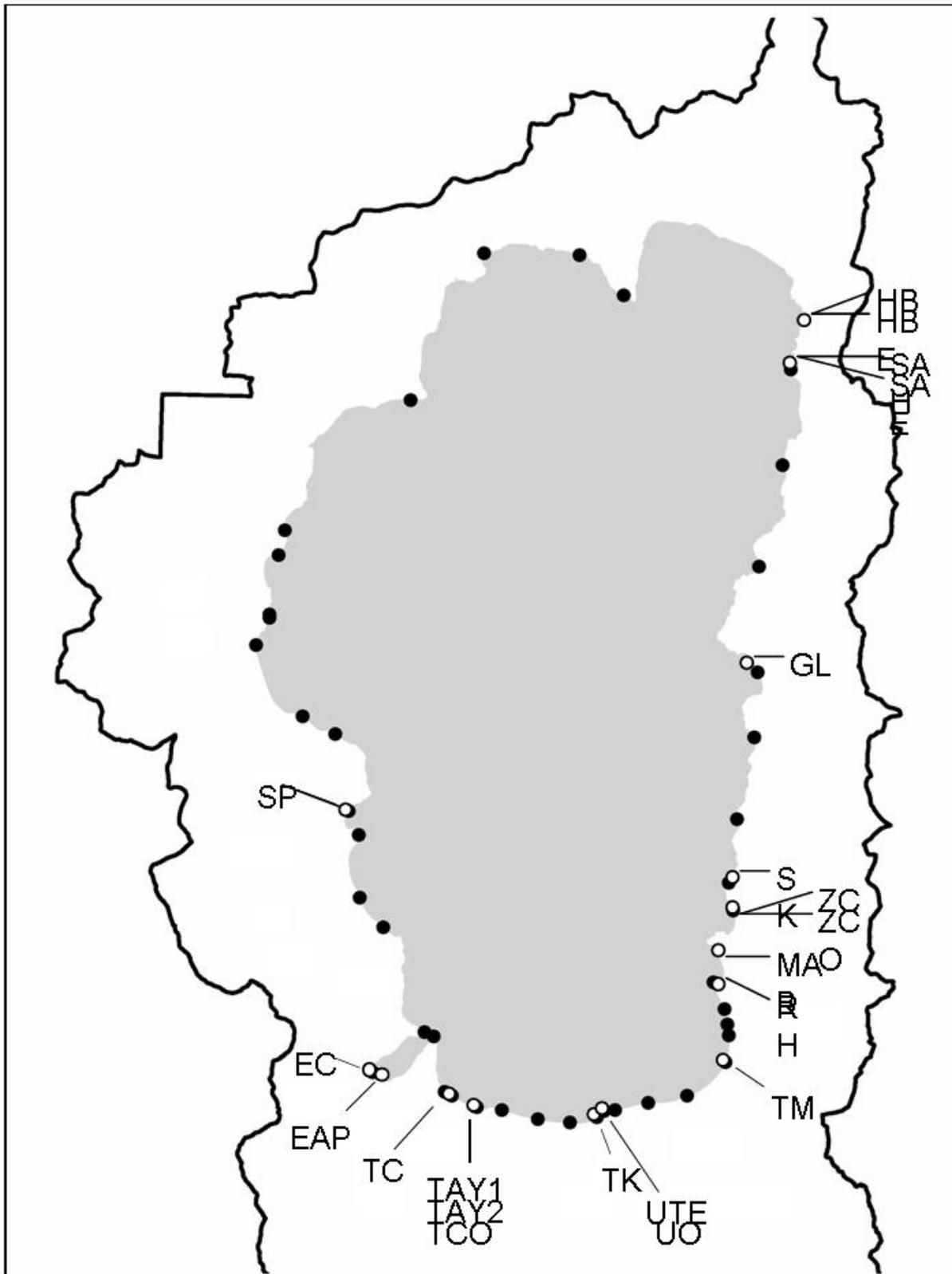


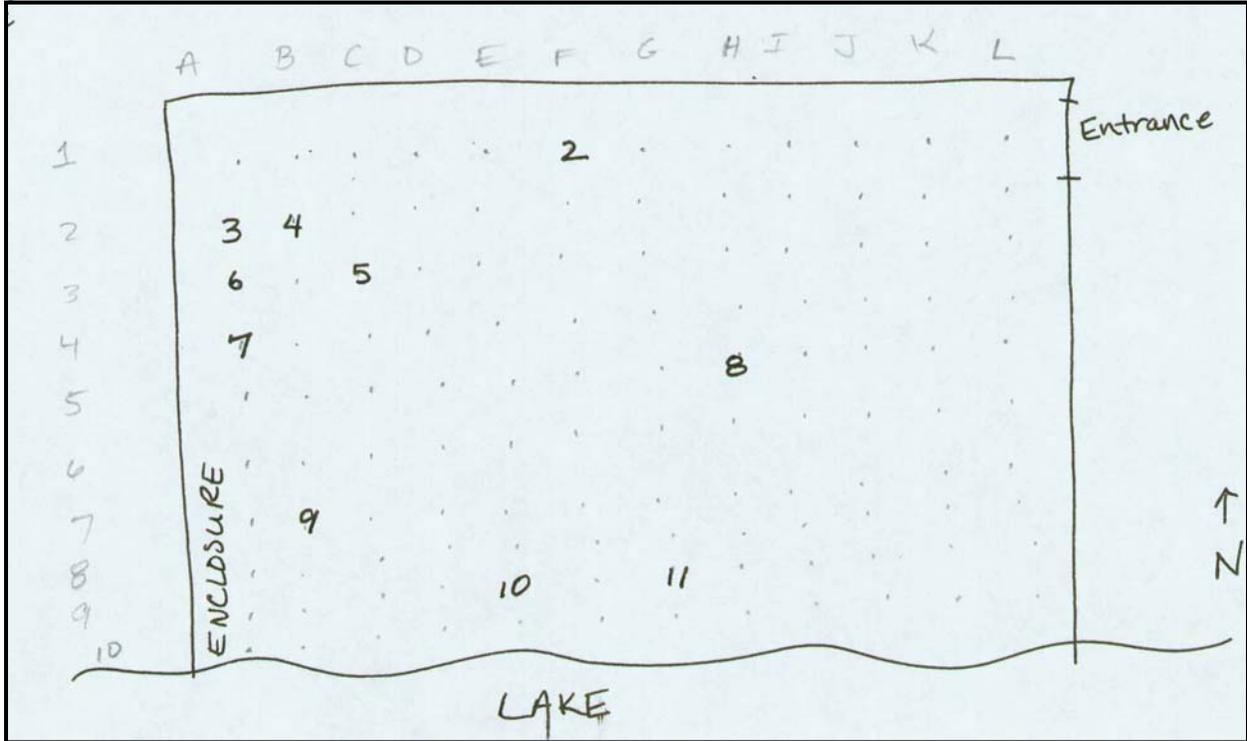
Figure 1. Location of *R. subumbellata* sites sampled in 2005. Open circles represent those populations sampled, with abbreviations identifying each site. Solid gray circles represent historic locations of populations. From Pavlik *et al.* 2002.

Appendix A. Allele frequencies for those two loci displaying variation in the 2005 collections. N is the mean number of samples at a locus.

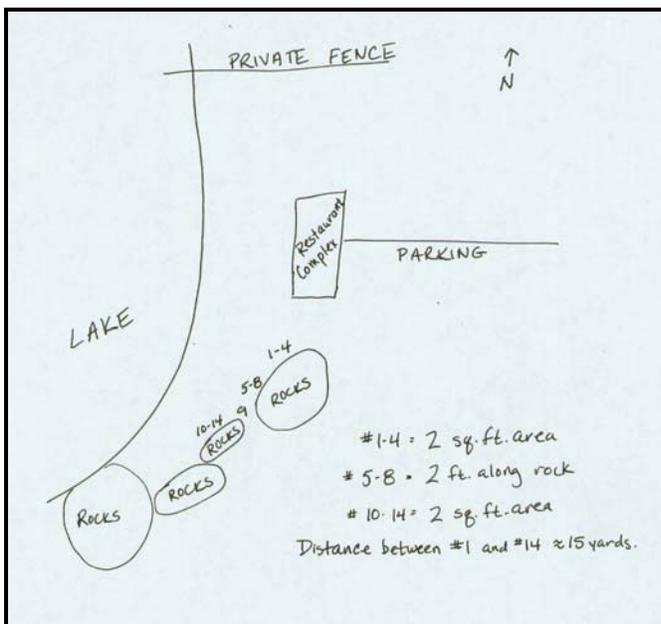
Population	N	UGPP1-1	UGPP1-2	DIA1-1	DIA1-2
Eagle Creek	23	1		1	
Emerald Bay Avalanche, Planted	30	1		1	
Glenbrook	31	1		1	
Hidden Beach	11	0.955	0.045	1	
Marla Bay	2	1		1	
Round Hill	14	1		0.929	0.071
Sand Harbor Enclosure	30	0.95	0.05	1	
Sand Harbor Native	6	1		1	
Skyland	11	1		1	
Sugar Pine	18	1		0.964	0.036
Tahoe Keys	24.5	0.923	0.077	1	
Tahoe Meadows	39	0.988	0.013	0.987	0.013
Tallac Creek	3	0.667	0.333	1	
Taylor Creek Enclosure 1	13	1		1	
Taylor Creek Enclosure 2	30	1		0.967	0.033
Taylor Creek Outplanted	28	1		1	
Upper Truckee East	30	1		1	
Upper Truckee East Outplanted	29.5	1		1	
Zephyr Cove	17	1		1	
Zephyr Cover Outplanted	30	1		1	

Appendix B. Location of *R. subumbellata* samples containing genetic variation at eight sites. Numbers represent sample identification and location. Maps are not to scale.

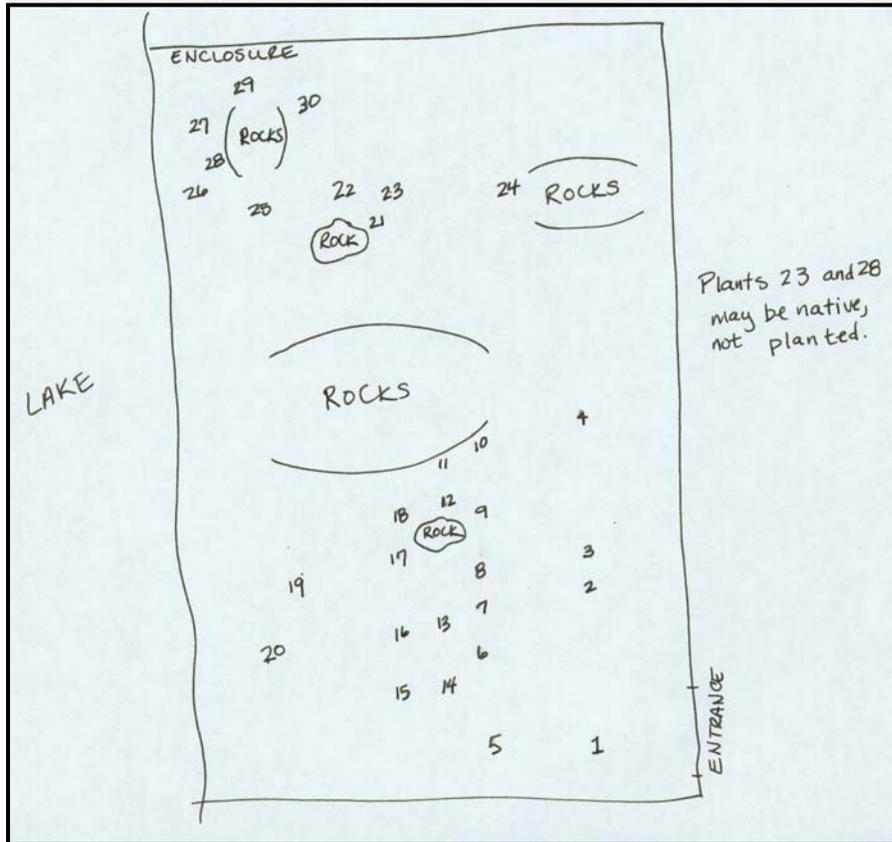
Site: Hidden Beach Enclosure. Genetically variable plant: #11 (Site mapped by J. DeWoody)
Grid coordinates assigned by NFGEL.



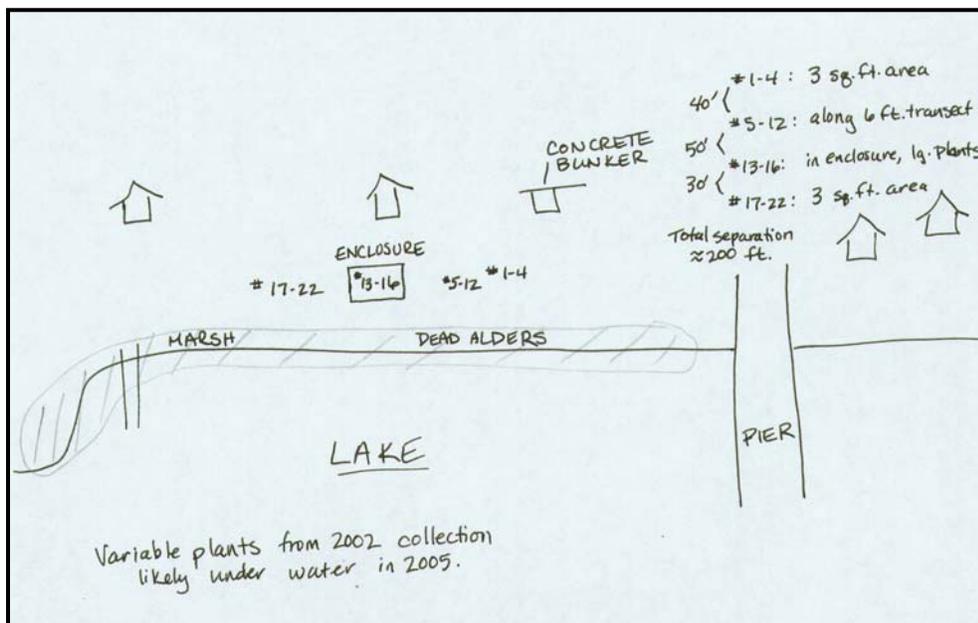
Site: Round Hill. Genetically variable plants: #9, #13 (Site mapped by V. Hipkins)



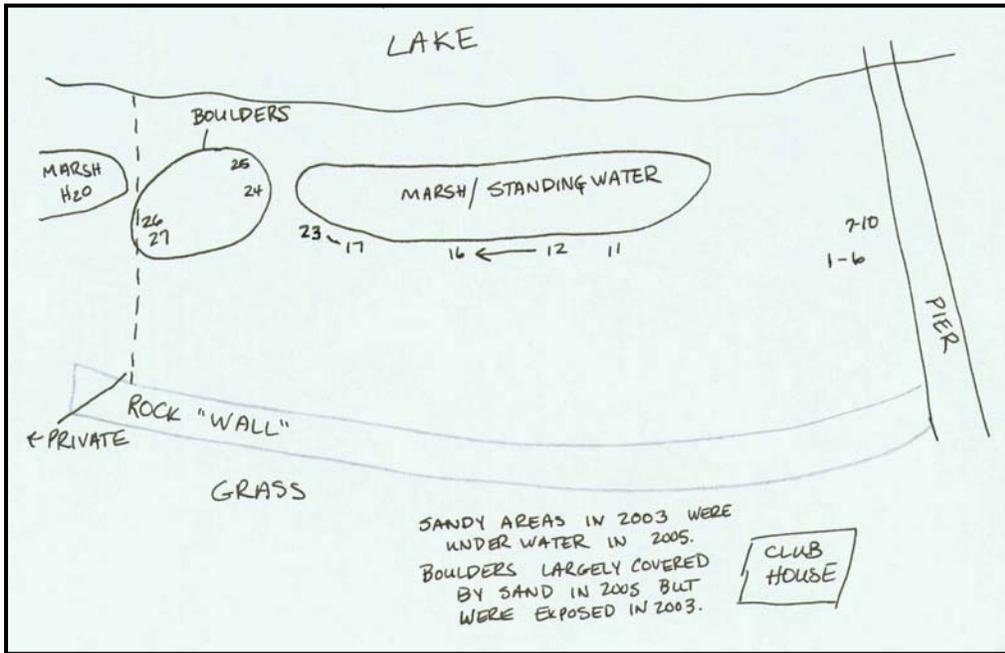
Site: Sand Harbor Enclosure. Genetically variable plants: #8, #11 (Site mapped by R. Hernandez)



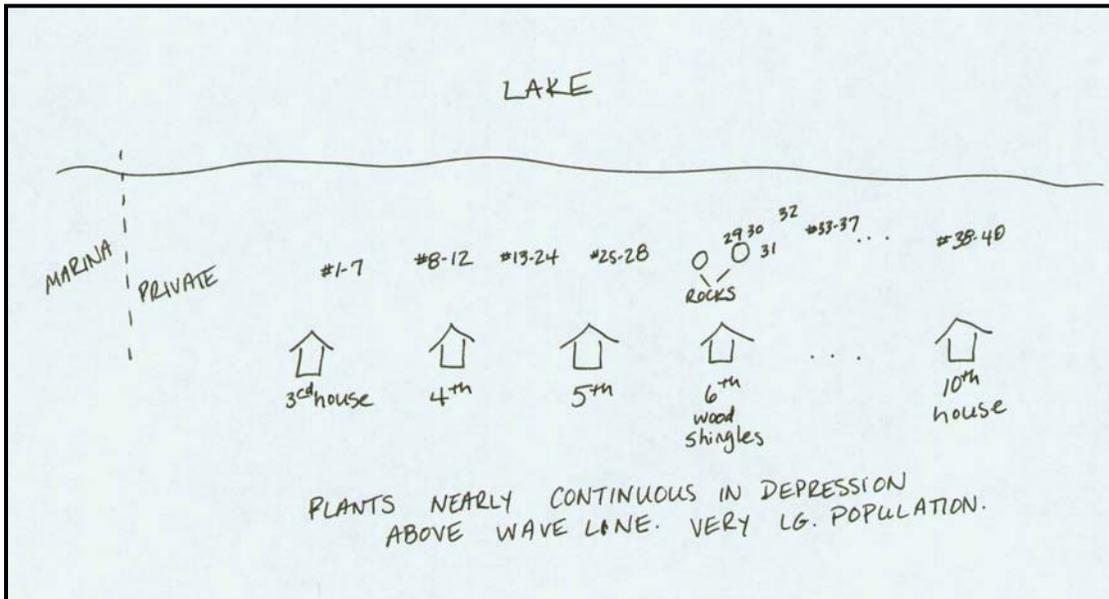
Site: Sugar Pine. Genetically variable plant: #9 (Site mapped by V. Hipkins)



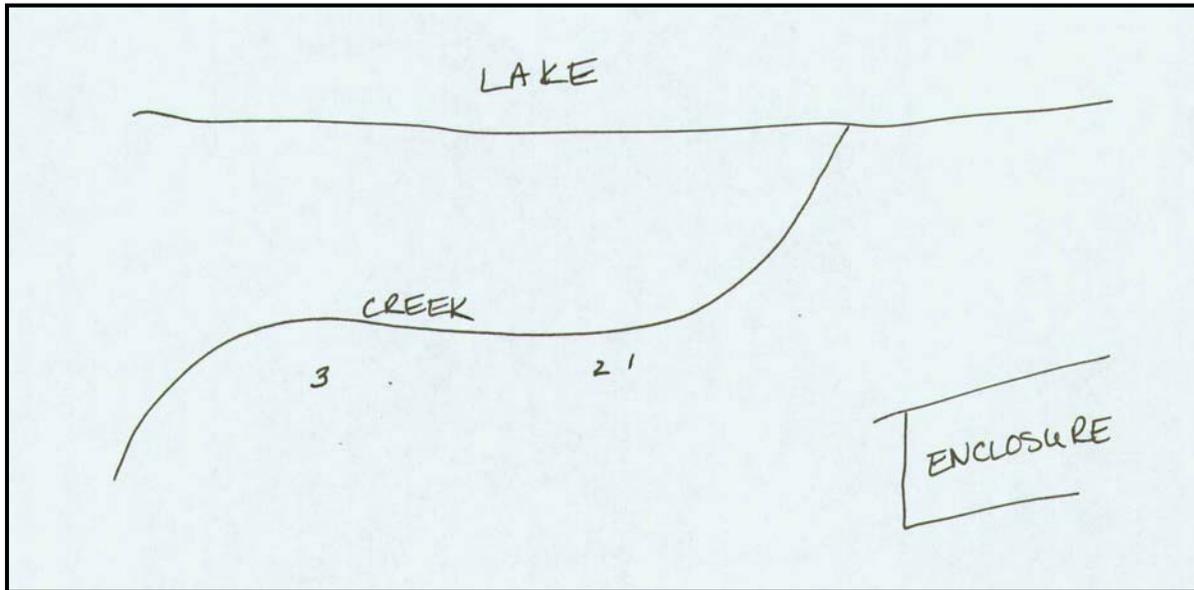
Site: Tahoe Keys. Genetically variable plants: #13, #26 (Site mapped by V. Hipkins)



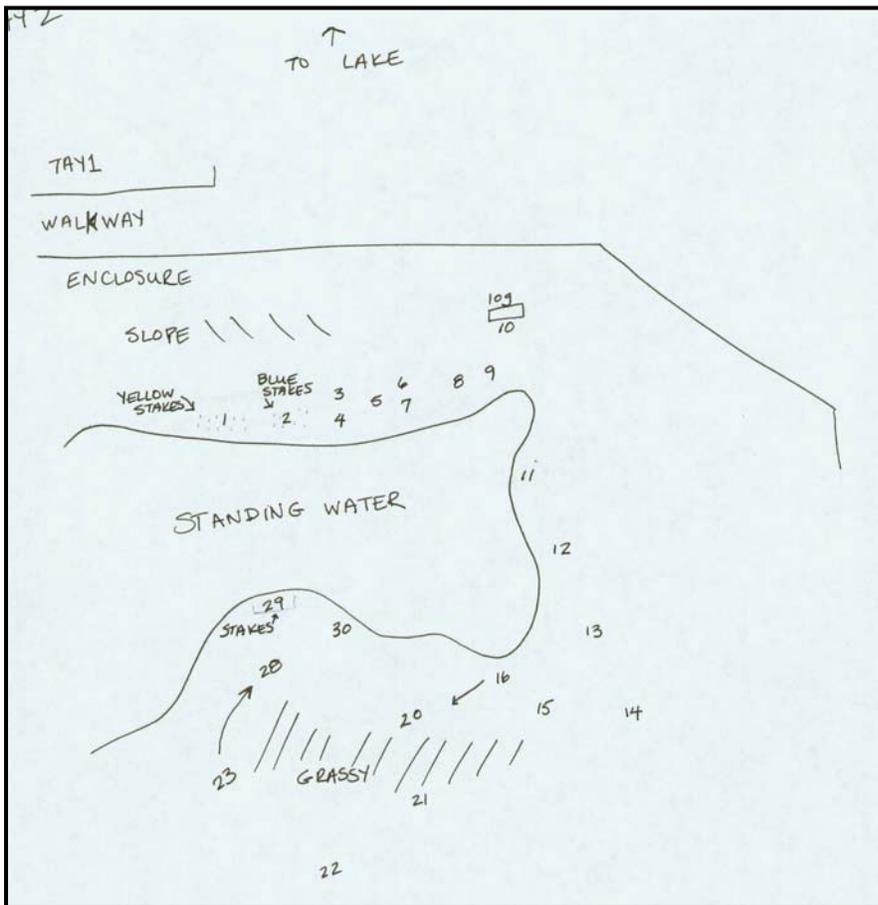
Site: Tahoe Meadows. Genetically variable plant: #39 (Site mapped by J. DeWoody)



Site: Tallac Creek. Genetically variable plant: #3 (Site mapped by J. DeWoody)



Site: Taylor Creek Enclosure 2. Genetically variable plants: #5, #27
(Site mapped by J. DeWoody)



Appendix C. Reconciling site names used by NFGEL and BMP Ecosciences.

NFGEL Site Name	Year/s Collected	BMP Ecosci. 2003 Site Name	Comments
4H	2003		
Baldwin	1999, 2002, 2003		Notes indicate NFGEL's site is distinct from Stanton's "Baldwin Beach".
Blackwood North	2003	Blackwood Creek N	
Blackwood South	1999, 2002, 2003	Blackwood Creek S	
Cascade West	2003	Cascade	
Eagle Creek	2003, 2005		
Edgewood	1999	Edgewood	
Emerald Bay Avalanche, Native	2002, 2003		
Emerald Bay Avalanche, Planted	2003, 2005		
Emerald Point	2002, 2003		
Glenbrook Bay	2005		
Hidden Beach, Native	2005		
Hidden Beach, Enclosure	2005		
Kahle/Nevada ^A	1999		
Lighthouse	1999, 2002, 2003	Lighthouse	
Marla Bay	2005		
Meeks Bay	2002, 2003	Meeks Bay Enclosure	Historic outplanting, 1988.
Pope Beach	2003	Pope Beach	
Regan/Al Tahoe	2002		
Round Hill	2005		
Rubicon	2002		Stanton indicates multiple areas in Rubicon Bay containing Rorippa Unclear which one was sampled.
Sand Harbor Enclosure	2005		NFGEL did not observe "native" plants observed by Stanton outside the Enclosure.
Sand Harbor Native	2005	Sand Harbor	NFGEL collection corresponds to Stanton's sighting on NW end of beach.
Skyland	2005		
Sugar Pine	2002, 2005		
Tahoe Keys	2003, 2005		
Tahoe Meadows	1999, 2003, 2005	Tahoe Meadows	NFGEL collections may cover a larger area than Stanton indicates
Tallac Creek	2002, 2005	Tallac Creek (outside of enclosure)	
Tallac Enclosure	1999	Tallac Enclosure	Historic outplanting, 1988.
Taylor Creek East	2002	Taylor Creek	
Taylor Creek Enclosure	1999	Taylor Creek Enclosure	Permanent enclosure
Taylor Creek Enclosure 1 Native	2005	Taylor Creek?	Not clear a distinction is made between the native plants and the outplanted plants in the temporary enclosure at Taylor Creek.
Taylor Creek Enclosure 2	2005	Taylor Creek Enclosure	Same as Taylor Creek Encl. (1999)
Taylor Creek Mouth	2002	Taylor Creek	
Taylor Creek Outplanted	2005		Temporary enclosure, removed after growing season.
Taylor Creek West	2002	Taylor Creek	
Upper Truckee East	1999, 2002, 2005	Upper Truckee East	
Upper Truckee East Outplanted	2005		
Upper Truckee West	1999, 2002	Upper Truckee West	
Zephyr Cove ^B	2005		Same site as Zephyr Spit 2002
Zephyr Cove Outplanted ^B	2005		
Zephyr Spit	2002		

^A Large outplanting at Kahle/Nevada exists, but was unknown to NFGEL during collections, thus not sampled in 2005.

^B Stanton uses Zephyr Cove for plants on private land, Zephyr Spit for USFS beaches.

Appendix D. Seed sources for outplantings sampled in 2005. Adapted from A. Stanton, BMP Ecosciences, personal communication.

Seed Source:	Blackwood	Cascade	Taylor Creek	Tallac	Lighthouse	Upper Truckee East	Regan Al Tahoe	Tahoe Meadows	Edgewood
Ouplanted Population:									
Avalanche	X		X			X		X	X
Taylor Creek (both enclosures)		X	X	X	X	X	X	X	
Upper Truckee East	X		X	X	X	X	X		X
Zephyr Cove	X		X			X		X	X
Sand Harbor		X	X	X	X	X	X	X	
Hidden Beach						X			