Report on genetic analyses of *Eriogonum corymbosum* populations in Las Vegas Valley

Mark Ellis and Paul Wolf, January 16, 2007

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INTRODUCTION

The *Eriogonum corymbosum* Benth. complex comprises a group of buckwheats distributed widely across the western U.S., from southwestern Wyoming, through Utah and southwestern Colorado, to northern Arizona, northern New Mexico, and southern Nevada (Reveal 2005). A putative history of dispersion, isolation, divergence, hybridization and introgression may have contributed to this morphologically and geographically diverse group (Reveal 2002). Currently eight varieties of *E. corymbosum* are recognized (Reveal 2005). Until recently, populations in Clark County, Nevada, have historically been treated as the wide ranging *E. corymbosum* var. *glutinosum* (Reveal 2002) as well as *E. corymbosum* var. *aureum* (Reveal 1985). In this report, we examine *E. corymbosm* samples from Clark County populations and assess their genetic similarity to other taxa in the region.

Reveal (2004) concluded that the Clark County populations are morphologically and ecologically distinct from *E. corymbosum* var. *glutinosum* and from var. *aureum*, and he proposed the new varietal designation *E. corymbosum* var. *nilesii* Reveal. Here we examine populations in the *E. corymbosum* complex and related *Eriogonum* species in the subgenus *Eucycla* to address the genetic distinctness of the populations of var. *nilesii* found in Clark County, Nevada.

We analyzed the DNA extracted from samples by generating Amplified Fragment Length Polymorphism (AFLP) profiles of the nuclear genome as well as sequences of chloroplast DNA. We examined the AFLP data using an unweighted-pair-group method analysis (UPGMA) and principal components analyses (PCAs). Chloroplast DNA sequences of an intergeneic spacer region were obtained and analyzed for phylogenetically informative markers. The results of these analyses of AFLP and sequence data add further support to the conclusion in our previous report and to Reveal's (2002, 2005) conclusion (based on morphological research) that the Clark County populations of *E. corymbosum* have diverged genetically enough to warrant taxonomic recognition.

METHODS

Collections

We collected leaf samples of 10-15 plants from each of 51 populations (Table 1, Table 2, Figs. 1 and 2) and dried them on silica gel inside sealed plastic bags immediately upon collection for rapid desiccation. These included *E. corymbosum* var. *nilesii* samples from 11 populations (Fig. 2), nine of which were located in and around Las Vegas, Nevada (referred to as the Las Vegas plants). Samples of a tenth population were collected from White Basin in the Muddy Mountains region (referred to as the White Basin plants), about 40km east of North Las Vegas. Samples from an eleventh population were

Table 1. Seventeen *Eriogonum* taxa sampled from 51 sites (abbreviated site name and number of sites in parentheses).

E. corymbosum var. *aureum* **(Eva 4)**	*E. hylophilum* **(Eh 2)**
E. corymbosum var. *corymbosum* **(Ecc 5)**	*E. lancifolium* **(El 1)**
E. corymbosum var. *glutinosum* **(Ecg 3)**	*E. leptocladon* **(Ele 2)**
E. corymbosum var. *nilesii* **(Ecn 11)**	*E. loganum* **(Elo 1)**
E. corymbosum var. *orbiculatum* **(Eco 5)**	*E. microthecum* **(Em 2)**
E. corymbosum var. *velutinum* **(Ecv 3)**	*E. nummulare* **(En 1)**
	E. racemosum **(Er 1)**
E. effusum **(Ee 2)**	*E. smithii* **(Es 2)**
E. brevicaule **(Eb 1)**	*E. thompsoniae* **(Et 5)**

collected in Lincoln County, Nevada (referred to as the Lincoln County plants), about 15km west of Utah's southwest corner and 100km northeast of North Las Vegas. Genomic DNA was extracted from the dried leaf samples using either the Qiagen DNeasy 96 Plant Kit or the Qiagen DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA), following the manufacturer's

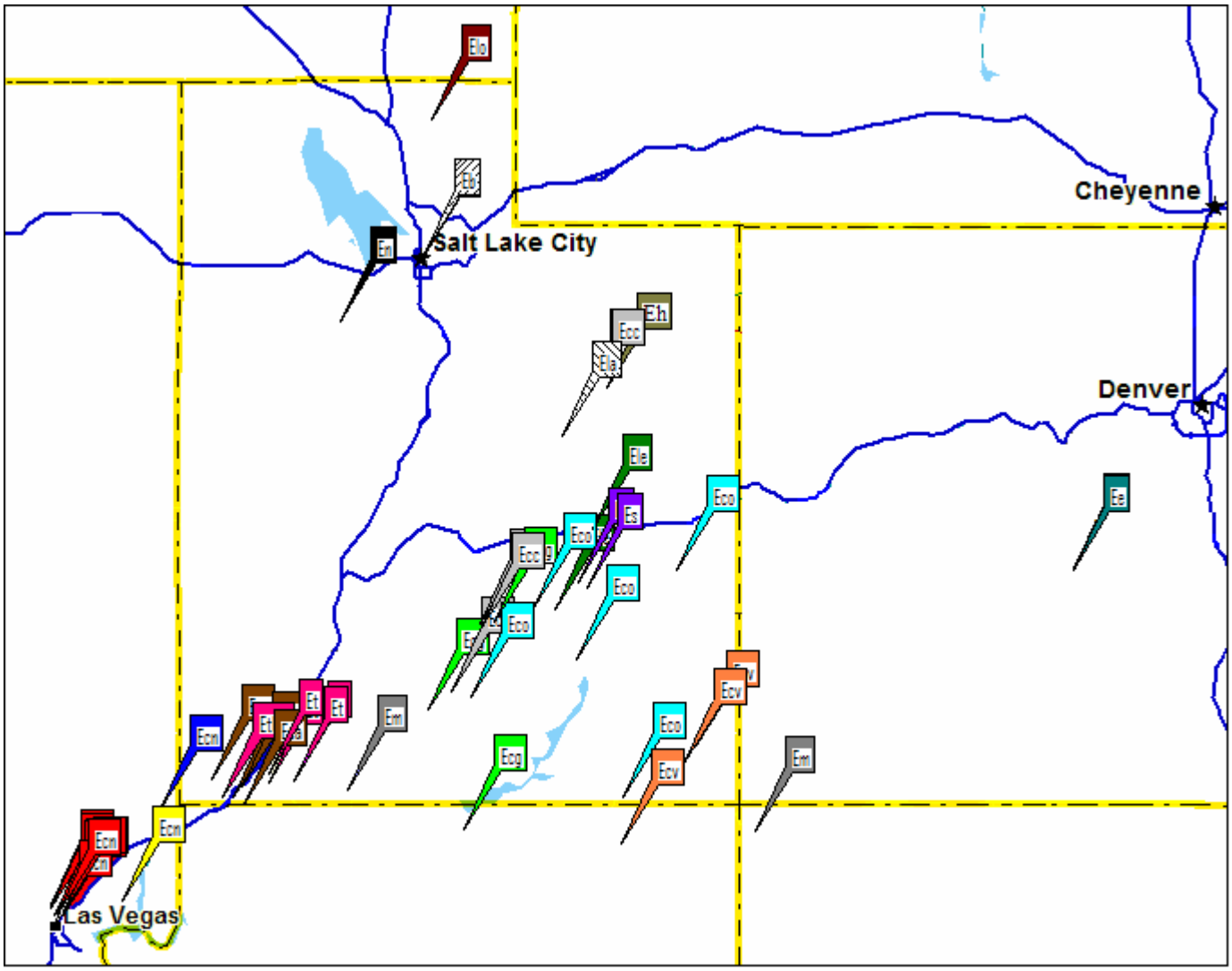

Fig. 1. Collection sites, with 11 in NV, 2 in AZ, 1 in NM, and 35 in UT. See Table 1 for key to abbreviations.

Table 2. Eriogonum populations collected and analyzed.

Site	Taxon	Location	Latitude and Longitude
U07	*E. brevicaule*	City Canyon, Salt Lake Co, UT	N40 48 29.9 W111 52 2.3
U01	*E. corymbosum aureum*	Shivwits, Washington Co, UT	N37 10 53.5 W113 46 09.2
U34	*E. corymbosum aureum*	Washington, Washington Co, UT	N37 7 51.9 W113 29 9.4
U36	*E. corymbosum aureum*	1Km E of Bloomington, Washington Co, UT	N37 3 4.2 W113 34 25.4
U35	*E. corymbosum aureum*	Behive Dome, Washington Co, UT	N37 0 35.4 W113 28 7
U09	*E. corymbosum corymbosum*	Escalante, Garfield Co, UT	N37 47 6.9 W111 37 52.5
U12	*E. corymbosum corymbosum*	Grover, Wayne Co, UT	N38 13 36.9 W111 20 48.2
U13	*E. corymbosum corymbosum*	3.5Km NW of Grover, Wayne Co, UT	N38 15 1.7 W111 22 26.5
U22	*E. corymbosum corymbosum*	Middle 9-Mile Canyon, Carbon Co, UT	N39 46 32.9 W110 28 47.1
U23	*E. corymbosum corymbosum*	Middle 9-Mile Canyon, Carbon Co, UT	N39 46 30.2 W110 27 21.1
U08	*E. corymbosum glutinosum*	16Km NE of Henrieville, Garfield Co, UT	N37 38 20.4 W111 50 37.8
U14	*E. corymbosum glutinosum*	2Km S of Fruita, Wayne Co, UT	N38 15 59.4 W111 14 41.3
A01	*E. corymbosum glutinosum*	9Km S of Page, Coconino County, AZ.	N36 50 15.5 W111 30 31.1
N01	*E. corymbosum nilesii*	N Las Vegas, Clark Co, NV	N36 17 29.4 W 115 11 47.1
N02	*E. corymbosum nilesii*	N Las Vegas, Clark Co, NV	N36 18 52.1 W115 11 35.8
N03	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 14 54.5 W115 09 20.6
N04	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 14 16.9 W115 09 34.7
N05	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 14 35.4 W115 04 45.3
N06	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 15 30.2 W115 04 22.7
N07	*E. corymbosum nilesii*	S Las Vegas, Clark Co, NV	N36 06 14.9 W115 12 29.7
N08	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 15 58.2 W115 04 43.5
N09	*E. corymbosum nilesii*	NW Las Vegas, Clark Co, NV	N36 14 59.6 W115 08 07.0
N10	*E. corymbosum nilesii*	White Basin, Clark County, NV	N36 20 26.7 W114 33 37.3
N11	*E. corymbosum nilesii*	22Km nw of Mesquite, AZ in Lincoln Co, NV	N36 57 42.1 W114 13 5.8
U11	*E. corymbosum orbiculatum*	14Km E of Escalante, Garfield Co, UT	N37 44 58.6 W111 26 38.0
U16	*E. corymbosum orbiculatum*	15Km W of Hanksville, Wayne Co, UT	N38 21 56.9 W110 53 11.9
U17	*E. corymbosum orbiculatum*	Three Forks, Wayne Co, UT	N38 00 2.5 W110 30 46.4
U30	*E. corymbosum orbiculatum*	Arches, Grand Co, UT	N38 37 3.8 W109 37 5.4
U31	*E. corymbosum orbiculatum*	22Km S of Mexican Hat, San Juan Co, UT	N37 3 21.5 W110 5 27.3
U28	*E. corymbosum velutinum*	17Km NE of Bluff, San Juan Co, UT	N37 25 5.8 W109 26 47.1
U29	*E. corymbosum velutinum*	Bluff, San Juan Co, UT	N37 17 21.8 W109 32 53.6
A02	*E. corymbosum velutinum*	13Km E of Kayenta, Navajo Co, AZ	N36 43 48.7 W110 6 42.0
C01	*E. effusum*	12Km NW of Salida, Chaffee Co, CO	N38 37 12.5 W106 4 42.1
C02	*E. effusum*	13Km NW of Salida, Chaffee Co, CO	N38 37 59.4 W106 4 45.7
U20	*E. hylophilum*	Upper 9-Mile Canyon, Duchesne Co, UT	N39 52 57.9 W110 13 51.1
U21	*E. hylophilum*	Upper 9-Mile Canyon, Duchesne Co, UT	N39 52 57.2 W110 14 6
U24	*E. lancifolium*	8Km E of Wellington, Carbon Co, UT	N39 32 48.3 W110 38 34.6
U25	*E. leptocladon*	20Km SW of Green River, Emery Co, UT	N38 54 30.3 W110 22 10.0
U15	*E. leptocladon leptocladon*	3Km S of Hanksville, Wayne Co, UT	N38 20 40.1 W110 42 23.6
U06	*E. loganum*	Logan, Cache Co, UT	N41 44 25.2 W111 48 25.5
NM1	*E. microthecum*	20Km W of Shiprock, San Juan Co, NM	N36 49 4.7 W108 54 44.2
U18	*E. microthecum simpsonii*	6Km N of Kanab, Kane Co, UT	N37 6 16.0 W112 32 55.6
U10	*E. nummulare*	17Km N of Dugway Proving Ground, Toole Co, UT	N40 20 12.7 W112 36 47.0
U05	*E. racemosum*	N Salt Lake City, Salt Lake Co, UT	N40 47 28.5 W111 51 42.7
U26	*E. smithii*	Little Flat Top, Emery Co, UT	N38 32 15 W110 29 38.5
U27	*E. smithii*	N Texas Hill, Emery Co, UT	N38 30 2.6 W110 24 47.8
U32	*E. thompsoniae*	4Km W of Bloomington Hills, Washington Co, UT.	N37 03 16.2 W113 39 54.7
U33	*E. thompsoniae*	4Km SE of Hurricane, Washington Co, UT	N37 08 53.2 W113 15 20.6
U03	*E. thompsoniae*	La Verkin, Washington Co, UT	N37 13 8.6 W113 15 0
U04	*E. thompsoniae*	Rockville, Washington Co, UT	N37 9 42.4 W113 1 56.6
U19	*E. thompsoniae matthewsiae*	SW Zion Ntl Park, Washington CO, UT	N37 11 47.4 W112 59 33.7

protocols. The concentrations of DNA in the extracted sample solutions were quantified with the NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware) following the manufacturer's instructions.

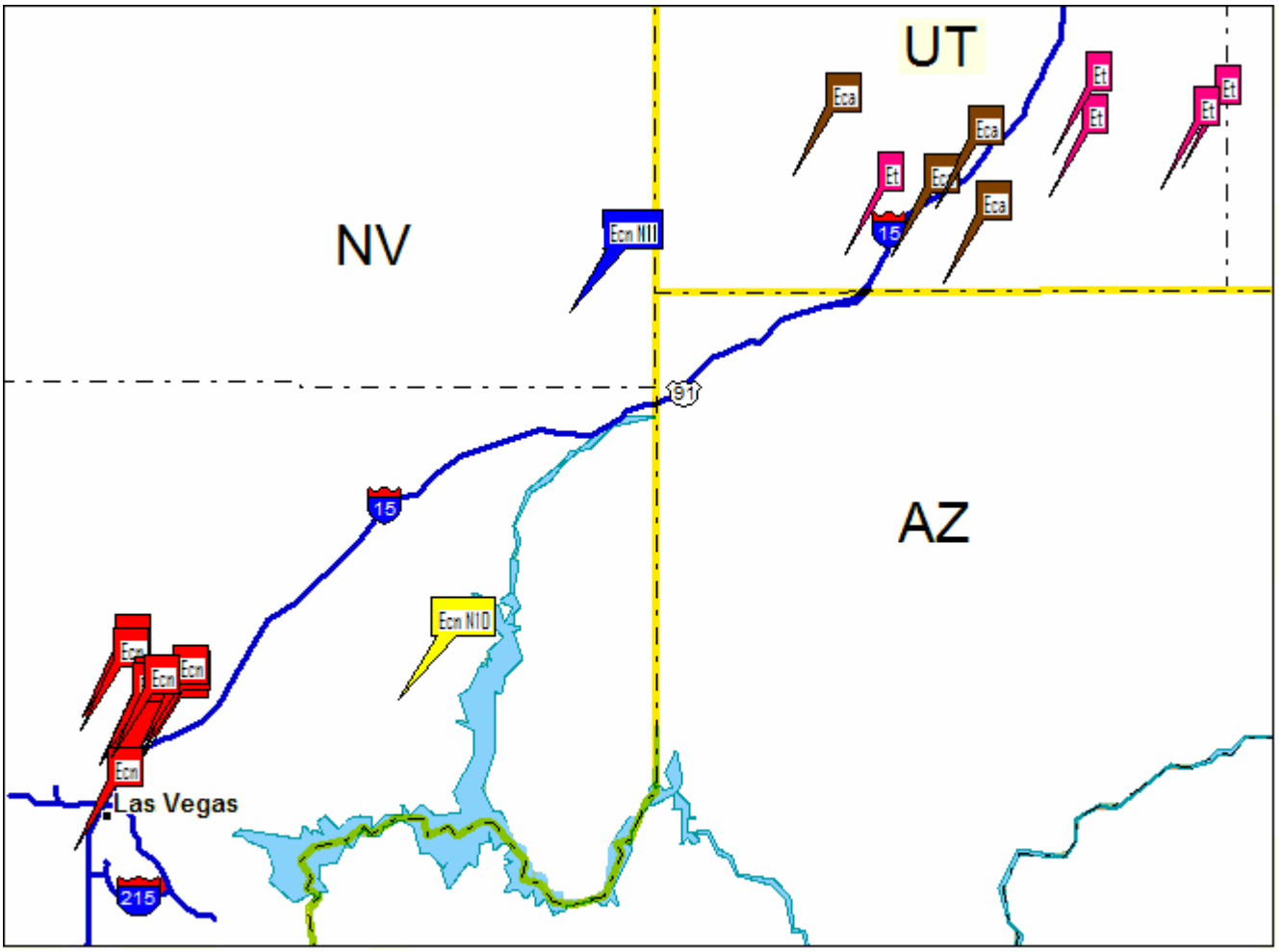

Fig. 2. Range of *E. c. nilesii* (Ecn, red, yellow, & blue), *E. c. aureum* (Eca, brown), and *E. thompsoniae* (Et, pink) collected.

AFLPs

An Amplified Fragment Length Polymorphism (AFLP) profile was generated for each DNA sample using a modified version of the AFLP protocol by Vos, et al. (1995). The genome was digested with restriction enzymes (rare cutter *Eco*RI and frequent cutter *Mse*I) and a subset of the fragments was amplified by PCR. We amplified the fragments with the following two selective primer pair sets: *Eco*RI-ACG with *Mse*I-ACT, and *Eco*RI-ACC with *Mse*I-AGC. The amplified restriction fragments were separated via capillary electrophoresis and recorded using Applied Biosystem's ABI 3730 DNA Analyzer with LIZ-500 size standards. The AFLP profile generated from each DNA sample was visualized and scored using Genographer v1.6.0 (Benham 2001).

The AFLP technique selects regions of the genome randomly and many polymorphisms can be found, representing variation among individuals via presence or absence of restriction sites. Using the two selective primer pair sets, we generated 228 polymorphic loci, providing the markers on which we based our analyses.

The scored AFLP data were then analyzed using the computer software SPSS v13 (SPSS Inc. 2005) to run a UPGMA – a multivariate procedure for detecting categories in data. In this case, we used a hierarchical approach to group the samples, which is based on genetic distance measures of the binary profile of each individual's AFLP fingerprint. Using the method of average linkage between groups, the computer program proceeds in a step-wise manner, joining pairs of individuals (based on overall similarity in AFLP profiles), then pairs of clusters, until all the data are combined within a single cluster encompassing all subclusters found.

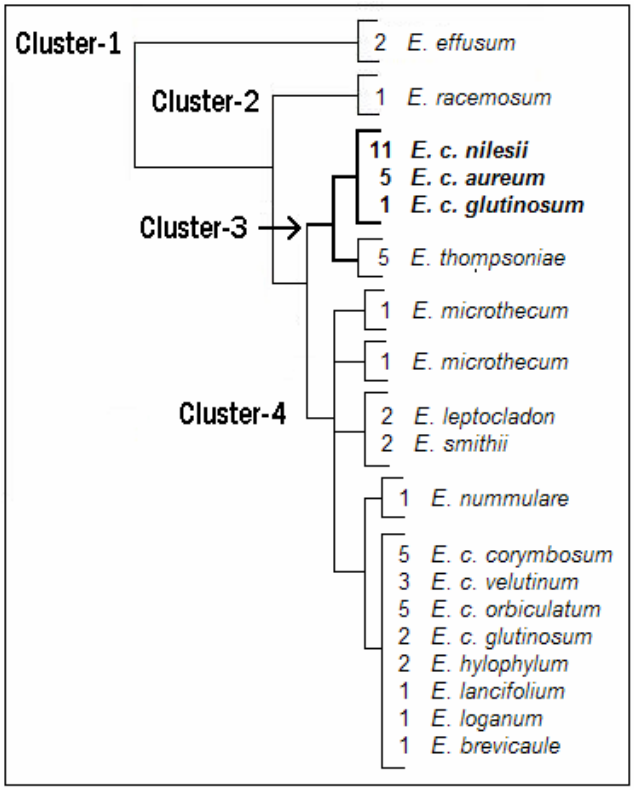

Fig. 3. Simplified UPGMA dendrogram of AFLP data showing relationships of populations

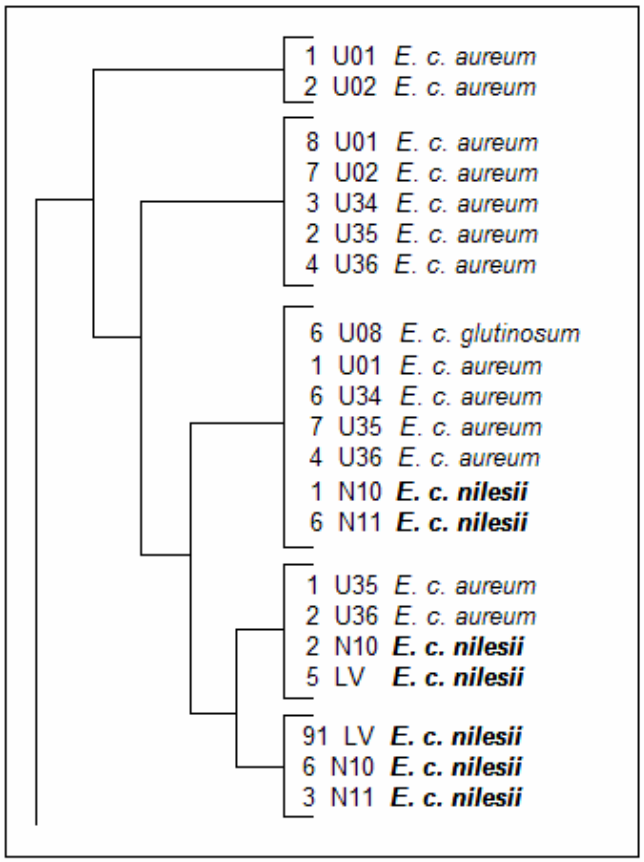

Fig. 4. Detail of subcluster within Cluster 3 showing numbers of individuals per population.

We also ran PCA analyses on the AFLP data with the computer software NTSYSpc v2.10t (Rohlf 2000). Using the AFLP matrix of presence and absence of amplified fragments (loci) among *Eriogonum* samples, three eigenvectors are extracted for each sample in the correlation matrix, and the combination of eigenvectors separates individual samples graphically in three-dimensional space. The locations of the samples in the resulting 3-D plots provided corroboration for the UPGMA results and for the sequence results.

Sequencing and Phylogenetic Analysis

We amplified and sequenced the *trnS*^{UGA}-*trnfM*^{CAU} cpDNA intergenic spacer region for 54 individuals from 13 different *Eriogonum* taxa using primers described by Shaw et al. (2005). We designed a third internal primer to ensure base clarity throughout the length of the sequence. We amplified this region using polymerase chain reactions (PCRs) in 50uL solutions with 400ng/uL BSA buffer, 1x Mg free buffer, 1.5mM $MgCl_2$, 0.20mM dNTPs (each), 2.5U *Taq* polymerase, 0.25uM of each primer, and 2.0ng DNA template in each reaction. The thermal cycler protocol began at 94°C for 2 minutes, followed by 35 cycles of 94°C for 1 minute (denature), 62°C for 1 minute (anneal), and 72°C for 2 minutes (extend). The last cycle was followed by a 7 minute hold at 72°C and then a continuous hold at 4°C.

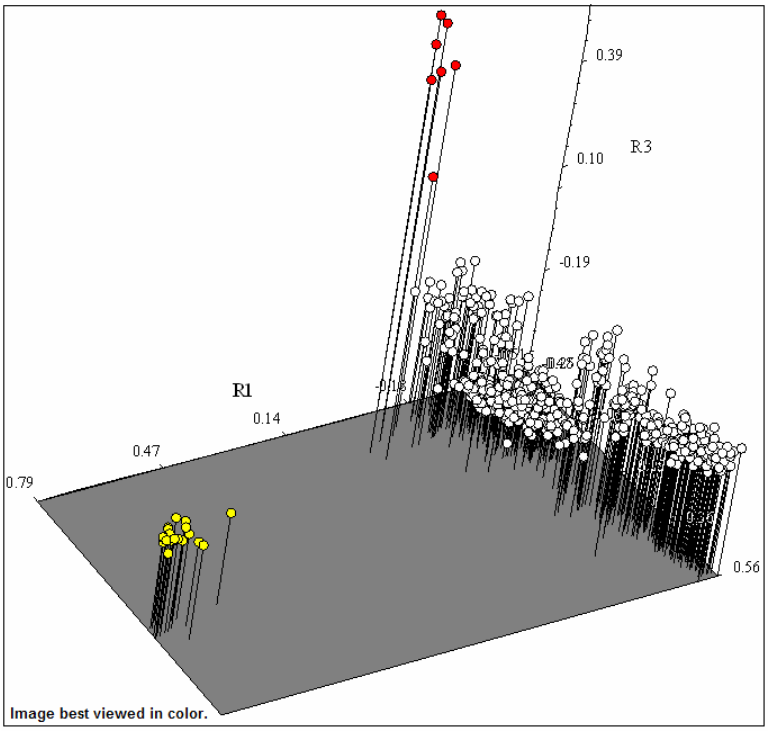

Fig. 5. PCA image of 2 *E. effusum* populations (yellow), *E. racemosum* (red), and all other samples collected (white).

The amplified PCR products were purified using Qiagen's Qiaquick Purification Kits (Qiagen, Inc., Valencia, CA), following the manufacturer's instructions. Sequencing reactions of the purified PCR products were run in both directions in separate reactions for each sample with each primer and Amersham's ET Dye Terminator. The thermal-cycler protocol was 30 cycles at 95°C for 20 seconds, 50 °C for 15 seconds, 60°C 3 minutes. The last cycle was followed by a continuous hold at 10°C.

The products of the sequencing reactions were purified through hydrated Sephadex and then run

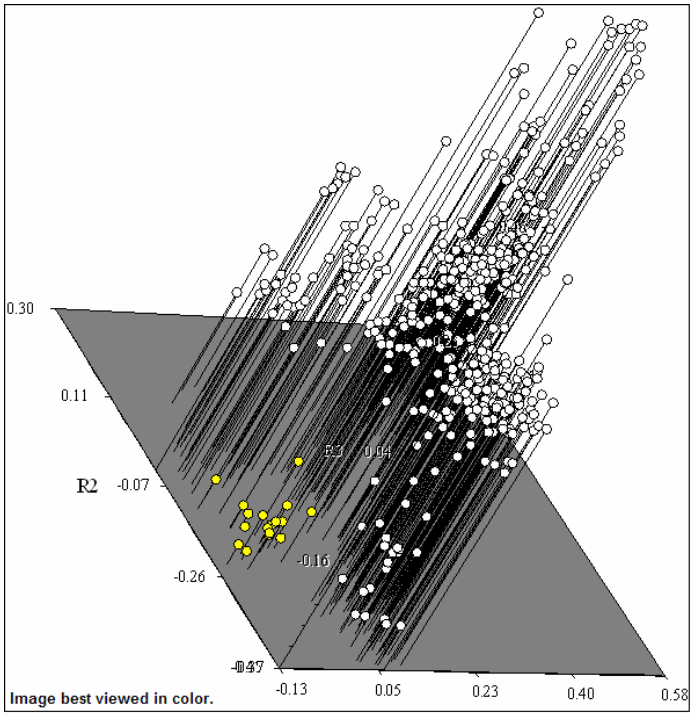

Fig. 6. PCA image of *E. leptocladon* (yellow) and the remaining collected samples (white).

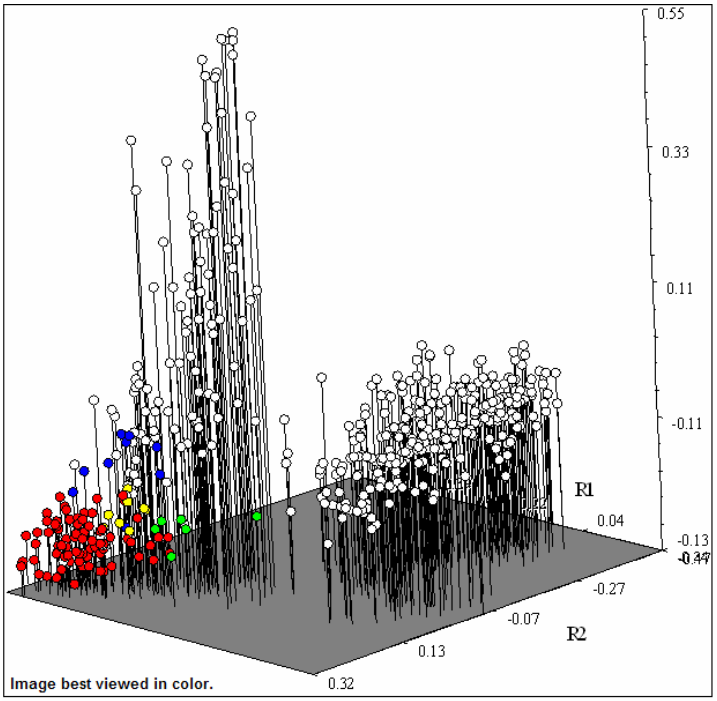

Fig. 7. PCA image of *E. c. nilesii* populations N01-09 (Las Vegas) in red, N10 (White Basin) in yellow, and N11 (Lincoln Co) in blue; *E. c. glutinosum* (U08) is in green; remaining collected samples in white.

on the ABI 3100 automated capillary sequencer (Applied Biosystems), producing sequences over 1200bp long. The initial sequence alignment was obtained with the ClustalW Multiple Alignment option in the BioEdit alignment program (Hall 1999). The final alignment was obtained manually, creating a trimmed sequence matrix with 1,235 characters. Although the gaps within a sequence were coded as missing data, the indels were recoded as a 5th character state appended to the end of each sequence as presence or absence of a gap (1 = gap present, and 0 = gap absent). Thus, each indel was assumed to have occurred by a single event.

The aligned sequences were imported into PAUP v4.0b10 (Swofford, 2002) and analyzed under the parsimony optimality criterion. All characters (bases and gap presence or absence) were analyzed as equal in weight and unordered. Of the variable characters, 5 were parsimony-uninformative, whereas 32 were parsimony-informative (reduced to 20 when the two *E. effusum* populations are excluded from the analysis). Tree space was examined with a heuristic search with simple addition sequence, the TBR (tree-bisection-reconnection) branch-swapping algorithm, and the MulTrees option in effect. MaxTrees was set at 200, and set to auto-increase by 100 if 200 were exceeded.

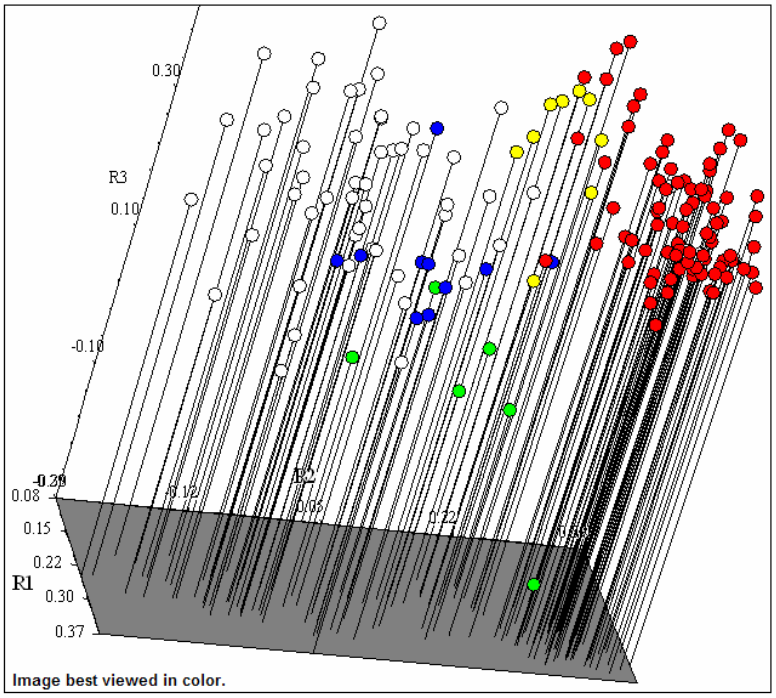

Fig. 8. PCA image of *E. c. nilesii* populations N01-09 (Las Vegas) in red, N10 (White Basin) in yellow, and N11 (Lincoln Co) in blue; *E. c. glutinosum* population U08 in green; and *E. c. aureum* populations in white.

RESULTS

AFLPs: UPGMA Analysis

The dendrogram from the UPGMA analysis of AFLP data for 472 individuals from 51 populations shows 4 major clusters (Fig. 3). Cluster-1 consists of the two *E. effusum* populations in Chafe County, Colorado. Although plants assigned to this taxon are spreading, woody shrubs with other morphological similarities to *E. corymbosum*, our AFLP profiles suggest that *E. effusum* is very different genetically. Cluster 2 consists solely of *E. racemosum*, an herbaceous species included as an outgroup. Cluster-3 includes all 11 populations of *E. corymbosum* var. *nilesii*, the five populations of var. *aureum* (all found in Washington County, Utah, and within a 20km radius of St. George), 1 population of var. *glutinosum*, and (in a separate subcluster) the five populations of *E. thompsoniae*. All remaining samples are in Cluster-4, including a subcluster that includes the remaining varieties of *E. corymbosum*. This separation highlights the genetic divergence of variety *nilesii* from most taxa in the *E. corymbosum* complex. Close examination of Cluster-3 (Fig. 4) shows subclustering that largely isolates the Las Vegas populations of *E. corymbosum* var. *nilesii*, with 82 of 87 samples (94%) grouping together as a unit separate from all other taxa. Included with them are 6 samples from the White Basin population, and 3 samples from the Lincoln County population. Also found within this subcluster are six samples from the White Basin population and three from the Lincoln County population. An adjacent subcluster includes the other five Las Vegas samples, and three samples from two *E. corymbosum* var. *aureum* populations. The rest of the sublusters in Cluster 3 include the *E. corymbosum* var. *aureum* samples from five populations and all members of one population of *E. corymbosum* var. *glutinosum*. Although there is overlap, the majority of the White Basin samples of variety *nilesii* clustered with the Las Vegas plants, while the majority of those from Lincoln County are associated with samples of variety *aureum*.

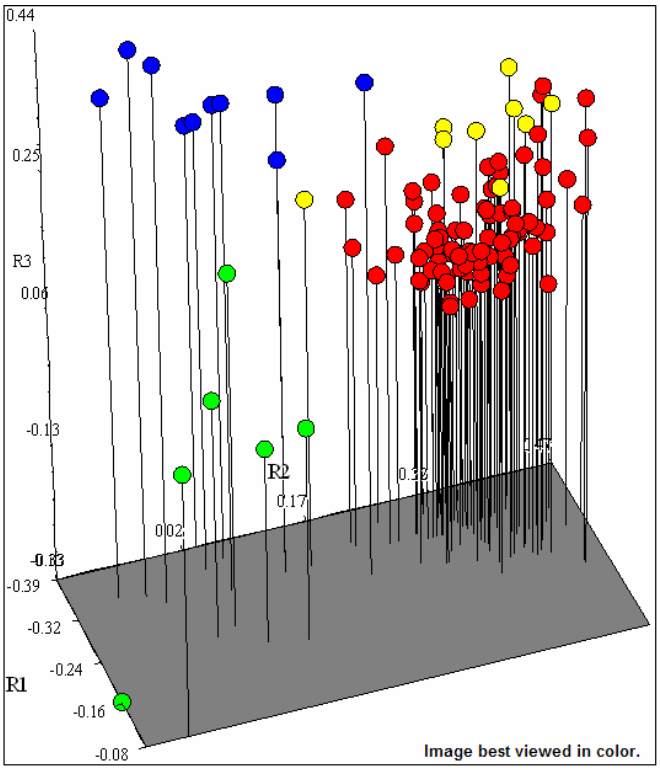

Fig. 9. PCA image of *E. c. nilesii* populations N01-09 (Las Vegas) in red, N10 (White Basin) in yellow, and N11 (Lincoln Co) in blue; *E. c. glutinosum* (U08) is in green.

AFLPs: PCA Analyses

PCA 3-D graphs derived from the AFLP data matrix show details that corroborate UPGMA results. The distant relationship of *E. effusum* to the other taxa tested, as well as the distant relationship of *E. racemosum*, are both apparent in Figure 5. With those two taxa removed from the data matrix, a PCA analysis shows *E. leptocladon* to be an outlying taxon (Fig. 6) as well as *E. nummulare* (not shown). A PCA analysis with those 4 outlying taxa removed from the data matrix shows the general relationship of *E. corymbosum* var. *nilesii* to the rest of the samples examined (Fig. 7). As in the UPGMA analysis, *E. corymbosum* var. *nilesii* is most closely associated in PCA graphs to the subspecific taxon *E. corymbosum* var. *aureum*. When the relationship of variety *nilesii* is examined with the most closely associated varieties shown in the UPGMA anaylis – *aureum* and one population of *glutinosum* (U08) – the separation of the Las Vegas plants can be seen (Fig. 8). The White Basin samples are most closely associated with the Las Vegas samples, while the overlapping Lincoln County plants are more closely associated with those from populations of variety *aureum*. This relationship between samples from Las Vegas, White Basin, and Lincoln County is demonstrated when the populations of variety *aureum* are removed from the analysis (Figs. 9 and 10). The U08 population of variety *glutinosum* groups separately (Fig. 9).

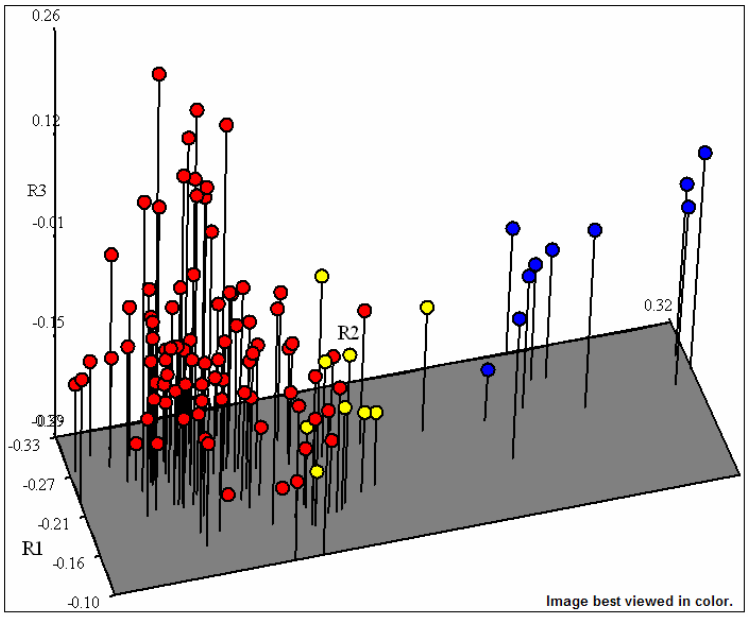

Fig. 10. PCA imgage of *E. c. nilesii* populations N01-09 (Las Vegas) in red, N10 (White Basin) in yellow, and N11 (Lincoln Co) in blue.

Sequencing

Among the 13 taxa and 54 individuals we successfully sequenced, there were 17 different haplotypes due to 24 substitutions, 7 insertions, and 3 deletions. Although he sequences provided considerably less polymorphic data than the AFLPs, and the subsequent sequence analyses were necessarily less informative, the results were corroborative. This can be seen in one of the most parsimonious sequence trees (Fig. 11) and in the consensus tree (Fig. 12). All Las Vegas samples of *E. corymbosum* var. *nilesii* had identical sequences, and shared this haplotype with two populations of *E. corymbosum* var. *aureum* and one population of *E. thompsoniae* (U32). The two samples of variety *nilesii* from Lincoln County (N11) shared their sequence haplotype with *E. corymbosum* var. *glutinosum* (U08) and two populations of *E. thompsoniae* (U19 and U33).

These results mirror closely the results from the AFLP data, with *E. corymbosum* var. *nilesii* showing genetic separation from all taxa except *E. corymbosum* var. *aureum* and *E. thompsoniae*, and a close association with one population of *E. corymbosum* var. *glutinosum* (U08). The overlap of variety *nilesii* with variety *aureum* is among members of the Lincoln County population (N11).

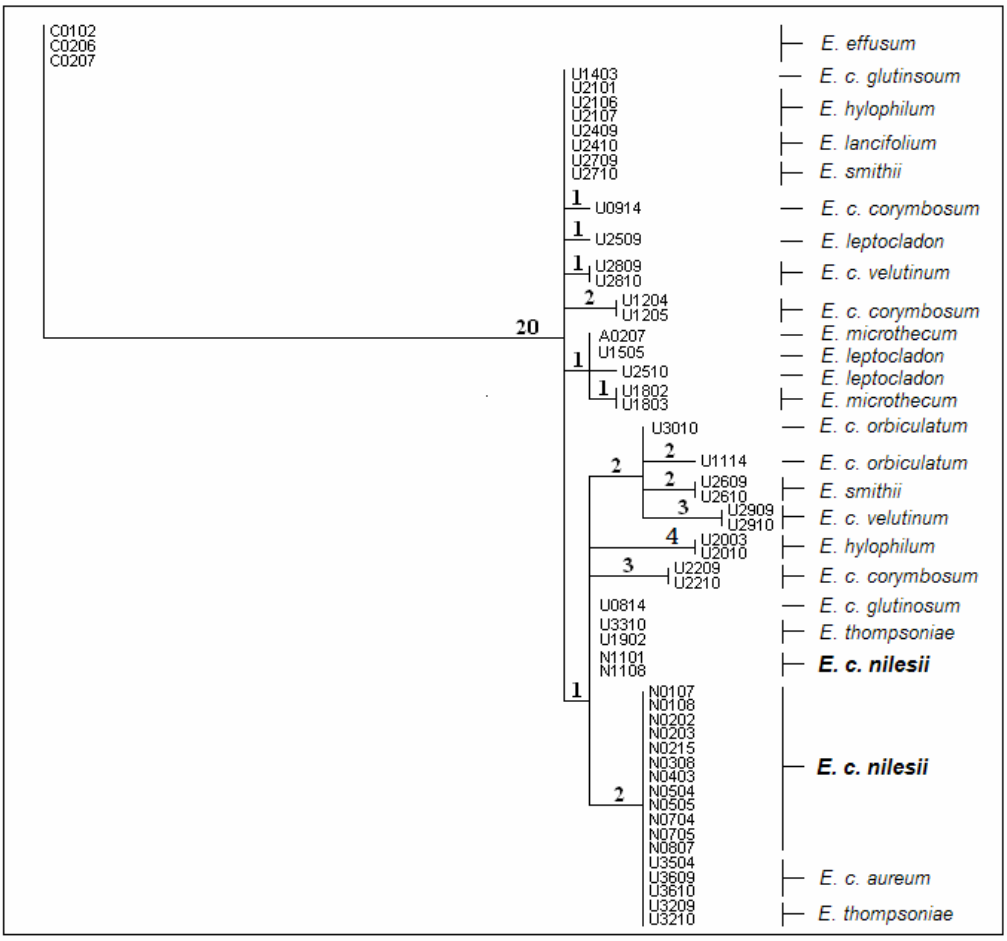

Fig. 11. Phylogram from a parsimony analysis of 13 *Eriogonum* taxa based on cpDNA sequences of the *trnS/trnfM* intergenic spacer. Branch numbers designate sequence changes.

DISCUSSION

All three analyses (UPGMA and PCA analyses of AFLP data, and parsimony analysis of sequence data) show the Las Vegas Valley populations of *E. corymbosum* (including the White Basin population) to be genetically similar to each other. UPGMA and PCA analyses separate the Las Vegas Valley samples from all other samples tested, although grouping them most closely to the Lincoln County population (N11) and to populations of *E. corymbosum* var. *aureum*, *E. thompsoniae*, and one population of *E. corymbosum* var. *glutinosum*. The sequence analysis separates the Clark County samples from all taxa except *E. corymbosum* var. *aureum* and one population of *E. thompsoniae*. Because there are only 20 parsimony-informative characters in the sequence analysis when the two *E. effusum* populations are excluded, the parsimony results are not as discriminatory as the analyses of AFLP data. Yet, the consensus tree supports the AFLP results by demonstrating the cohesiveness of the Las Vegas samples as well as their close relationship with *E. corymbosum* var. *aureum* and *E. thompsoniae*.

The nearest *E. corymbosum* var. *aureum* population (U01) to site N11 is about 50km, as is the nearest *E. thompsoniae* population (U03). We hypothesize that hybridizations involving these 3 taxa have occurred in this region.

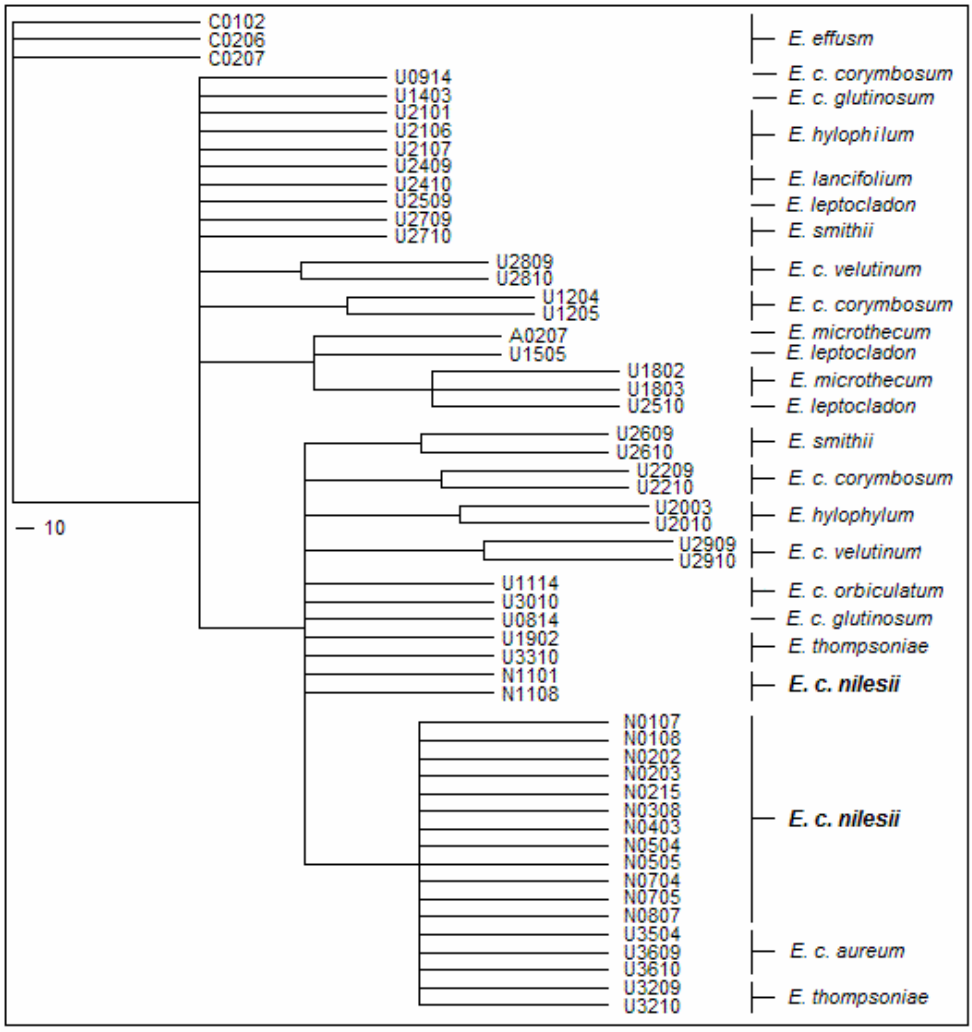

Fig. 12. Consensus phylogram of 13 *Eriogonum* taxa based on cpDNA sequences of the *trnS/trnfM* intergenic spacer region.

The genetic distinctness of *E. corymbosum* var. *nilesii* sampled from Clark County, Nevada shown in our analyses (particularly those using the AFLP data) suggests the metapopulation in this region has embarked on its own divergent hereditary trajectory. Although we found some genetic cohesiveness among the samples from the White Basin population, the individuals sampled from the nine 'populations' in and around Las Vegas showed close genetic similarity to each other but little site fidelity in our analyses. This suggests that Nevada's sites 1-9 were likely contiguous in the past, and can be considered a single population.

A comparison of the *E. corymbosum* varieties tested (excluding the putative hybrids variety *aureum* and the Lincoln County population, N11, of variety *nilesii*) shows *E. corymbosum* var. *nilesii* to be the most genetically distinct of the 4 varieties (Fig. 13). The variety most closely associated with variety *nilesii* in our tests was one of three populations of variety *glutinosum*. Yet the other two populations of variety *glutinosum* we tested showed more genetic similarity to other *E. corymbosum* varieties. The lack of genetic cohesion between the three populations of variety *glutinosum* is apparent in Fig. 13.

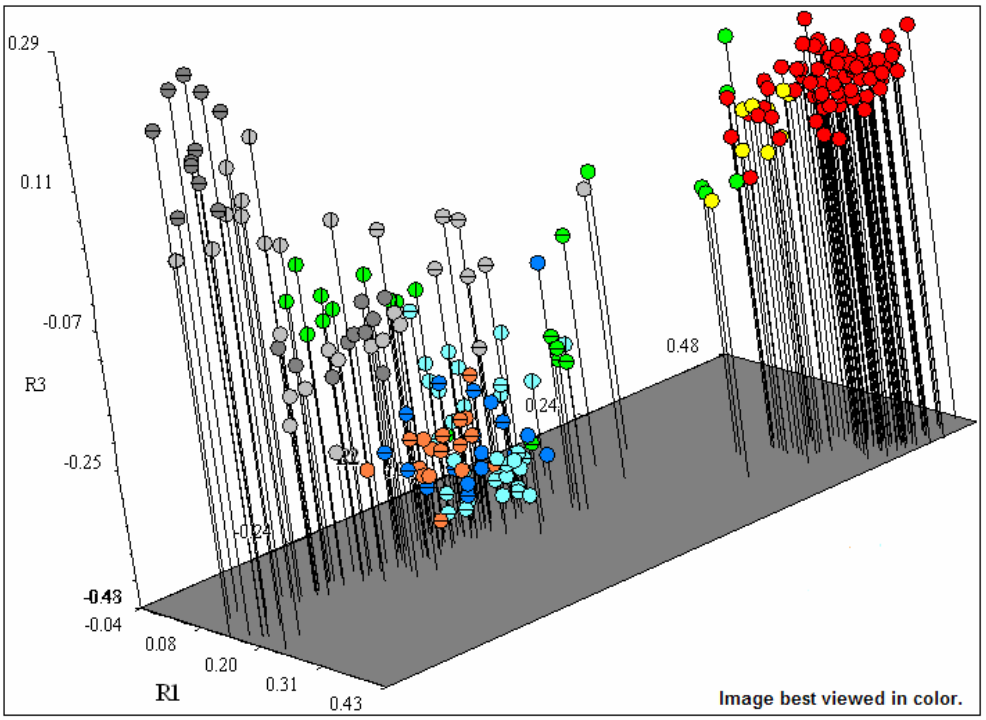

Fig. 13. PCA image of *E. corymbosum* varieties (without *aureum* or the Lincoln Co *nilesii*): ●● = *nilesii*, ●◐⊖●⊖ = *corymbosum*, ●◐⊖●⊖ = *orbiculatum*, ●◐⊖ = *glutinosum*, ●⊖ = *velutinum*.

Because our findings demonstrate that the Clark County populations of *E. corymbosum* show levels of genetic similarity to each other as well as divergence from the other varieties in the *E. corymbosum* complex that equal or exceed those aspects of the other accepted varieties, the designation of populations in this region as a separate variety is supported. The morphological characters common to variety *nilesii* make field identification relatively easy. Indeed, field identification based on the set of morphological characteristics that separate this varietal taxon from others (predominantly yellow flowers combined with densely pubescent inflorescence branches and adaxial leaf surfaces: Reveal 2006) may well be a function of heredity rather than edaphic or other environmental factors. Thus, our results complement the morphological study by Reveal and support his decision to designate the Clark County populations as variety *nilesii*.

REFERENCES

Benham, J. J. 2001. Genographer Version 1.6.0. http://hordeum.oscs.montana.edu/genographer.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Reveal, J. L. 1985. An annotated key to *Eriogonum* (Polygonaceae) of Nevada. Great Basin Naturalist 45(3):493-519.

——. 2002. A review of *Eriogonum corymbosum* Benth. with emphasis on the species in southern Nevada. USFW, Las Vegas, Nevada.

——. 2004. New entities in *Eriogonum* (Polygonaceae: Eriogonoideae). *Phytologia* 86: 121-159.

——. 2005. *Eriogonum corymbosum*. In: Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 12+ vols. Oxford University Press, Oxford, UK.

——. 2006. In: Flora of North America Editorial Committee. Flora of North America. Vol. 5. Magnoliophyta: Caryophyllidae, part 2. Oxford Univ. Press, Oxford, U.K.

Rohlf, F. J. 2000. NTSYSpc: Numerical Taxonomy System, ver. 2.1t. Exeter Publishing, Ltd., Setauket, NY.

Shaw, J., E. B. Lickey, J. T. Beck, S. B. Farmer, W. Liu, J. Miller, K. C. Siripun, C. T. Winder, E. E. Schilling, and R. L. Small. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92: 142-166.

SPSS Inc. 2005. SPSS v 13 for Windows. SPSS Inc., Chicago IL.

Swofford D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10 (Alvitec). Sinauer Associates, Sunderland, Massachusetts.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van De Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeau. 1995. AFLP: a new technique for DNA Fingerprinting. Nucleic Acids Research 23: 4407-4414.