Genetic Distinctness and Variability of *Sedum integrifolium ssp. leedyi* Final Report

BY

Joel P Olfelt' and Glenn R. Furnier[2]

Departments of Plant Biology' and Forest Resources[2]
University of Minnesota
1530 N. Cleveland Ave.
St. Paul, MN 55108-6112

TO:

Richard Baker
Natural Heritage and Nongame Research Program
Minnesota Department of Natural Resources
500 Lafayette Rd.
St. Paul, MN 55155-4007

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Abstract

Leedy's roseroot *(Sedum integrifolium ssp. leedyi) is* on the Minnesota endangered, and federally threatened species lists. The federal status depends on Clausen's (1975) description of the taxon as distinct, however, his sample sizes are small, and his description is open to question. We have sampled plants from Minnesota, New York, and western S. *integrifolium* populations, and analyzed them using random amplified polymorphic DNA (RAPD) markers as well as morphological markers. We report progress in techniques we plan to use for a common garden experiment as well as preliminary analyses of genetic distances and variation within and among S. *integrifolium* taxa. These analyses suggest that there are significant differences between Leedy's roseroot and the other subspecies of S. *integrifolium,* and they show little genetic variation in Leedy's roseroot. The variation and genetic distances within *S. integrifolium* are compared with genetic distances and variation within and among *S. rosea, S. rhodanthum* ,and S. *integrifolium.* These parameters are important for decisions of whether and how to protect Leedy's roseroot populations.

Introduction

Sedum integrifolium is a 10 to 40 cm tall plant that generally grows on moist rocky slopes or cliffs. Robert Clausen (1975), an authority on the *Sedum* of North America, divides *Sedum integrifolium* into four distinct subspecies. *Two* of these subspecies are widespread at high elevations in western North America. A third western subspecies is known to occur only on Sierra Blanca, a so-called sky island, in New Mexico. The fourth subspecies, Leedy's roseroot, *(S. integrifolium ssp. ieedyi) is* known to occur at low elevations in four southeastern Minnesota locations, and at Seneca Lake in New York state. Leedy's roseroot is on the Minnesota endangered, and the federally threatened species lists. The threatened status of Leedy's roseroot depends on Clausen's description of the taxon as distinct from the other subspecies. His description of the four subspecies is based on morphological data collected in the field, as well as on a very small common garden experiment (as few as 1 plant for some characters). Thus Clausen's work is based primarily on data that may only reflect environmental differences rather than genetic differences. This makes the division of *S. integrifolium* into four subspecies questionable, and has led wildlife workers to call for more data on Leedy's roseroot's genetic distinctness as well as its variability (U.S Fish and Wildlife Service 1993). These data can be useful in setting conservation priorities both for the taxon, and for its individual populations.

The taxon is interesting not only because of the conservation problems it poses, but also because of its distribution, which is a natural experiment in plant evolution. All of the Leedy's roseroot populations are more than 1000 km from the nearest populations of western *S. integrifolium* subspecies. Thus gene flow between Leedy's roseroot and the western subspecies must be negligible, and the groups can evolve independently. Also, since Leedy's roseroot grows at low elevations, it may be subject to different selective pressures than the western subspecies.

Both DNA based molecular markers and morphological markers are used in this study. DNA based RAPD (random amplified polymorphic DNA) markers are useful genetic markers that require only a few nanograms of DNA per assay (Williams et. a1.1990). With them, large numbers of genetic markers are relatively easy to obtain so that
precise estimates of variation and distance can be made. The small amount of DNA needed makes them a good choice for studies of protected plants such as Leedy's roseroot where only small amounts of tissue can be collected from each plant. In general, DNA based molecular markers, such as RAPDs, reveal information about parts of a genome which are not under selective pressure, so they are especially useful in the measurement of genetic drift, gene flow between populations, and population structure. That is, random genetic changes in small isolated populations, and reproductive information such as clonal structure in populations can be detected with molecular markers.

Morphological markers, in contrast with molecular markers, are more likely to be under selective pressure. This property gives a tool that can reveal differences among populations due to environmental adaptation, and that can complement the advantages of molecular markers. They are generally more difficult to obtain than molecular markers, and if they are measured over varying environments, they may only reflect environmental differences between populations rather than genetic differences.

Our goal in this study is to evaluate both morphological and molecular genetic data to obtain estimates of genetic variability and distance within and among Leedy's roseroot and other taxa of S. *integrifolium.* We also hope to gain insights into the genetic structure and evolution of natural plant populations. The purpose of this final report is to communicate our progress and results to date. As we stated in our proposal, the goals of this project will be completed at the

defense of Joel Olfelt's thesis. The results and conclusions presented there will be made available to the Minnesota Natural Heritage Program.

Materials and Methods

Plant Sampling

We collected the leaves, fruits and seeds analyzed in this report from 17 S. *integrifolium* populations, 2 S. *rosea* populations, and 4 S. *rhodanthum* populations in July, August, or September 1994. The S. *integrifolium populations* are partitioned as follows: 5 S. *integrifolium ssp. leedyi* (Leedy's roseroot) populations; 4 S. *integrifolium ssp. integrifolium* populations; 6 *S. integrifolium ssp. procerum* populations; and 2 S. *integrifolium ssp. neomexicanum* populations. The numbers of individuals assayed for RAPID markers are given in Appendix 1 and in Table 1. The numbers of individuals assayed for leaf, seed, and fruit characters are given in the legends of Tables 3 and 4. Many of the fruits that we collected in Colorado and New Mexico during 1994 contained aborted seeds, perhaps because of the very dry summer that year. Also, some of our seeds were destroyed by mold during storage. Because of these losses, we returned to the Colorado and New Mexico populations in September 1995 for more seeds. We haven't yet been able to fully evaluate our collections, but we believe that we have seeds from at least 60 individuals per western subspecies. We plan to use these seeds in a common garden experiment in the next several months to complement the molecular marker data reported here.

Table 1

Leedy's Roseroot Sampling Statistics

		Plants Assayed by PCR	
Population	**Total # Plants**	**# Plants 1 or more meters Apart**	**# Clusters of Plants**
Deer Creek	38	30	4
Simpson Cliffs	51	40	7
Whitewater Wildlife Management Area	41	27	4
Glenora Cliffs	45	33	4
Glenora Falls	4	3	1

In Minnesota we used rappelling equipment, ladders, and rock scrambling techniques to reach plants. In New York, on Seneca lake, plants were accessible either by rock scrambling, or via the many staircases that give access from the cliff top to the beach below. We sampled accessible plants from along the entire length of each Leedy's roseroot population, and we sampled plants at the cliff tops, bases, and at intermediate heights. To avoid the possibility of including multiple samples from a single individual in our diversity and distance analyses, we include only Leedy's roseroot individuals that are at least 1 meter apart. In each Leedy's roseroot population we sampled some sets of plants that are clustered less than 1 meter apart both to estimate the size of individuals and to test for clonal reproduction. In these cases, only one plant per cluster is included in the diversity and distance analyses. The minimum distance between sampled S. *rosea, S. rhodanthum,* and western S. *integrifolium (ssp. integrifolium, ssp. procerum,* and ssp. *neomexicanum*)plants was 3 meters.

We collected whole plants from western S. *integrifolium* populations to test methods of growing the plants in the greenhouse. In Minnesota and New York we collected only plants that had fallen from the cliffs, and used them along with the western subspecies to test greenhouse techniques.

Leaves were placed in plastic wire closure bags with wet paper towel, and stored for up to a week on ice in the field. The leaves were frozen at -20°C on arrival at our laboratory. Fruits were stored in plastic wire closure bags over ice in the field, and at 40C over desiccant in our laboratory Whole plants, or rootstocks were stored in ziplock bags on ice in the field, and at 40C in the laboratory until they could be planted.

Locating Populations

We located Leedy's roseroot populations with notes and instructions from state agencies, and we located other *Sedum* populations with site descriptions from herbarium sheets at the Cornell University herbarium. The *S. integrifolium* specimens at Cornell were identified to their subspecies by Clausen, so we could use them to identify populations that he had located and classified. The *Sedum* populations that we sampled, and their geographic coordinates are listed in appendix 2. Minnesota's Bear Creek population of Leedy's roseroot is not included among the sampled populations because the landowner denied us access to his property. Voucher specimens from the sampled Colorado and New Mexico populations are on deposit in the University of Minnesota's herbarium.

Plant Culture Conditions

We treated *Sedum* seeds for 1 day in 400ppm Gibberellic acid (GA3, Gibco/BRL Life Technologies), followed by 1 month at 4°C on moist filter paper and two weeks at room temperature. Seedlings and whole plants were planted in 50% sand, 50% soil mix (the soil mix is 2 parts soil, 2 parts peat, 1 part vermiculite, and 1 part perlite) and kept in the Department of Forest Resources greenhouse at the University of Minnesota during 1994. At the beginning of 1995 the plants were placed at 40C for several months and kept moist. On 30 June 1995 the plants were placed in the Plant Biological Sciences greenhouse.

Insects, especially aphids, occasionally have damaged the plants. We have attempted to control the aphids with insecticidal soap (Safer Inc., Eden Prairie, MN), and with water sprayed from a mist nozzle. We stopped using the soap when plants appeared to be damaged by it.

Preparation of DNA

We extracted total DNA from about 3 fresh or frozen leaves from each plant by grinding the leaves in 1.5 ml microcentrifuge tubes with hot extraction buffer using a modification of the procedure described by Torres et. al., (1993). When the extractions were complete, the DNA was diluted n 50,1 TE buffer (1.0 M Tris and 0.5M EDTA). Quantities of DNA were estimated 0 ng Hoechst dye, and a Hoefer TKO 100 fluorometer (Hoefer Scientific Instruments; San Francisco, CA). Working solutions of 5ng DNA/f,I in TE buffer were made, hand DNA solutions from individuals were I randomized before PCR reactions were begun.

Generation of RAPD Markers

Polymerase Chain Reactions (PCR) ~o generate RAPDs were conducted in a 15[1 volume. Each reaction mixture cont ined 1.51,1 5ng/wl *Sedum* DNA, a solution made of 7.7[t1 double distilled water, 1.5[,1 Ox buffer provided with the *Taq* polymerase, 1.5gi of nonacetylated bovine serum albumin (New England Biolabs), 1,5[t1 of a solution that was 2.5mM for each dNTP, 0.6,1 of a 5mM solution of a DNA decamer il primer (Operon Technologies), 0.6,1 f a 50mM solution of magnesium chloride, and 0.1upl of a 5 units/RI solution of *Taq p l*ymerase (GIBCOBRL, Life Technologies, Inc.). A master mix for each decamer primer, which contained all reaction components except DNA and *Taq* polymerase, was made so that reaction conditions for the primer would be as uniform as possible. Reaction mixes were prepared on ice by pipeting *Sedum* DNA into the wells of a 96 well polycarbonate microplate (Hybaid, Ltd.), adding 13.58.1 of master' mix into which an appropriate amount of *Taq* polymerase had been mixed, and by overlaying each reaction mix with a drop of mineral oil. Reactions were run in an Omnigene thermal cy ler (Hybaid, Ltd.) programmed for 1 min. 30sec. at 92.50C, followed by 45 cycles of 1 mi . at 92.50C, 2min. at 36.OOC, and 2min. at 72.OOC, followed by a final 7min. at 72.0°C. All the temperature transitions were run at the maximum speed except for the transition from 360C to 7200, which was run at 4 sec/10C. The thermal cycler was programmed to keep

the reaction mixes at room temperature once the temperature cycling was finished. The processed reaction mixes were kept at room temperature for up to several hours, and then stored at 40C to await gel electrophoresis. Reaction products were prepared for size fractionation by adding 3[1 of bromophenol blue buffer (0.5% w/v bromophenoi blue, 50% w/v sucrose) to reaction mixes. They were loaded into a 1.4% agarose gel and electrophoresed until the bromophenol blue dye front had migrated about 15cm. Gels were stained with ethidium bromide, and photographed with Polaroid Type 57 film (Polaroid Corporation; Cambridge, MA) under ultra-violet light. Each gel had three lanes of a 1 kb molecular weight marker (GIBCO/BRL, Life Technologies, Inc.) for use in estimating the sizes of PCR products. All individuals were assayed twice by repeating each PCR and only fragments that consistently amplified were scored.

RAPD Data Analysis

We scored RAPD bands as present or absent for each individual, grouped individuals according to Clausen's taxonomy, and constructed a data matrix of band frequencies in each taxon. From this band frequency matrix, a data matrix of average taxonomic distance was derived and analyzed using SIMINT and the unweighted pair -group method (UPGMA) of the SAHN clustering program of NTSYS-pc, version 1.80 (Exeter Software, Setauket, NY; Rohlf 1993)

Morphological Measures

For each sampled plant, we selected the tenth leaf from the plant's growing tip, measured its length and width; and counted the number of teeth on it. When fruits were present on an individual we collected and stored them. Fruit and seed measurements were made of 3 fruits and 9 seeds per plant using a Wild-Heerbrugg binocular microscope with an ocular scale. The leaf and seed data were entered into Microsoft Excel's version 5.0 spreadsheet (Microsoft Corporation), they were grouped according to Clausen's taxonomy of S. integrifolium, and

analyzed for significant differences using microsoft Excel's ANOVA package. We also analyzed for differences among Leedy's roseroot populations.

Results

Genetic Variation Shown by RAPDs

A total of 21 markers were scored as present or absent for each individual. The scored markers, their approximate sizes, the primers used to amplify them, and their frequencies in each poulation or taxon are listed in table 2. The data show that Leedy's roseroot populations, especially those in Minnesota, have very little variability The markers A, G, and L, are present in at least 96% of the Leedy's roseroot individuals, and with the exception of N, fewer than 5% of the assayed Leedy's roseroot individuals carry any of the other 17 markers. Glenora's populations, here analyzed as one population because of their nearness to each other and the small number of individuals from Glenora Falls, has the most variability of the Leedy's roseroot populations. The marker N is in 30% of its individuals, and a marker unique to the population (T) was detected in one individual at Glenora Falls. The western S. *integrifolium* subspecies have more variability than Leedy's roseroot, and carry several markers that are absent in Leedy's roseroot (B, F, J, K, Q, S, and *U). Sedum rosea* has one marker (G) that is diagnostic in our *Sedum* samples for the species, and two markers (B and J) that occur at a much higher frequency in *S. roses* than in any of the other taxa we assayed. There is much more variation in *S. rhodanthum* than in *S. integrifoiium* and *S. rosea*.

In the 20 clumps of Leedy's roseroot assayed, 8 clumps had an individual with different RAPID markers from its near neighbors. The distance between neighbors with different markers varied from about 6cm to about 80cm.

Table 2

Frequencies of RAPID Makers

	Primer			OPF - 01			OPF - 04			OPF -14											
Marker	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*J*	*K*	*L*	*M*	*N*	*O*	*P*	*Q*	*R*	*S*	*T*	*U*
Marker size (kb)	0.90	0.65	1.00	0.85	0.65	0.72	0.75	1.90	2.20	2.30	2.40	0.85	2.60	0.90	1.60	1.35	0.80	1.00	1.30	1.20	4.50
Taxonomic Unit																					
Deer Creek (ssp. leedyi)	*100*	*0*	*0*	*3*	*3*	*0*	*9*	*7*	*0*	*0*	*0*	*0*	*100*	*3*	*0*	*3*	*3*	*0*	*0*	*0*	*0*	*0*
Simpson Cliffs (ssp. leedyi)	*100*	*0*	*0*	*0*	*0*	*0*	*100*		*2*	*0*	*0*	*0*	*100*	*2*	*0*	*0*	*2*	*0*	*2*	*0*	*0*	*0*
Whitewater WMA (ssp. leedyi)	*100*	*0*	*0*	*0*	*4*	*0*	*96*		*0*	*0*	*0*	*0*	*100*	*4*	*0*	*0*	*4*	*0*	*0*	*0*	*0*	*0*
Glenora Cliffs & Falls (ssp. leedyi)	*100*	*0*	*5*	*3*	*0*	*0*	*100*		*0*	*0*	*0*	*0*	*100*	*0*	*30*	*3*	*0*	*0*	*0*	*0*	*3*	*0*
S. integrifolium ssp. integrifolium	*100*	*0*	*3*	*0*	*0*	*3*	*100*		*3*	*0*	*0*	*0*	*93*	*40*	*7*	*10*	*3*	*0*	*10*	*3*	*0*	*0*
S. integrifolium ssp. procerum	*100*	*3*	*0*	*0*	*0*	*0*	*100*		*0*	*0*	*0*	*6*	*97*	*23*	*19*	*0*	*0*	*3*	*6*	*0*	*0*	*0*
S. integrifolium ssp. neomexicanum	*100*	*3*	*0*	*6*	*6*	*3*	*91*		*0*	*0*	*3*	*0*	*97*	*27*	*6*	*3*	*18*	*0*	*0*	*0*	*0*	*3*
S. rosea	*100*	*100*	*0*	*0*	*0*	*0*	*100*		*0*	*100*	*97*	*0*	*100*	*0*	*50*	*0*	*0*	*0*	*3*	*0*	*0*	*0*
S. rhodanthum	*100*	*0*	*8*	*16*	*16*	*16*	*16*		*92*	*0*	*0*	*8*	*50*	*58*	*0*	*16*	*25*	*0*	*16*	*0*	*0*	*0*

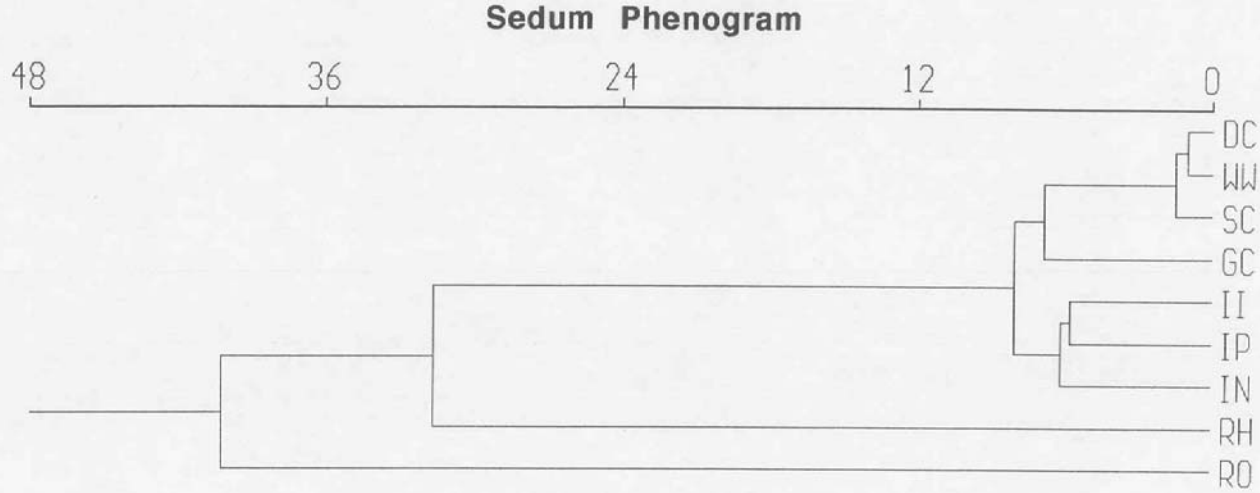
Genetic Distance Shown by RAPDs

The genetic distance of Leedy's roseroot from the other S. *integrifolium* subspecies is shown in Figure 1 relative to other distances estimated among the included *Sedum* taxa. The phenogram shows relationships among the *Sedum* taxa by using distances to pair groups that are most alike. The RAPD data, as analyzed here, place Leedy's roseroot in a branch that is distinct from the other S. *integrifolium* subspecies. Since the phenogram's branch lengths show relative genetic distances, a comparison of branch lengths can show the importance of divergences. Thus, the branch length that separates Leedy's roseroot from the other subspecies is about one fourth the branch length that separates the species S. *integrifolium,* and S. *rhodanthum.* This suggests a significant difference between Leedy's roseroot and the other S. *integrifolium* subspecies.

Plant Culture Conditions

The rootstocks and seedlings grown in the Forest Resources greenhouse all sprouted and grew vigorously for several months in 1994. During August 1994 aphids damaged the plants, and we applied insecticidal soap to the plants to control the aphids. Shortly after the soap applications, several seedlings, and a plant established from rootstock died, and many of the plants lost their leaves. The remaining plants showed very little, to no growth during the next few months.

In 1995, after treatment at 40C, all of the plants showed vigorous growth within two weeks of being placed in the Plant Biological Sciences (PBS) greenhouse. Within seven weeks, several of the Leedy's roseroot and ssp. *neomexicanum* plants had flowered, but the ssp. *procerum* and ssp. *integrifolium* plants had only what appeared to be aborted flower primordia. We observed aphids on the plants but were able to successfully control the aphids by spraying them with water from a mist nozzle.



DC = Deer Creek. GC = Glenora Cliffs and Falls combined. II = *S. integrifolium* ssp. *integrifolium*. IN = *S. integrifolium* ssp. *neomexicanum*. IP = *S. integrifolium* ssp. *procerum*. SC = Simpson Cliffs. WW = Whitewater Wildlife Management Area. DC, GC, SC, and WW are populations of subspecies *leedyi*.

Morphological Similarities and Differences

The morphological measurements we took from leaves and seeds don't support the division of S. *iniegrifolium* into the four subspecies that Clausen described. The morphological similarities and differences we found are summarized in Tables 3 and 4. Clausen reported "highly significant" differences among wild populations in seed length, leaf width, and number of teeth per leaf, and "significant" differences for leaf and fruit length among wild populations (1975). In contrast, we find no differences among populations in seed length. For leaf length and width we find some differences among populations, and one population differs from the others in number of teeth per leaf. The groups that have similar means for a character do not correspond to Clausen's scheme of four subspecies. For example, for the character leaf length, Leedy's roseroot plants from Simpson cliffs in Minnesota are very similar to S. *integrifolium* ssp. *integrifolium* plants from Colorado (Table 3), but they are different from other populations of Leedy's roseroot

Table 3

LEAF SIMILARITIES AND DIFFERENCES

Populations with a significant difference (a< 0.01) in the means for a character are separated by a comma.
Populations with no significant differences (a> 0.01) among them are grouped in brackets().

Length	(gc ww), (ii sc), dc, ii, inm, ip
Width	(dc, gc, ip, sc, ww), ii, inm
Teeth/Leaf	(dc, gc, ii, inm, ip, ww), sc

do = Deer Creek, MN (45 plants). gc = Glenora Cliffs, NY (64 plants). ii = subspecies integrifolium,CO and NM (39 plants). inm = subspecies neomexicanum, NM (45 plants). ip subspecies procerum, CO (55 plants). sc = Simpson Cliffs, MN (41 plants). ww = Whitewater Wildlife Management Area, MN (51 plants). dc, gc, sc, and ww are populations of subspecies leedyi. Leaf measurements are from the tenth leaf from a growing tip of each plant.

Table 4

SEED AND FRUIT SIMILARITIES AND DIFFERENCES

Populations with a significant difference (a< 0.01) in the means for a character are separated by comma. Populations with no significant differences (a> 0.01) among them are grouped in brackets().

Seed Length	(gc/sc, ii, inm, ip)
Seed Width	(gc/sc, ii, inm, ip)
Fruit Length	(gc/sc, ii, inm), ip
Fruit Width	(gc/sc, ii, inm), ip

gc/sc = Glenora Cliffs, NY and Simpson Cliffs, MN combined (25 plants). ii - subspecies integrifoliumCO and NM (13 plants). inm = subspecies *neotnexicanurn,* NM (16 plants). ip -subspecies procerum, CO (i6 plants). dc, gc, sc, and ww are populations of subspecies *leedyi.* These analyses are based on three fruits, and nine seeds per plant.

Discussion

Genetic Variation Shown by RAPDs

The low variability of Leedy's roseroot in Minnesota is an expected result given that the populations are small and isolated. Low variability in this sort of population may be due to limited variation in founding individuals, genetic drift, or because of bottlenecks in population size during the population's history. New York's population, because of its larger size, is expected to be less affected by genetic drift or by bottlenecks and to have more variation than the Minnesota populations. This expectation is met. It is surprising that S. *rhodanihum* shows more variation than the western subspecies of S. *integrifolium.* Both species are common in the Rocky mountains, and would both be expected to have much variation there. This may mean that S. *integrifolium* has relatively little genetic variability

The differing genotypes within Leedy's roseroot clumps shows that clumps are not necessarily clonal. Since the amount of variation among Leedy's roseroot individuals is low, it is impossible

to know, from the data that we have, whether the clumps with no detected variation are clones, or sexually reproduced individuals with no detected variation.

Genetic Distance shown by RAPDs

The genetic distances estimated between Leedy's roseroot and the other taxa of *Sedum* investigated in this study supports the idea that Leedy's roseroot is genetically distinct. The inclusion of the genetic distances among S. *rosea, S. rhodanthum, and S. integrifolium* give a sort of ruler against which the genetic distances among the S. *integrifolium* subspecies can be compared, and suggest that the genetic distance between the S. *integrifolium* subspecies is important. However, this result should be considered as preliminary both because it is based on only 21 RAPD markers and may be subject to unacceptably large statistical variance, and because it represents only a first attempt to organize the data. The method used to analyze the data has been used by others for RAPD data in similar studies, (Harrison, 1994) but we have not yet been able to evaluate other methods that may better accommodate the properties of our data set.

Plant Culture Conditions

The work we have done with plants in the greenhouse demonstrate that a common garden experiment is feasible. Thus we can get morphological data that are minimally influenced by differences in environment. Our observation in the greenhouse that several Leedy's roseroot and S. *integrifolium* ssp. *neomexicanum* plants flowered, but that no plants of the other subspecies flowered suggests that the plants are differently adapted to the greenhouse conditions we had during July and August 1995. One reasonable explanation is that the plants from New York, Minnesota, and Sierra Blanca (the southernmost population) are better adapted to high temperatures such as we had in July and August 1995.

Morphological Similarities and Differences

The results we have from leaf and seed measurements contrast with Clausen's results. Our sample sizes are all at least as large, and in many cases several times as large as Clausen's, so we should be able to detect differences with at least similar sensitivity. One likely explanation for the discrepancy is that the measured differences among populations are due to differences in environment that change from year to year, making it difficult or impossible to understand taxonomic relationships within *S. integrifolium* based on field morphological data alone. These results should thus be given very limited weight compared with RAPD data, and with data from the common garden experiment that we are planning.

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Appendix 1

Number of Plants Assayed per Population

Population	Taxon	State	Leaves
Deer Creek	*S. integrifolium* ssp. *leedyi*	MN	38
Simpson Cliffs	"	MN	51
Whitewater Wildlife Management Area	"	MN	41
Glenora Cliffs	"	NY	45
Glenora Falls	"	NY	
	Total ssp. *leedyi*		**179**
Milner Pass	*S. integrifolium* ssp. *integrifolium*	CO	6
Wolf Creek Pass	"	CO	10
Santa Fe Ski Area	"	NM	6
Sandia Crest	"	NM	8
	Total ssp integrifolium		**30**
Elwood Pass	*S. integrifolium* ssp. *procerum*	CO	5
Platoro	"	CO	4
Eureka Gulch	"	CO	5
Picayune Gulch	"	CO	7
Animas River	"	CO	2
Molas Pass	"	CO	8
	Total ssp procerum		**31**
Ice Springs	*S. integrifolium* ssp. *neomexicanum*	NM	19
Ski Apache	"	NM	14
	Total ssp. neomexicanum		**33**
Mount Horrid	S. *Rosea*	VT	19
Wildcat Ravine	"	NY	17
	Total S. rosea		**36**
Elwood Pass	S. *Rhodanthum*	CO	3
Mary Jane Ski Area	"	CO	1
Milner Pass	"	CO	4
Long's Peak	"	CO	
	Total S. rhodanthum		**12**

Appendix 2

Locations of Sampled Populations

Population	Taxon	State	Geographic Coordinates*
Deer Creek	S. *integrifolium ssp. leedyi*	MN	Lat. 43°43'55" N, Long. 92°20'30"W
Simpson Cliffs	"	MN	Lat. 43°52'55" N, Long. 92°24'15"W
Whitewater Wildlife Management Area	"	MN	Lat. 44° 06'05" N, Long. 92°08'00"W
Glenora Cliffs	"	NY	Lat. 42°30'50" to 42°29'35" N, Long. 76°54'55"W
Glenora Falls	"	NY	Lat. 42°29'20" N, Long. 76°55'00"W
Milner Pass	S. *integrifolium ssp. integrifoliurn*	CO	Lat. 40°24'45" N, Long. 105°49'00"W
Wolf Creek Pass	"	CO	Lat. 37°30'00" N, Long. 106°48'15"W
Santa Fe Ski Area14	"	NM	Lat. 105°47'40" N, Long. 35°47'30"W
Sandia Crest	"	NM	Lat. 35°12'40" N, Long. 106°27'00"W
Elwood Pass	S. *integrifolium ssp. procerum*	CO	Lat. 37°24'15" N, Long. 106°39'50"W
Platoro	"	CO	Lat. 37°21'00" N, Long. 106°30'00"W
Eureka Gulch	"	CO	Lat. 37°52'50" N, Long. 107°34'30"W
Picayune Gulch	"	CO	Lat. 37054'40" N, Long. 107°33'40"W
Animas River	"	CO	Lat. 37054'40" N, Long. 107°33'15"W
Molas Pass	"	CO	Lat. 37°43'30" N, Long. 107°44'15"W
Ice Springs	S. *integrifolium ssp. neomexicanum*	NM	Lat. 33°24'00" N, Long. 105°48'50"W
Ski Apache	"	NM	Lat. 33°23'50" N, Long. 105°48'30"W
Mount Horrid	S. *Rosea*	VT	Lat. 43°50'40" N, Long. 72°57'45"W
Wildcat Ravine	"	NY	Lat. 42°10'20" N, Long. 74003'50"W
Elwood Pass	S. *Rhodanthum*	CO	Lat. 37°24'15" N, Long. 106°39'50"W
Mary Jane Ski Area	"	CO	Lat. 39°52'40" N, Long. 105°45'35"W
Milner Pass	"	CO	Lat. 40°24'45" N, Long. 105°49'00"W
Long's Peak	"	CO	Lat. 40°16'30" N, Long. 105°34'30"W

* These coordinates should be considered accurate to plus or minus 5".

Photographs
of
the work toward estimating Leedy's roseroot's
distinctness and variability

Explanation of Slides

1. A greenhouse grown *Sedum integrifolium ssp. leedyi* (Leedy's roseroot) plant along with its scientific and common navies . The plant is in flower.

2. A Leedy's roseroot plant at Minnesota's Whitewater Wildlife Management Area.

3. The specialized habitat that Leedy's roseroot occupies".

4. Leedy's roseroot habitat at Whitewater Wildlife Management Area. The pale green plants that Joel Olfelt is pointing to are Leedy's roseroot plants.

5. The known distribution of Leedy's roseroot. The subspecies is known only in New York, and in Minnesota. The estimated total number of individuals is between 9500 and 15,000 plants (U.S. Fish and Wildlife Service 1993)

6. A Leedy's Roseroot plant on Glenora Cliffs at Seneca Lake in New York.

7. The approximate distributions of the four subspecies of *S. integrifoliurn.* (Clausen, 1975)

8. *S. integrifolium ssp. procerum* plants near Elwood pass in the San Juan mountains of Colorado.

9. *S. iniegrifolium ssp. procerum* plants near the Animas river, north of Silverton, Colorado.

10, 11, & 12. Joel Olfelt collecting seeds and leaves from Leedy's roseroot plants in Minnesota.

13. Joel Olfelt extracting DNA from plant tissue.

14. DNA from individual plants is loaded into individual wells of this tray along with enzymes in a reaction mix that will copy fragments of the plant DNA. These DNA fragments will make a DNA fingerprint.

15. The tray is loaded into this thermal cycler. The thermal cycler provides a programed cycle of temperatures that allows the enzymes to copy fragments of DNA.

16. After processing in the thermal cycler, reaction mixes from individual plants are loaded onto a gelatin-like slab called a gel. Copied DNA fragments are separated by size when an electric current is passed across the gel. The gel is stained, and placed over an ultra-violet light source to reveal the DNA fingerprints.

17. A stained gel with DNA fingerprints from Minnesota and western *Sedum integrifolium* plants. Bands that form a column are an individual's DNA fingerprint.

18. Plants can be grown from seed in a greenhouse, so features like leaf shape or color can be compared in the common environment of a greenhouse. We plan to grow plants from Minnesota, New York, Colorado, and New Mexico in a common environment. Information

* This diagram is redrawn from p.11 of *Sedum infegrifolium ssp. leedyi* (Leedy's Roseroot) Recovery Plan (U.S. Fish and Wildlife Service, 1993)

from the common garden experiment should supplement what we learn from DNA fingerprinting.

19, & 20. Some human pressures on Leedy's roseroot habitat at Seneca Lake in New York. This study is designed to gather information about the genetic distinctness of Leedy's Roseroot from more common subspecies of *S. integrifolium.* This information can be used in decisions of whether, and how Leedy's roseroot populations should be protected.

References

Clausen, R.T Sedum of North America North of the Mexican Plateau. Ithaca, NY: Cornell University Press; 1975.

U.S. Fish and Wildlife Service. 1993 Sedum iniegrifolium ssp. leedyi (Leedy's Roseroot) Recovery Plan. Technical/Agency Draft. U.S. Fish and Wildlife Service, Ft. Snelling, Minnesota. vi + 31 pp.