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WHAT DATA DETERMINE WHETHER A PLANT TAXON IS DISTINCT ENOUGH TO MERIT LEGAL PROTECTION? A CASE STUDY OF *SEDUM INTEGRIFOLIUM* (CRASSULACEAE)[1]

JOEL P. OLFELT,[2,5] GLENN R. FURNIER,[3] AND JAMES J. LUBY[4]

[2]Department of Biology, St. Olaf College, 1520 St. Olaf Avenue, Northfield, Minnesota 55057-1098 USA;
[3]Hohokum Middle School, 7400 Settler Avenue, Tuscon, Arizona 85746 USA; and
[4]Department of Horticultural Science, University of Minnesota, 1970 Folwell Avenue, St. Paul, Minnesota 55108 USA

Measures of molecular and morphological genetic variation are often used to set conservation priorities and design management strategies for plant taxa. Evaluated together they can give insights into a taxon's evolutionary status that neither data type alone can achieve. We investigated the distinctness and variability of *Sedum integrifolium* ssp. *leedyi*, a federally and state-listed taxon, from its conspecific relatives using 33 random amplified polymorphic DNA (RAPD) markers (253 plants) and 37 morphological characters from 1308 common-garden-grown plants. We included *S. integrifolium* ssp. *leedyi* (four populations), its conspecific relatives (11 populations), and *S. rosea* and *S. rhodanthum* populations in our study. The morphological and molecular data correspond in showing that *S. integrifolium* ssp. *leedyi* populations are highly distinct. However, the data sets differ in their estimates of the relatedness of some *S. integrifolium* ssp. *leedyi* populations and in the percentage variation detected due to differences among them (25 and 9–13% for the molecular and morphological data, respectively) suggesting little gene flow among populations and some differentiation, possibly from selective pressures. Given our data, we recommend that *S. integrifolium* ssp. *leedyi* merits protection under the U.S. Endangered Species Act and that its populations be managed as distinct units.

Key words: common garden; conservation biology; Crassulaceae; genetic variation; *Sedum integrifolium* ssp. *leedyi*; RAPDs; rare plant.

Conservation biologists often use information about the distribution of genetic variation within and among populations to set conservation priorities and plan management strategies (Schonewald-Cox et al., 1983; Hamrick et al., 1991; Frankel, Brown, and Burdon, 1995; Schmidt and Jensen, 2000). Administrative and legal structures, such as the federal Endangered Species Act in the USA, regulate the sets of organisms that may be protected using limited public monies, and, in general, more distinct groups of organisms are thought to warrant higher levels of effort than those that are less distinct (Holsinger and Gottlieb, 1991; Rojas, 1992; Given, 1994; Frankel, Brown, and Burdon, 1995; Meffe and Carroll, 1997). Thus, the way distinctness is defined and the methods used to estimate levels of variation and differentiation are often critical elements in decisions of whether a set of organisms becomes a public conservation priority or not (Holt, 1987; Fergus, 1991; Waples, 1991, 1994; Nowak, 1992).

The definition of which biological units are rare, imperiled, and distinct is often not straightforward (Drury, 1974, 1980; Fiedler, 1986; Eisner et al., 1995). Plants, for example, often form interfertile complexes among groups that are morphologically, ecologically, or cytologically divergent and defy attempts to delimit biologically meaningful groups (Levin, 1979; Fiedler, 1986; Mace and Lande, 1991). Plant species with wide ranges generally have significantly higher levels of genetic variation than their geographically restricted congeners, but variation levels in some restricted species are similar to levels in their widespread congeners (Karron, 1987; Soltis and Soltis, 1991; Ge et al., 1999). Because there is no sure way to predict a group's level of genetic variation and divergence from its closest relatives, many cases are investigated individually (e.g., Wyatt, Evans, and Sorenson, 1992; Mymudes and Les, 1993; Walters, 1993; Baskauf, McCauley, and Eickmeier, 1994; Van Buren et al., 1994; Gemmill et al., 1998; Ayres and Ryan, 1999).

Evidence commonly used to estimate levels of differentiation and variation within and among rare and endangered plant groups can be divided into three categories: (1) morphological data from diverse field environments, (2) morphological data from plants grown in a uniform environment, and (3) molecular genetic data. Data from each category can yield information about different aspects of an investigated group and require different investments of time, equipment, and money. Morphological data collected from field-grown plants have long been useful in plant taxonomy (Stuessy, 1990), but are limited by their inability to distinguish morphological differences due to environmental effects from those due to genetic differences. Growing plants in a uniform environment can, in theory, eliminate variation due to these environmental effects, so any morphological differences among the plants are attributable to underlying genetic differences. The characters typically evaluated in common garden studies are those that interact directly with the environment and may have been shaped by the selective regime in the plant's area of origin (Clausen, Keck, and Heisey, 1948; Rehfeldt, 1993; Podolsky and Holtsford, 1995). Uniform environment experiments are dependent

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[5] Author for reprint requests (olfelt@stolaf.edu).

on successful propagule collection and cultivation of the study plants, are limited by the finite number of readily available morphological markers, and are labor-intensive and time-consuming.

Molecular genetic markers, such as isozymes and DNA-based polymorphisms, are widely used to infer relationships among plant groups and estimate their levels of genetic variation (Hamrick, 1989; Chase et al., 1993; Karl and Avise, 1993). These markers are generally thought to be useful for detecting the action of nonselective evolutionary forces, such as gene flow and drift (Nei, 1987). They are relatively convenient, can yield large numbers of useful markers, and often require very small amounts of plant tissue. For reasons such as these many rare plant studies rely exclusively on molecular genetic markers. However, reliance on molecular markers alone, without information about how their distributions are influenced by selection, can be misleading because they are unlikely to yield information about adaptively important character traits that may be critical to a population's survival in its habitat. In some cases, plant groups with very low levels of molecular genetic differentiation among populations show significant levels of morphological genetic differentiation (Wheeler and Guries, 1982; Furnier et al., 1991; Karhu et al., 1996).

The U.S. Fish and Wildlife Service (1993, 1998) has been considering what conservation efforts, if any, should be made to protect populations of the federally listed *Sedum integrifolium* ssp. *leedyi*, a member of a widespread succulent perennial species with four described subspecies. A pivotal issue in these considerations has been the level of differentiation between *S. integrifolium* ssp. *leedyi* and its conspecific taxa. We used a combination of morphological and molecular genetic marker data to address this problem and to compare the results from the two types of data.

MATERIALS AND METHODS

We collected materials from populations of *S. integrifolium* ssp. *integrifolium*, ssp. *procerum*, ssp. *neomexicanum*, and ssp. *leedyi* to estimate levels of variation within and differentiation among *S. integrifolium* populations. We also included materials from *S. rosea* and *S. rhodanthum*, the other two North American representatives of *Sedum* subgenus *Rhodiola* (Clausen, 1975), to generate a scale for evaluating relative differences among the subspecies. Plant populations were located from herbarium label descriptions (Cornell University and University of Minnesota herbaria) and identified by location and with keys (Clausen, 1975).

We collected leaves from 34 or more plants from each of four of the five known *S. integrifolium* ssp. *leedyi* populations (MN1, MN2, MN3, NY1). To avoid multiple samples from a single clone in these populations, most (134) of the samples were taken from plants separated by >1 m. In the cases (28) in which sampled plants were <1 m from their nearest neighbor we used DNA fingerprint data to ensure that no clonal replicates were included in our analyses (Olfelt, Furnier, and Luby, 1998). We collected leaves from a total of 28 *S. integrifolium* ssp. *integrifolium* plants from four populations (I1, I2, I3, I4), 33 *S. integrifolium* ssp. *procerum* plants from five populations (P1, P2, P3, P4, P5), 30 *S. integrifolium* ssp. *neomexicanum* plants from the single known population (M),12 *S. rhodanthum* plants from four populations (H1, H2, H4, H5), and 38 *S. rosea* plants from two populations (R1 and R2) (Fig. 1, Table 1). Plants sampled from the western North American populations were at least 3 m apart, and plants from R1 and R2 were at least 1 m apart. Leaves from population R2 were a generous contribution from Steve Young (New York Natural Heritage Program). Leaves from all populations were placed on moist tissue paper in the field, stored on ice for up to 1 wk, and then frozen at −20°C until DNA extraction. We applied the different distance limits between sampled plants in the western and eastern populations because of different edaphic conditions. We used different sample sizes because of limiting population sizes and inaccessibility of plants. The western populations occur in remote mountain areas, often in gravel soils with no clear limit between individuals that may be large (covering an area >1 m^2). Plants in the eastern populations grow on cliffs, often in crevices with clear limits between smaller individuals (Clausen, 1975; Olfelt, Furnier, and Luby, 1998; Olfelt, personal observation).

We collected seeds from MN3, NY1, R2, H2, H3, H4, and H5 in 1994 and from MN1, MN2, H1, I1, I2, I3, I4, I5, P1, P2, P3, P4, P5, and M in 1995 (Fig. 1, Table 1). Seeds were placed on ice in the field and stored at 4°C over desiccant in the laboratory until germination. Voucher specimens of plants collected in the field from populations H1, H2, H3, H4, H5, I1, I2, I3, I4, I5, P1, P2, P3, P4, P5, and M and grown from seed collected at MN1, MN2, MN3, NY1, and R1 are deposited at the University of Minnesota (MIN); a voucher for population R2 is deposited at the New York State Museum (NYS).

DNA was extracted from all plants by the method for chickpeas of Davis et al. (1995) slightly modified as described in Olfelt, Furnier, and Luby (1998). We used polymerase chain reactions to generate 33 randomly amplified polymorphic DNA (RAPD) markers from five decamer DNA primers as described in Olfelt, Furnier, and Luby (1998). Each individual was assayed twice in random order to test repeatability of the amplification and to avoid investigator bias in scoring. Only bands that could be consistently scored across all individuals were used. We chose RAPD markers because they can yield an essentially unlimited number of markers, require the use of only very small quantities of plant tissue, and are relatively inexpensive (Welsh and McClelland, 1990; Williams et al., 1990; Liu and Furnier, 1993; Peakall, Smouse, and Huff, 1995). These characteristics made RAPDs an excellent choice for use in studying the rare and endangered *S. integrifolium* ssp. *leedyi*. Uniformity in the developmental stage of plants at the time of sampling is of less concern with RAPDs than for isozymes (Wendel and Weeden, 1989), the small amount of plant tissue required was likely to have only a low impact on each sampled plant, and we could obtain a large number of useful markers within the constraints of our available budget.

Plants from 126 open-pollinated maternal families were grown from seed as described in Olfelt, Furnier, and Luby (1998). One month after sowing, two plants from each seed family were placed in random order in each of six experimental blocks on a single greenhouse bench. Data for 37 morphological characters were taken between 2 and 7 mo after sowing (Table 2). Characters were chosen for one or more of the following reasons; because Clausen (1975) found them taxonomically informative (especially the leaf lengths, and widths and flower morphology), because they seemed likely to be adaptively important (especially the developmental characters), because our observations of the plants in the common garden suggested that the characters might be taxonomically informative. The characters used had means that differed at a level of $P < 0.0005$ between one or more of the operational taxonomic units (OTUs; Table 1), and were correlated with other characters at a level of <0.91, or were not logically correlated. Plants within the same block were measured for each character in a single day to minimize experimental error, except for flower and cotyledon characters and leaf number at stem elongation, which are comparable only at discrete developmental stages. Flowers were collected from one block only at the time of pollen shed or nectar production and were measured immediately or stored at 4°C overnight on moist paper towels before measuring. Cotyledons were excised with a scalpel and pressed when they were beginning to senesce or plants had approximately four leaves, whichever occurred earlier. The number of leaves at stem elongation was counted when internodes on annual stems were first visible. Pollen and nectar production were monitored daily during flower production, cotyledon status was monitored weekly, and stem elongation was monitored every 3–4 d. Flower parts were measured with a Wilde binocular microscope under 60× magnification using a 100-unit ocular scale. Healthy, fully expanded leaves were excised with a scalpel from plants in blocks 1–6 and pressed at 127, 128, 129, 135, 140, and 142 d after sowing, respectively. Pressed leaf and cotyledon parts were measured using calipers with a precision of ±0.1 mm. Leaf chroma, lightness, and hue were measured across the adaxial midrib of each of three healthy fully expanded leaves from each plant in block 1 using a Minolta model CR 200 colorimeter, and means for each plant were

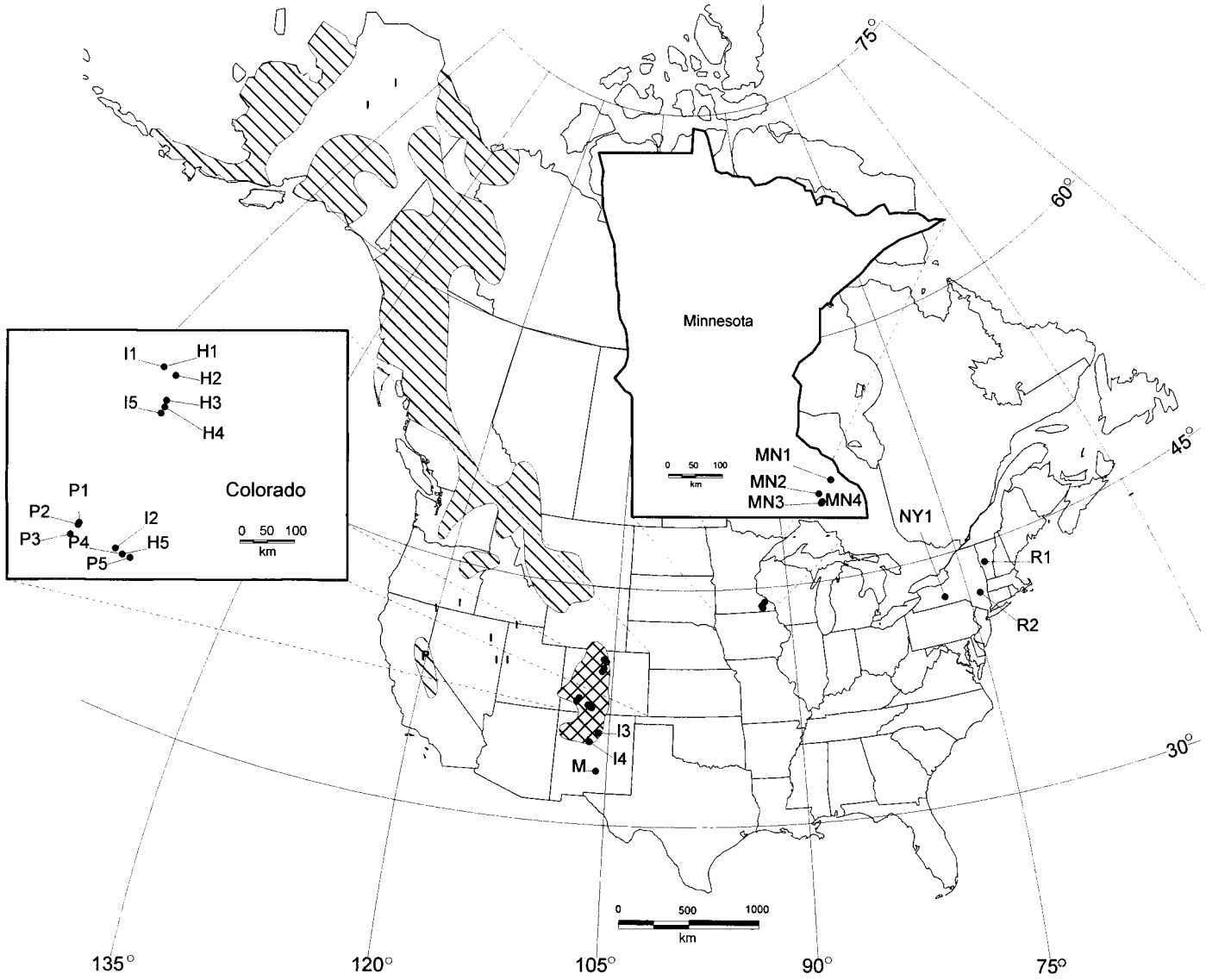

Fig. 1. Location of sampled *Sedum integrifolium* ssp. *leedyi* (MN1–4 and NY1), ssp. *integrifolium* (I1–I5), ssp. *procerum* (P1–P5), ssp. *neomexicanum* (M), *S. rhodanthum* (H1–H4), and *S. rosea* (R1 and R2) populations and approximate known ranges of *S. integrifolium* ssp. *integrifolium* (\\\ or I for isolated populations) and ssp. *procerum* (/// or P for the isolated population). *Sedum integrifolium* ssp. *leedyi* and *neomexicanum* are known from only five and one population(s) respectively. Ranges are from Clausen (1975) and U.S. Fish and Wildlife Service (1993).

used in subsequent data analyses. Color measurements for plants with leaves too narrow to span the color probe were taken from the adaxial surfaces of leaves placed side by side.

For each morphological character and RAPD marker, we tested for differences among operational taxonomic units (Table 1). Differences among OTUs in morphological traits were tested with contrasts or *t* tests using data that were transformed when appropriate to better fit normality assumptions of the SAS GLM procedure for unbalanced data sets (SAS, 1990) (Tables 2 and 3). From untransformed data for each morphological character, we obtained Type IV least squares means for each OTU using the SAS GLM procedure (SAS, 1990). Least squares means for each group were standardized by subtracting the character mean over all OTUs and dividing by the standard deviation among OTUs. RAPD marker frequencies in each OTU were calculated but not standardized. Taxonomic distance matrices were constructed from standardized morphological and RAPD frequency data and used to build UPGMA phenograms using the SIMINT distance and SAHN clustering programs of NTSYS (Rohlf, 1993). Subsets of the data (the first ten RAPD markers obtained, the vegetative morphological data, and the floral morphological data) were initially analyzed separately, then combined with the full molecular and morphological data sets, respectively, to compare preliminary and final results. The distance matrices were also evaluated using the WPGMA, UPGMC, Complete, and FLEXI cluster methods for comparison with the UPGMA phenograms.

Pearson correlation matrices, principal components, and eigenvalues were derived from standardized morphological and unstandardized RAPD data using the SAS PRINCOMP procedure (SAS, 1990), and factor scores for the first three principal components were plotted using the PROJ and MOD3D programs of NTSYS (Rohlf, 1993).

Molecular genetic variation within and among OTUs was evaluated using the AMOVA program of the Arlequin population genetics software package (Schneider et al., 1997). RAPD marker presence or absence data for each individual were entered and used in this program to construct a matrix of distances among all individuals. The variation in these distances was then analyzed to examine the levels of variation within and among OTUs, with

TABLE 1. Number of plants (RAPD marker analyses) and seed families (common garden analyses) sampled. MN1-MN3 and MY1 = *Sedum integrifolium* ssp. *leedyi*, I = *S. integrifolium* ssp. *integrifolium*, P = *S. integrifolium* ssp. *procerum*, M = *S. integrifolium* ssp. *neomexicanum*, H = *S. rhodanthum*, R = *S. rosea*, ND = no data.

Pop.	OTU	RAPD	Common garden
MN1	MN1	39	3
MN2	MN2	50	18
MN3	MN3	34	4
NY1[a]	NY1[a]	39	17
I1	I	6	3
I2	I	10	4
I3	I	4	6
I4	I	8	5
I5	I	ND	5
Total	I	28	23
P1	P	8	5
P2	P	3	5
P3	P	5	4
P4	P	6	5
P5	P	11	5
Total	P	33	24
M1	M	30	20
H1	H	3	ND
H2	H	5	1
H4	H	1	ND
H5	H	3	3
Total	H	12	6
R1	R	22	11
R2	R	16	ND
Total	R	38	11
Total overall		303	126

[a] Includes three plants from a subpopulation of NY1 at Glenora Falls.

the significance of differences among OTUs tested by bootstrap resampling the data 1000 times. A nested ANOVA was performed to estimate the levels of morphological variation within and among *S. integrifolium* populations using the restricted maximum likelihood method of the SAS VARCOMP procedure (SAS, 1990). Diversity for continuous vegetative morphological characters 1–17 (Table 2) for each *S. integrifolium* population was estimated from the mean of within-seed-family standard deviations. Seed-family standard deviations were used instead of population standard deviations because seed family sizes were constant (12 individuals), and differences among their standard deviations are not confounded with differences in sample size. OTU-family mean standard deviations for these characters were standardized by subtracting the mean for a character over all OTUs and dividing by the standard deviation among OTUs. Absolute values of the standardized data were averaged over the 17 characters to obtain within-OTU estimates of morphological genetic diversity.

RESULTS

The UPGMA phenogram based on RAPD data shows that the *S. integrifolium* ssp. *leedyi* populations are clearly distinct from the western *S. integrifolium* populations, that NY1 and MN1–MN3 are only distantly related, and that the genetic distances between MN1–MN3 and the other *S. integrifolium* OTUs approached those among species (Fig. 2). The analyses using subsets of data and alternative clustering methods yielded phenograms showing these same relationships. Distances among the *S. integrifolium* ssp. *leedyi* populations are similar to or higher than those among the named western subspecies (Fig. 2), and the differences among OTUs are highly significant as tested by AMOVA (all $P < 0.00000$, except for OTUs I vs. P, $P < 0.002$). Of the 33 RAPD markers evaluated, nine (6, 15, 16, 20, 21, 24, 25, 27, 28) were unique to *S. integrifolium*, one (9) unique to *S. rosea*, and one (3) unique to *S. rhodanthum*. Of the nine markers unique to *S. integrifolium*, one (28) was unique and two (6, 20) nearly unique to *S. integrifolium* ssp. *leedyi*, and one (21) was unique to the western subspecies (Table 4).

Of the 1721 plants placed in the experimental blocks, 1685 (98%) survived to the end of the experiment. *Sedum integrifolium* ssp. *leedyi* differed significantly from the western subspecies (Table 3) having, for example, longer leaves and rhizomes ($P < 0.0001$) and significantly less ($P < 0.003$) stamen adnation. All evaluated morphological characters showed significant differences ($P < 0.0005$) among taxa. In the UPGMA phenogram based on the morphological data NY1 clusters with MN1–MN3, and distances among MN1–MN3 and between M and I and P are more pronounced than in the RAPD-based phenogram. The morphology and RAPD-based phenograms are largely comparable in other respects (Fig. 2).

Principal components analysis revealed relationships generally similar to those shown by the UPGMA phenograms (Fig. 3). The first three principal components explained 78% of the RAPD and 78% of the morphological marker variation among the OTUs. The RAPD marker data show differences among the *S. integrifolium* ssp. *leedyi* OTUs as similar to or greater than those among the named western subspecies, and differences between MN1 and I that are similar to differences among the *Sedum* species (e.g., H vs. P, M, I, vs. R). The morphological marker data differ from the RAPD data in clustering NY1, more closely with MN1–MN 3, and in separating M from the other western *S. integrifolium* subspecies. In other respects the RAPD and morphology-based principal components analyses are similar.

The molecular diversity indices were highest for *S. integrifolium* ssp. *integrifolium* and ssp. *procerum*, which are widespread, intermediate for the large but isolated *S. integrifolium* ssp. *neomexicanum* and NY1 ssp. *leedyi* populations, and the lowest for MN1, MN2, and MN3, but the confidence intervals in these indices overlap extensively (Table 5). In contrast, morphological diversity estimated from the continuous morphological characters was highest for *S. integrifolium* ssp. *neomexicanum*, and higher for MN1, MN3, and NY1 than for *S. integrifolium* ssp. *integrifolium* and ssp. *procerum* (Table 5).

The percentage of the total variation in *S. integrifolium* due to differences among OTUs was 30, 29, and 19% for the RAPD and floral and vegetative morphological data, respectively. Considering only the data from *S. integrifolium* ssp. *leedyi*, those figures were 25, 9, and 13%, respectively. Of the 19 possible tests of each predetermined contrast among the *S. integrifolium* OTUs, up to 18 were significantly different ($P < 0.05$) for a contrast (MN1 vs. MN2 and P vs. M), and of 15 possible tests for each predetermined pairwise difference, up to 12 were significantly different ($P < 0.05$) (Table 3).

DISCUSSION

Adaptively important morphological traits can differ dramatically between closely related plants due to a small number of differing alleles and might not be detected in a typical molecular marker survey of variation. For example, differences at a very small number of loci explained most of the floral differences between *Mimulus cardinalis* and *M. lewisii* that adapt them for hummingbird and bumble bee pollination, re-

TABLE 2. Morphological traits measured in *Sedum* plants. Data for characters 25, 26, and 37 were square-root transformed for contrast and *t* test comparisons. All other data were log transformed.

Character	Description
1. Leaves 2	number of leaves at 97, 98, or 99 d in blocks 1, 2–3, and 5–6, respectively
2. Leaves 3	number of leaves at 104, 105, or 106 d in blocks 1–2, 3–4, and 5–6, respectively
3. Leaves 4	number of leaves at 118, 119, 120, 121, 122, and 123 d in blocks 1–6, respectively
4. Height 1	height at 86 or 87 d in blocks 1–2 and 3–6, respectively
5. Height 2	height at 90, 91, 92 or 95 d in blocks 1, 2, 3–5, and 6, respectively
6. Height 3	height at 143, 144, 146, and 147 d after sowing in blocks 1–2, 3, 4–5, and 6, respectively
7. Height 4	height at 163, 164, 167, and 168 d after sowing in blocks 1–2, 3, 4–5, and 6, respectively
8. Stem number	number of stems at 132, 133, and 134 d after sowing in blocks 1–2, 3–5, and 6, respectively
9. Rhizome height	rhizome height above soil (mm) at 86 and 87 d after sowing in blocks 1–2 and 3–6, respectively
10. Leaf length	length (mm) of sessile leaf blade
11. Leaf width 1	width (mm) at 1/4 leaf length from leaf blade base
12. Leaf width 2	width (mm) at 1/2 leaf length from leaf blade base
13. Leaf width 3	width (mm) at 3/4 leaf length from leaf blade base
14. Cotyledon blade length	length (mm) from cotyledon blade base to tip
15. Cotyledon blade width	width (mm) of cotyledon at widest point
16. Stem elongation date	days from sowing to stem elongation
17. Leaf number at stem elongation	number of leaves when annual stem was first visible
18. Leaf color (lightness)	average value from adaxial surface of 3 healthy median leaves from each plant in block 1
19. Leaf color (chroma)	same as 18
20. Leaf color (hue)	same as 18
21. Axillary buds	percentage of plants in group with macroscopic axillary bud or branch
22. Flowering	percentage of plants in group with flowers
23. Fleshy petals	percentage of flowering plants with fleshy petals
24. Flowering date	days from sowing to flowering
25. Sepal number	
26. Stamen number	
27. Sepal length	
28. Sepal thickness	
29. Sepal width	
30. Stamen/petal adnation	length of adnation from petal base
31. Length petaloid stamen	length of stamen from petal attachment to anther tip
32. Length sepaloid stamen	length of a stamen opposite a sepal
33. Length carpel	
34. Width carpel	
35. Length nectaries	
36. Width nectaries	
37. Sepal color	1 = green, 2 = green with red tips, and 3 = red
38. Flower color	superimposed on morphological phenogram

TABLE 3. Number of significantly different contrasts (of 19 continuous vegetative characters) and number of significantly different pairwise differences (of 13 continuous floral and two continuous vegetative characters) for predetermined comparisons.

Contrast	$P < 0.05$	$P < 0.01$	$P < 0.001$	Pairwise differences	$P < 0.05$	$P < 0.01$	$P < 0.001$
MN1–3 and NY1 vs. I	16	13	12	MN1 vs. I	4	2	1
MN1–3 and NY1 vs. P	17	15	15	MN1 vs. P	4	2	1
MN1–3 and NY1 vs. M	16	15	13	MN1 vs. M	5	5	2
				MN2 vs. I	11	7	5
				MN2 vs. P	10	8	5
				MN2 vs. M	11	8	7
				MN3 vs. I	6	3	2
				MN3 vs. P	4	2	2
				MN3 vs. M	8	5	3
MN1–3 and NY1 vs. I, P, and M	14	12	9	NY1 vs. I	11	11	9
				NY1 vs. P	12	9	6
				NY1 vs. M	10	10	8
MN1 vs. MN2	18	14	14	MN1 vs. MN2	2	1	1
MN1 vs. MN3	12	11	8	MN1 vs. MN3	1	0	0
MN2 vs. MN3	6	5	5	MN1 vs. NY1	0	0	0
MN1–MN3 vs. NY1	10	9	6	MN2 vs. MN3	3	2	1
				MN2 vs. NY1	4	3	2
				MN3 vs. NY1	3	2	1
I vs. P	11	9	6	I vs. P	5	1	0
I vs. M	14	14	13	I vs. M	8	7	6
P vs. M	18	18	17	P vs. M	9	6	4

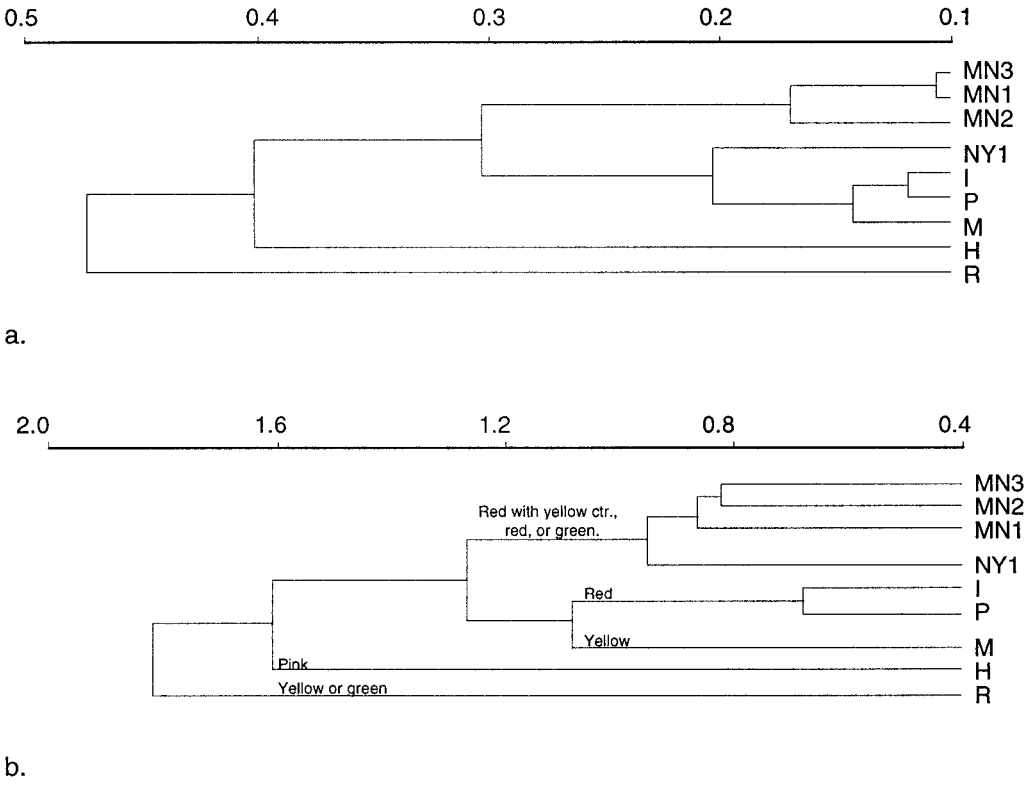

Fig. 2. UPGMA phenograms based on (a) average taxonomic distance among OTU RAPD fragment frequencies and (b) on average taxonomic distances among OTU least squares means of morphological traits. MN1–3 and NY1 = *S. integrifolium* ssp. *leedyi*, I = ssp. *integrifolium* P = ssp. *procerum*, M = ssp. *neomexicanum*, H = *S. rhodanthum*, R = *S. rosea*. The flower colors in each group are superimposed on (b).

spectively (Bradshaw et al., 1995, 1998). Few studies have estimated the number of loci that affect morphological characters, but morphological data that probably represent adaptively important traits often show differentiation that is not detectable using neutral molecular markers. Lodgepole pine (*Pinus contorta*), a western North American conifer with four described subspecies in a wide geographical and ecological range, shows little allozyme differentiation (3%) among its subspecies, but substantial differentiation (38%) for cone and seed traits (Wheeler and Guries, 1982). Furnier et al. (1991) found a similar pattern of differentiation among white spruce (*Picea glauca*) populations for allozymes (3.8%) vs. height growth in a common garden at ages 9 and 19 yr (48 and 54%, respectively). Harrison et al. (1997) described five distinct groups of wild North American strawberries (*Fragaria*) using morphological data from a common garden, but only three groups were distingushed using RAPD markers. Each of these studies reveal potentially selectively important morphological differentiation among populations, but gene flow among these populations is apparently extensive enough to prevent high levels of differentiation for selectively neutral molecular markers. Basing conservation decisions only on the molecular data would lead to the loss of important adaptive variation. The combination of morphological and molecular genetic data is valuable to effective gene and species conservation because it gives a superior understanding of a group's evolutionary status.

Significant adaptive differentiation is likely to evolve in ecologically and geographically widespread species, such as *S. integrifolium*. *Sedum integrifolium* ssp. *leedyi*, which is on the federal threatened and Minnesota and New York endangered species lists, occurs in isolated cool microhabitats on moist, north-facing limestone (MN) or east-facing shale (NY) cliffs between 274 and 378 m (MN) and 137 and 150 m (NY) above sea level. Historically, the major surrounding vegetation types were tallgrass prairie and oak savanna in Minnesota and northern hardwoods in New York (Barbour and Christensen, 1993; Brouillet and Whetstone, 1993; U.S. Fish and Wildlife Service, 1993, 1998). In contrast, the western subspecies are distributed in climates ranging from polar to cool temperate and are surrounded by vegetation ranging from tundra to western montane conifer and boreal forest (Barbour and Christensen, 1993; Brouillet and Whetstone, 1993). In the Colorado and New Mexico populations sampled for this study, plants occur on moist, rocky slopes above 3000 m. This suggests that the different *S. integrifolium* populations are evolving under different selective pressures. Our data support this, showing extensive differentiation among subspecies and among populations within *S. integrifolium* ssp. *leedyi* (Figs. 2 and 3, Table 3). The higher distance detected between M and P and I with morphological data than with RAPD data, reflected in differences such as leaf chroma, hue, length, widths, petal length ($P < 0.0001$), and flower color (Table 1, Fig. 2), suggests that M is differentiated from P and I because of selection, but that there is sufficient gene flow to avoid extensive differentiation in nonselective characters.

In contrast to the previously cited studies (Wheeler and Guries, 1982; Furnier et al., 1991; Harrison et al., 1997), the RAPD markers in general detect greater variation due to differences among the *S. integrifolium* subspecies and popula-

TABLE 4. RAPD marker frequencies (%) in each OTU.

Marker	Population								
	MN1	MN2	MN3	NY1	I	P	M	H	R
1	87	98	82	95	96	78	100	75	97
2	0	0	0	0	0	3	0	0	97
3	0	0	0	0	0	0	0	17	0
4	97	100	100	100	100	100	93	17	100
5	44	86	29	13	29	69	3	92	0
6	77	26	74	8	7	0	3	0	0
7	92	92	82	79	89	94	77	0	29
8	0	2	0	0	4	3	0	92	0
9	0	0	0	0	0	0	0	0	100
10	0	0	0	0	0	0	3	0	97
11	100	100	100	92	89	97	97	33	97
12	3	4	0	0	39	25	30	67	0
13	0	0	0	31	7	25	3	0	50
14	3	2	3	3	4	0	17	33	0
15	0	0	0	3	7	0	3	0	0
16	0	0	0	0	4	3	0	0	0
17	90	78	85	46	11	13	0	0	42
18	36	84	53	46	54	88	73	75	87
19	0	0	0	3	0	0	3	0	68
20	15	26	6	28	4	0	0	0	0
21	0	0	0	0	36	22	7	0	0
22	92	60	59	79	68	75	60	08	08
23	0	0	0	33	54	34	37	0	97
24	97	86	100	41	32	34	27	0	0
25	10	48	0	74	4	22	50	0	0
26	100	62	85	51	79	84	97	100	79
27	56	54	41	79	11	28	43	0	0
28	100	88	79	5	0	0	0	0	0
29	0	0	0	3	36	31	33	0	42
30	0	0	0	0	32	6	0	0	26
31	0	20	0	0	14	9	3	0	55
32	5	4	18	0	4	6	0	08	76
33	26	16	56	92	82	81	67	92	3

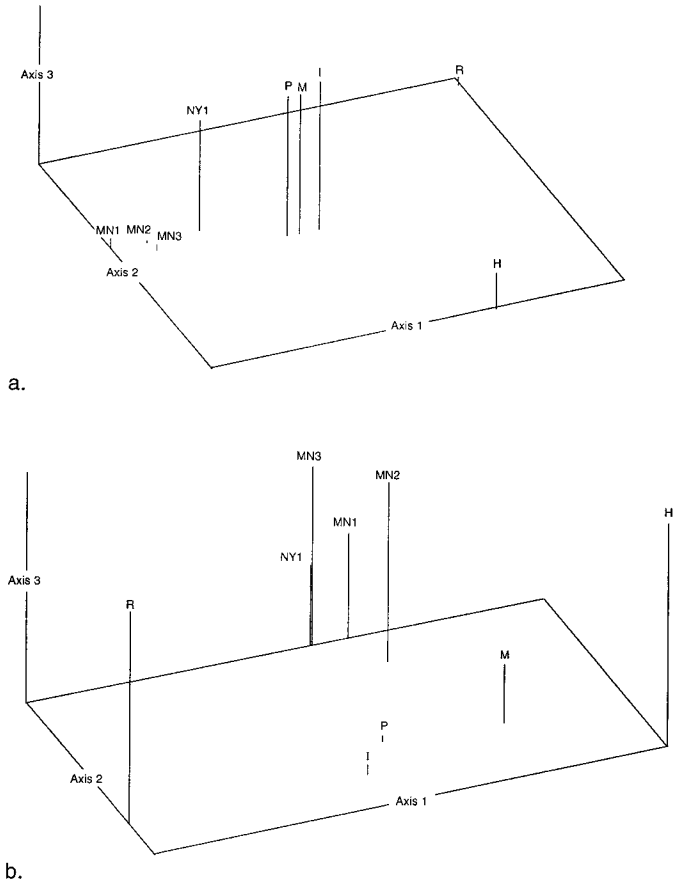

Fig. 3. Relationship of OTUs defined (a) by the first three principal components of RAPD frequencies for OTUs and (b) by the first three principal components of least squares means of morphological traits for OTUs. MN1–3 and NY1 = *S. integrifolium* ssp. *leedyi*, I = ssp. *integrifolium*, P = ssp. *procerum*, M = ssp. *neomexicanum*, H = *S. rhodanthum*, and R = *S. rosea.*

tions than the morphological markers. The level of RAPD differentiation among the *S. integrifolium* ssp. *leedyi* populations (25%) is comparable to levels of allozyme variation reported among populations of animal-pollinated plant species with mixed breeding systems (mean ± 1 SE = 21.6% ± 2.4%), but is high in comparison with the levels of variation among populations with wind dispersed seeds (mean ± 1 SE = 14.3% ± 2.0%) (Hamrick and Godt, 1990). These relatively high levels of molecular differentiation, especially among populations within *S. integrifolium* ssp. *leedyi*, suggest low levels of gene flow among populations. The *S. integrifolium* plants have seeds adapted for wind dispersal and are usually functionally dioecious, but occasionally have perfect, self-compatible flowers. They can be pollinated by hover flies (Syrphidae), bees (superfamily Apoidea), and in our common garden study, lady bird beetles (*Hippodamia convergens*) (Clausen, 1975; U.S. Fish and Wildlife Service, 1993; Olfelt, personal observation). Given these pollinators, the pollen dispersal range would likely be much less than 1000 m (Levin, 1979; Schmitt, 1980; Pleasants, 1991), suggesting very little current gene flow between any of the *S. integrifolium* ssp. *leedyi* populations and between those populations and the western subspecies, placing them each on separate evolutionary trajectories.

There are differences between the cluster patterns for NY1 shown by the morphological and molecular data (Fig. 2) that may reflect the differing effects of selective and nonselective evolution on the *S. integrifolium* ssp. *leedyi* populations. The RAPD marker data separate MN1, MN2, and MN3 more widely from NY1 than the morphological data do. MN1, MN2, and MN3 are currently much smaller (748, 445, and 278, respectively) than NY1 (>6000) (Olfelt, Furnier, and Luby, 1998; U.S. Fish and Wildlife Service, 1993, 1998), have smaller areas of potential habitat, and may have experienced more significant population bottlenecks leading to differentiation from NY1 through genetic drift. The absence of RAPD markers 13, 15, 23, and 29 in MN1, MN2, and MN3 and their presence in NY1 and the western *S. integrifolium* subspecies (Table 4)

TABLE 5. Genetic diversity estimates: number of polymorphic RAPD sites within each OTU; mean number of RAPD marker differences between all possible pairs of plants in each OTU; average diversity over loci; average standardized standard deviation of continuous morphological characters.

OTU	No. of polymorphic RAPD sites	RAPD pairwise differences	Average RAPD diversity	Morphological diversity
NY1	22	5.8 ± 2.8	0.17 ± 0.09	0.843
MN1	16	3.8 ± 1.9	0.11 ± 0.06	0.909
MN2	20	5.2 ± 2.5	0.15 ± 0.08	0.446
MN3	14	4.8 ± 2.4	0.14 ± 0.08	0.839
I	27	6.9 ± 3.3	0.20 ± 0.11	0.681
P	23	6.2 ± 3.0	0.18 ± 0.10	0.643
M	22	5.7 ± 2.8	0.17 ± 0.09	1.101

suggest that these markers may have been present in ancestral *S. integrifolium* populations, but lost in MN1, MN2, and MN3, possibly through genetic drift. Using RAPD data alone, we might overestimate the differentiation between NY1 and MN1–MN3. Or, given the similarities between NY1 and the western *S. integrifolium* subspecies according to RAPD marker data, we might underestimate the distinctness of NY1, perhaps suggesting a somewhat lower conservation priority for the population. These examples illustrate that a data set consisting only of selectively neutral markers may fail to reveal adaptively important variation formed through natural selection, and can lead to biologically unsound management strategies.

The trends in our molecular genetic diversity estimates within OTUs are generally what would be expected, with the highest diversity estimates for the wide-ranging subspecies and the lowest estimates for the smallest and most narrowly distributed OTUs. An exception to this pattern is that although MN1 is the largest *S. integrifolium* ssp. *leedyi* population in Minnesota, it has the lowest estimate for mean number of pairwise differences and average diversity over loci and an estimate of number of polymorphic sites intermediate to those in MN2 and MN3 (Table 5). It also has low flowering, germination, and seed set rates (Olfelt, Furnier, and Luby, 1998), suggesting that it may be experiencing inbreeding depression. The morphological data, in contrast, yield higher diversity estimates for most of the narrowly distributed OTUs and lower estimates for the more widely distributed *S. integrifolium* ssp. *integrifolium* and *procerum* OTUs, again illustrating that selectively neutral and morphological genetic data may differ and suggesting that any population bottlenecks in MN1 and MN3 have not been so severe as to exhaust morphological genetic variation.

The disjunct occurrence of *S. integrifolium* ssp. *leedyi* populations in Minnesota and New York can be explained either as relatively recent long-distance dispersal events from the west, or as populations that have been separated from the western subspecies for more than several thousand years as relicts of the tundra or boreal vegetation that occupied eastern North America after the Wisconsin glaciation (Wright, Winter, and Patten, 1963; Davis, 1983; Delcourt and Delcourt, 1987). The first hypothesis predicts little or no detectable differentiation between *S. integrifolium* ssp. *leedyi* and the western subspecies because of recent gene flow. Such is the case for *Cirsium canescens* and *C. pitcheri*, and for *Howellia aquatilis* populations. These taxa and populations are thought to have become isolated $<$11 000 yr ago and have very low levels of allozyme differentiation (Lesica et al., 1988; Loveless and Hamrick, 1988). This first hypothesis is supported by pollen core data that show maxima in prairie graminoid and forb pollen $\sim$7000 to 8000 yr ago and low lake levels in east-central Minnesota (Wright, Winter, and Patten, 1963; McAndrews, 1966), suggesting that the climate would have been too warm and arid in southeastern Minnesota during that period for a habitat specialist such as *S. integrifolium* ssp. *leedyi*. However, our molecular marker data show significant differentiation between *S. integrifolium* ssp. *leedyi* and the western subspecies and relatively high levels of molecular genetic variation within population NY1. These observations argue against recent colonization from the west and suggest that the *S. integrifolium* ssp. *leedyi* populations have been isolated from the western subspecies since well before the arid warm period, perhaps surviving the warm and arid conditions, or dispersing from possible eastern refugia.

The Recovery Plan for *S. integrifolium* ssp. *leedyi* calls for formal protection of the three privately owned Minnesota populations (MN2, MN3, MN4) and a portion of NY1 if the taxon is determined to be distinct from the western *S. integrifolium* subspecies (U.S. Fish and Wildlife Service, 1993, 1998). Our data show unequivocally that *S. integrifolium* ssp. *leedyi* is distinct and we recommend that the populations be formally protected. Genetic distances between the *S. integrifolium* ssp. *leedyi* populations are high and exceed or are comparable to the distances between the named western subspecies (Fig. 2), emphasizing further the need to protect all of them since they each appear to represent independent evolutionary units. We also recommend that the populations be considered as distinct, noninterchangeable entities and that genebanks for each population should be managed separately.

The morphological and molecular data sets show that the OTUs are diverging, probably through selective and nonselective pressures and that the populations are highly differentiated from one another. Together the data sets give us a very high degree of confidence in our results and recommendations. We urge plant conservation biologists to consider both morphological and molecular genetic evidence when setting conservation priorities and practices for rare and endangered plants. If time, funding, or other constraints do not allow the assembly of such complete evidence, we urge cognizance that sole reliance on molecular or morphological data may tell an incomplete story.

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