The Taxonomic Designation of Eriogonum corymbosum var. nilesii (Polygonaceae) is Supported by AFLP and cpDNA analyses

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The Taxonomic Designation of *Eriogonum corymbosum* var. *nilesii* (Polygonaceae) is Supported by AFLP and cpDNA Analyses

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**Abstract**—We examined populations of perennial, shrubby buckwheats in the *Eriogonum corymbosum* complex and related *Eriogonum* species in the subgenus *Eucycla*, to assess genetic affiliations of the recently named *E. corymbosum* var. *nilesii*. The known populations of this variety are all located in Clark County, Nevada, U. S. A. We compared AFLP profiles and chloroplast DNA sequences of plants sampled from populations of *E. corymbosum* var. *nilesii* with those of plants representing other *E. corymbosum* varieties and related *Eriogonum* species from Colorado, Utah, northern Arizona, and northern New Mexico. We found evidence of genetic cohesion among the Clark County populations as well as their genetic divergence from populations of other *E. corymbosum* varieties and species. The genetic component uncovered in this study supports the morphological findings upon which the nomenclatural change was based, attesting to the taxonomic distinctness of this biological entity.

**Keywords**—buckwheat, chloroplast sequences, Colorado Plateau, Mojave, principal components analysis, Structure 2.2.

Polygonaceae has been a large and diverse angiosperm family comprising 48 known genera with approximately 1,200 recognized species (Freeman and Reveel 2005). Several studies have demonstrated the monophyly of the family (Chase et al. 1993; Cuenoud et al. 2002; Lamb-Frye and Kron 2003; Sanchez and Kron 2008) but sorting out taxonomic relationships within Polygonaceae has been a long-standing challenge. It has been suggested that these difficulties, particularly in the subfamily Eriogonoideae, may be due to relatively recent and rapid diversification (Welsh et al. 2003; Sanchez and Kron 2008).

Polygonaceae has been variously subdivided based on morphological characters into subfamilies in numerous circumscriptions (e.g. Arnott 1832; Luerssen 1882; Roberty and Vautier 1964; Reveal 1978, 1989; Freeman and Reveel 2005). Molecular studies (Chase et al. 1993; Lledo et al. 1998; Cuenoud et al. 2002) failed to support the earlier classifications. More recently, Lamb-Frye and Kron (2003) analyzed Polygonaceae with chloroplast *rtcl* sequences and Sanchez and Kron (2008) included additional cpDNA sequences along with portions of the nuclear gene *LEAFY*. Findings of both studies supported monophyly of Polygonaceae and its subdivision into two redefined clades—Polygonoideae and Eriogonoideae.

Eriogonoideae sensu Sanchez and Kron (2008) includes several examples of endemism in the geographic region of the southwestern United States (Archibald et al. 2001; Meyer 1986; Reveal 2004b; Welsh 1978). This area appears to comprise a zone of adaptive radiation, with endemics from other plant families found there as well (Douglas and Manos 2007; Meyer 1986; Nickrent and Wiens 1989; Sivinski and Knight 1996). The narrow ranges for some endemic taxa may point to recent diversification in this arid region of North America (Reveal 1989, 2005; Sanchez and Kron 2008). Edaphic factors, including gypsum-rich soils, may play a role in plant diversification and endemism in North America’s southwestern deserts (Douglas and Manos 2007; Drohan et al. 2006; Maschinski et al. 2004; Moore and Jansen 2007). Glacial and postglacial climate change may also have contributed to taxonomic divergence through range contraction and expansion, fragmentation, and hybridization of taxa (Comes and Kadereit 1998; Fehlberg and Ranker 2009). This putative history of recent divergence may explain past difficulties in determining relationships within Eriogonoideae.

The *Eriogonum corymbosum* Benth. complex (Eriogonoideae), as currently recognized, comprises a group of buckwheat taxa distributed across the western U. S. A., from southwestern Wyoming, through Utah and southwestern Colorado, to northern Arizona, northern New Mexico, and southern Nevada (Reveal 2005). Across the range, these woody shrubs vary in size, leaf shape and surface structure, predominant flower color, and overall habit, among other aspects of morphology. A putative history of dispersion, isolation, divergence, hybridization, and introgression may have contributed to the group’s morphological and geographical diversity (Reveal 2002; Welsh et al. 2003). The group has been revised on several occasions (Jones 1903; Reveal 1967, 2002, 2005), with eight varieties currently recognized.

Until recently, predominantly yellow-flowered populations of *E. corymbosum* in Clark County, Nevada, have been treated either as the wide ranging *E. corymbosum* var. *glutinosum* (Reveal 2002) or the more narrowly distributed *E. corymbosum* var. *aurium* (Reveal 1983, 1985). Identification of *E. corymbosum* var. *glutinosum* had previously hinged primarily on flower color (with predominantly yellow-flowered plants identified as variety *glutinosum*). However, Reveal (2002) pointed out that flower color in the predominantly yellow-flowered *Eriogonum* Michx. taxa can vary among individuals within populations, which often include white or cream-colored members. In our field observations we also found this to be the case. Although yellow flower color is useful, in combination with other traits, for identifying sub-specific taxa within *Eriogonum*, flower color alone is not definitive for any variety.

The only known population of *E. corymbosum* var. *aurium* is located north of St. George, Utah (Appendix 1; Figs. 1, 2), a predominantly yellow-flowered variety distinguishable from variety *glutinosum* based on glabrous branches of the inflorescence (Reveal 2005). This trait also separates variety *aurium* from the predominantly yellow-flowered plants in the *E. corymbosum* populations of Clark County, Nevada, whose flowering branches tend to be silvery-pubescent. Both sides of the leaves of the Clark County plants are silvery-pubescent, separating them from varieties *glutinosum* and *aurium*. Based on these traits, as well as ecological considerations, Reveal (2004a) concluded that the Clark County populations are
Fig. 1. Collection sites (11 in Nevada, two in Arizona, one in New Mexico, 35 in Utah). See Table 1 for key to abbreviations.
Fig. 2. Sites sampled in Clark County, Nevada (E. corymbosum var. nilesii), Lincoln County, Nevada (E. corymbosum N11), and Washington County, Utah (E. corymbosum var. aureum and E. thompsoniae).
morphologically and ecologically distinct and geographically disjunct from both *E. corymbosum* varieties *glutinosum* and *aurum*, and he proposed the new varietal designation *nilesii* (Niles’s wild buckwheat). The known populations of *E. corymbosum* var. *nilesii* are mainly in and around Las Vegas and the Muddy Mountains region of Clark County, Nevada (Reveal 2005).

Concerns have been expressed regarding the loss of the Clark County populations of *E. corymbosum* var. *nilesii* as a result of development and off-road vehicle recreation, and questions about its taxonomic status have been raised (Reveal 2004a). *Eriogonum corymbosum* var. *nilesii* is currently listed by the Bureau of Land Management (BLM) as a sensitive species (Boettger et al. 2007) and the U.S. Fish and Wildlife Service (USFWS) recently selected it as a candidate for protection under the Endangered Species Act of 1973 (ESA) (USFWS 1983; Perkins and Samargo 2008). Further evidence demonstrating the taxonomic distinctness of *E. corymbosum* var. *nilesii* would lend support to an ESA listing, whereas evidence to the contrary might suggest that the Clark County populations are part of a more widespread taxon.

Here we examine populations in the *E. corymbosum* complex and related *Eriogonum* species in subgenus *Eucycla* to address the genetic distinctness of the populations of *E. corymbosum* var. *nilesii* found in Clark County, Nevada. By analyzing a combination of amplified fragment length polymorphism (AFLP) and chloroplast DNA (cpDNA) data, we evaluate the conclusion of Reveal (2004a) that the Clark County populations of *E. corymbosum* have diverged in a manner and to an extent that warrants this varietal recognition.

### Materials and Methods

**Collections**—Based on recorded locations from herbarium specimens and personal communications with numerous field biologists, we surveyed for *Eriogonum corymbosum* and related *Eriogonum* taxa from southern Nevada, northern Arizona, and northern New Mexico north through Utah and into Colorado. We collected leaf samples of 10–15 plants from each of 51 populations representing 12 *Eriogonum* species within subgenus *Eucycla* (Appendix 1; Table 1; Figs. 1, 2). Each collection site, referred to as a population, comprises a geographically bounded and relatively isolated group of potentially interbreeding individuals.

Leaves were dried rapidly on silica gel inside sealed plastic bags. Sampled populations included individuals from six varieties of *E. corymbosum*. We sampled 10 populations that we identified morphologically as *E. corymbosum* var. *nilesii* (Fig. 2), nine of which were located in and around Las Vegas, Nevada. Samples of the tenth population (N10) were collected from White Basin in the Muddy Mountains region about 40 km east of North Las Vegas. These 10 sites were all located in Clark County (referred to as variety *nilesii*). Additionally, samples from an eleventh Nevada population (referred to as N11) that appeared phenotypically similar to variety *nilesii* were collected in Lincoln County about 15 km west of Utah’s southwest corner and 100 km northeast of the Las Vegas plants (Fig. 2). Although there is only one confirmed population of variety *aurum* (which we sampled), we located and sampled three additional populations that key most closely to variety *aurum*, and in this paper we refer to them as such.

A protocol was followed at each collection site to avoid bias in the sample-selection process. After surveying to determine the general boundaries of a population, a central transect was marked through the length of the population. Plants were sampled by walking the transect and selecting consecutive plants near the transect that were at least 5 m apart (in order to avoid selecting clones). If too few plants were sampled following this method, plants were sampled further from the transect, while again ensuring they were at least 5 m from any other sampled plant. Ten to 15 leaves were collected per plant (more for taxa with very small leaves). Individual plants were sampled if they had enough healthy leaves (60 or more) to ensure that sampling would not be likely to cause lasting damage to the plant. Plants were not, however, selected based on size, apparent age, or other morphological features. Plant vouchers for each collection site were deposited at UTC.

**DNA Extraction**—Genomic DNA was extracted from the dried leaf samples using the Qiagen DNeasy 96 Plant Kit and the Qiagen DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, California) following the instructions of the manufacturer. Concentrations of DNA in the extracted sample solutions were quantified with the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). Extracted DNA solutions were stored at −80 °C until use.

**Amplicred Fragment Length Polymorphisms**—An AFLP profile was generated for each DNA sample using a modified version of the protocol by Vos et al. (1995). The extracted DNA was digested with restriction enzymes (with the rare cutter EcoRI and the frequent cutter Msel) then ligated with forward and reverse adaptors. A subset of the fragments was amplified by polymerase chain reaction (PCR) using an EcoRI/Msel primer pair set, with an additional selective nucleotide on each primer: EcoRI-A and Msel+. This +1 PCR reaction was followed by a +3 PCR reaction in which 2 additional selective nucleotides were added to the primer pair sets. We used two different +3 primer pair combinations with the +1 PCR product: EcoRI-ACG and Msel-ACG. 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). We replicated 32 (8.2%) of the samples to determine the error rate in band scoring.

The AFLP technique amplifies regions of the genome randomly and many polymorphisms can be found, representing variation among individuals via presence or absence of restriction sites and the selective nucleotides. From the scored fragments generated by the +3 primer pair sets, we selected 103 polymorphic loci based on data quality and bimodality of signal across the dataset, varying in size from 66–476 bp, and obtained AFLP profiles from an average of eight plants per population (on which we based our analyses).

We examined the AFLP data with Principal Components Analysis (PCA) using the program NTSyspc v. 2.10 (Rohlf 2000). This multivariate analysis constructs a set of three orthogonal coordinate axes from the first three eigenvalues (derived from the presence/absence data) and projects the individual samples as points in a scatter plot within these three axes such that variance is maximized in as few dimensions as possible. Representing the variance extracted by each axis, eigenvalues can be summed as a percentage of the total variance. This exploratory approach rapidly provides graphical 3-D correlation matrices that demonstrate potential clustering.

For further insight into the data, we analyzed the AFLP sample profiles using a model-based method. The program Structure 2.2 utilizes a Bayesian approach to infer related clusters (K) of individuals from multilocus genotype data while also evaluating the strength of evidence for

### Table 1. Seventeen *Eriogonum* taxa sampled from 51 populations. Identifications following Reveal (2005).

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Map Code &amp; # of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>aurum</em> (M. E. Jones)</td>
<td>Eca-4</td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>corymbosum</em> Benth.</td>
<td>Ecc-5</td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>glutinosum</em> M. E. Jones</td>
<td>Ecg-3</td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>nilesii</em> Reveal</td>
<td>Ec-10</td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>orbiculatum</em> (S. Stokes)</td>
<td>Eco-5</td>
</tr>
<tr>
<td>Reveal &amp; Brotherson</td>
<td></td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> var. <em>velutinum</em> Reveal</td>
<td>Ec-3</td>
</tr>
<tr>
<td><em>Eriogonum corymbosum</em> (variety undetermined)</td>
<td>N11–1</td>
</tr>
<tr>
<td><em>Eriogonum effusum</em> Nutt.</td>
<td>E-2</td>
</tr>
<tr>
<td><em>Eriogonum breviculae</em> Nutt.</td>
<td>Eb-1</td>
</tr>
<tr>
<td><em>Eriogonum hyophyllum</em> (Reveal &amp; Brotherson)</td>
<td>Eh-2</td>
</tr>
<tr>
<td>S. L. Welsh</td>
<td></td>
</tr>
<tr>
<td><em>Eriogonum lancifolium</em> Reveal &amp; Brotherson</td>
<td>Ea-1</td>
</tr>
<tr>
<td><em>Eriogonum leptocladon</em> Torr. &amp; A. Gray</td>
<td>El-2</td>
</tr>
<tr>
<td><em>Eriogonum longum</em> A. Nelson</td>
<td>Eel-2</td>
</tr>
<tr>
<td><em>Eriogonum microthecum</em> Nutt.</td>
<td>Em-2</td>
</tr>
<tr>
<td><em>Eriogonum nanumulae</em> M. E. Jones</td>
<td>En-1</td>
</tr>
<tr>
<td><em>Eriogonum racemosum</em> Nutt.</td>
<td>Er-1</td>
</tr>
<tr>
<td><em>Eriogonum smithii</em> Reveal</td>
<td>Ee-2</td>
</tr>
<tr>
<td><em>Eriogonum thompsoniae</em> S. Watson</td>
<td>Et-5</td>
</tr>
</tbody>
</table>
the inferred clusters (Falush et al. 2007; Pritchard et al. 2000; Pritchard et al. 2007). Structure starts with an arbitrary parameter configuration that iteratively updates via a Markov Chain Monte Carlo (MCMC) algorithm until converging on the posterior distribution of all the parameters based on the data available (Pritchard et al. 2000). Each individual is assigned to a cluster according to its genetic makeup, represented by a vector $q$, which gives the percentages of that genetic makeup (if any) originating from each of the populations recognized by Structure. Although Structure was developed for diploid genotypic data that provide allelic information, Falush et al. (2007) extended the MCMC algorithm to account for the partial information provided by dominant markers (such as AFLPs).

We analyzed sampled populations of *E. corymbosum* var. *nilesii*, *E. corymbosum* var. *aureum*, *E. thompsoniae*, and population N11 with Structure set to the admixture model. We tested for the number of genetic clusters by running five replicates for each of six simulations, from $K = 1$ to $K = 6$, with 100,000 MCMC iterations after a burn-in of 30,000 (following Pritchard et al. 2000 and Pritchard et al. 2007).

**Chloroplast Sequencing and Phylogenetic Analysis**—We amplified and sequenced the *trnS*-*trnF* 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 cpDNA sequences. We amplified this region using PCRs in 50 μl solutions and purified the PCR products using Qiagen’s Quiaquick Purification Kits (Qiagen, Inc.). 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 products of the sequencing reactions were purified through hydrolyzed Sephadex and then run on the ABI 3100 automated capillary sequencer (Applied Biosystems). Contigs were assembled and sequences confirmed using Sequencher v3.1.1 (Gene Codes, Ann Arbor, Michigan). The initial alignment for all sequences was obtained with the Clustal-W Multiple Alignment option in the BioEdit alignment program (Hall 1999). The final alignment was obtained manually, creating a trimmed sequence matrix with 1,222 characters. Nucleotide sequences are deposited in GenBank as accession numbers FJ204255 through FJ204308.

The aligned sequences were imported into PAUP v4.0b10 (Swofford 2002) and analyzed under the parsimony optimality criterion. All characters were analyzed as equal in weight and unordered, with gaps treated as missing. Tree space was examined with a heuristic search with simple addition sequence, the tree-bisection-reconnection (TBR) branch-swapping algorithm, and the MulTrees option in effect, keeping all trees. We used *E. effusum* samples to root the tree. Additionally, bootstrap values were calculated for 1,000 replicates and plotted onto the parsimony tree to evaluate relative branch support (Felsenstein 1985). The dataset for this phylogenetic analysis was submitted to TreeBASE (study number S2268).

### RESULTS

**AFLP Reproducibility**—Before running analyses on the AFLP matrix, we examined the similarity of the 32 AFLP replicates to assess the reproducibility of the data. The results from the replicated samples showed a locus-scoring error rate of 1.09% over 103 loci within the AFLP data matrix. This error rate is relatively low for AFLP studies (when rates are reported) and it should not bias our analytical results.

**AFLPs: Principal Components Analyses**—Principal components analysis 3-D graphs derived from the AFLP data matrix demonstrate genetic similarities among populations. The graphical distances of *E. effusum* and *E. racemosum* from the other taxa tested are apparent in Fig. 3. With those two taxa removed from the data matrix, a PCA analysis shows two large clusters (Fig. 4). One of these two main clusters is a broadly connected group composed of three clusters: a subcluster of *E. corymbosum* var. *nilesii* samples at one end, a more loosely associated subcluster of *E. thompsoniae* samples at the other, and spread between these two subclusters are all the sampled individuals identified as *E. corymbosum* var. *aureum* as well as the Nevada population N11 from Lincoln County. Three individuals identified as *E. corymbosum* var. *glutinosum* are also part of this large cluster, most closely associated with the *E. corymbosum* var. *nilesii* subcluster. The second large cluster includes all other varieties of *E. corymbosum* (including 16 samples of *E. corymbosum* var. *glutinosum* from two populations) along with the remaining *Eriogonum* species.

To bring further clarity to the main cluster containing *E. thompsoniae* and *E. corymbosum* varieties *nilesii* and *aureum*, we ran another PCA with those individuals only, excluding the three members of *E. corymbosum* var. *glutinosum* (which will be examined more closely in another PCA). In Fig. 5, the samples of variety *nilesii* from the vicinity of Las Vegas (from populations N1-N9) and White Basin (population N10) can be seen as a tightly packed and separate cluster. The adjacent cluster, formed by members of variety *aureum* and the Lincoln...
County plants (N11), bridges the gap between variety *nilesii* and *E. thompsoniae*. The first three principal components captured 44.1, 8.6, and 3.3% of the variance in the data set respectively.

Although there were too few samples of *E. corymbosum* var. *glutinosum* from U8 to draw any conclusions with confidence, we examined the graphical relationship between the three samples from U8 and the samples of *E. corymbosum* var. *nilesii* from populations N1-N10 in a separate PCA analysis (Fig. 6). The samples of variety *glutinosum* were peripheral to the cluster of variety *nilesii* samples.

### AFLPs: Structure 2.2 Analyses

Using the program Structure 2.2 (Pritchard et al. 2007), we analyzed the AFLP profiles of all individuals sampled from populations designated as *E. thompsoniae*, *E. corymbosum* var. *aureum*, *E. corymbosum* var. *nilesii*, and those from population N11. In our simulation tests to determine *K*, each increasingly larger *K*-value had a higher probability, but even at *K* = 6 it was vanishingly small. We attributed this to the putative complexity of the populations in the St. George region of Washington County, Utah and population N11 in adjacent Lincoln County, Nevada. Such results are not infrequent when data sets are not all from genetically discrete populations, and in such cases it is recommended to choose a biologically reasonable value for *K* that also appears to capture most of the structure (Pritchard et al. 2000; Pritchard et al. 2007). We therefore selected *K* = 3 as a realistic estimation, given that three taxa were presumed to be involved. As with the PCA analyses, no population information was given for any of the individual AFLP profiles. Structure assigned each individual probabilistically to one of three clusters, but a number of apparently admixed individuals had affiliations with more than one cluster.

With the Structure output arranged with the individuals in order by presumed taxon (Fig. 7), the cluster of all individuals from the Clark County populations of *E. corymbosum* var. *nilesii* showed little evidence of admixture from the other two groups. However, many of the individuals in the second group (composed of all the *E. corymbosum* var. *aureum* samples and the samples from population N11) showed admixture from both of the other two clusters. The third cluster, composed of samples identified as *E. thompsoniae*, included some that showed admixture as well, especially from members of *E. corymbosum* var. *aureum* populations.

**Chloroplast Sequence Analyses**—Among the 13 taxa and 54 individuals we successfully sequenced, there were 17 different haplotypes due to 24 substitutions and 10 indels. Of the 24 variable characters in the PAUP parsimony analysis, 21 were parsimony-informative.

The analysis found a single most parsimonious tree (Fig. 8). All Clark County, Nevada samples of *E. corymbosum* var. *nilesii* had identical sequences, and shared their haplotype with individuals from two populations of *E. corymbosum* var. *aureum* (U35 and U36) and one population of *E. thompsoniae* (U32). The haplotype most similar, but not identical, to that of the Clark County, Nevada samples of *E. corymbosum* var. *nilesii* was shared by the two samples from population N11 (Lincoln County, Nevada), an *E. corymbosum* var. *glutinosum* sample from U08, and members of two other populations of *E. thompsoniae* (U19 and U33).

### Discussion

The populations of *E. corymbosum* in Clark County, Nevada, have been grouped at different times in the past with two established varieties, either variety *glutinosum* or variety *aureum*, based on habit and flower color, among other phenotypic traits (Reveal 1967, 1985, 2002). Recently, Reveal (2004a) argued that the Clark County populations were morphologically and ecologically distinct enough to warrant a new taxonomic designation, which he named variety *nilesii*. Our comparison of the Clark County populations, using AFLP markers and cpDNA sequence data, supports Reveal’s conclusions. We found demonstrable genetic cohesion among *E. corymbosum* individuals sampled from populations in Clark County, Nevada. Not only did the sampled individuals form a tight group isolated from sampled populations of other *E. corymbosum* varieties and related species in both PCA and Structure analyses, the Structure analysis also demonstrated
that they are independent from their most closely related taxa (demonstrating little or no evidence of introgression).

Eriogonum corymbosum var. aureum, the taxon which we attributed to a number of populations in and around St. George, Utah, appears to be the closest relative of E. corymbosum var. nilesii. We also found an apparent relationship between the predominantly herbaceous species E. thompsoniae and E. corymbosum var. nilesii, linked by their mutual relationships to E. corymbosum var. aureum. Although a geographically distant and morphologically distinct population of E. corymbosum var. glutinosum with only three individuals tested (U8 in Appendix 1) needs further examination, all other Eriogonum varieties and species that we tested (including two other populations of variety glutinosum) were members of more distantly related clusters in our analyses.

The relationships of E. corymbosum var. nilesii to variety aureum and E. thompsoniae are demonstrable in our AFLP and sequence results. Although variety nilesii forms a tight and separate cluster in our PCA analyses (Figs. 4–6) and Structure analysis (Fig. 7), the association of this taxon with the loosely formed cluster that includes variety aureum and E. thompsoniae may be the result of its past migration and hybridization with Eriogonum taxa in Utah. Given that the Structure and NTSYS programs employ different algorithms to group the genetic profiles of samples, the similar clustering arrangements they provided add corroborative support to our conclusions. The sequence analysis adds additional evidence that E. corymbosum var. nilesii is a distinct taxon whose nearest relative is variety aureum. These results indicate that variety aureum may be a hybrid and repository of genes from both variety nilesii and E. thompsoniae, thereby providing a conduit for introgression between them. In that regard, the Structure analysis shows introgression by attributes characteristic of E. corymbosum var. nilesii into both E. corymbosum var. aureum and E. thompsoniae, but no obvious evidence of the reverse (Fig. 7).

While PCA analyses show a clear genetic separation of E. corymbosum var. nilesii and E. thompsoniae (Figs. 4, 5), the Structure analysis suggests that a number of E. thompsoniae individuals share genetic characters with E. nilesii (Fig. 7). Interestingly, the E. thompsoniae population that appears in our AFLP tests to be least influenced by E. corymbosum var. nilesii or var. aureum (U32; Fig. 7) shares the cpDNA haplotype common to all the Clark County samples of variety nilesii (Fig. 8), echoing the Structure findings. So even this distinctive E. thompsoniae population may have a historical relationship with E. corymbosum var. nilesii.

The loose relationships between the samples of E. corymbosum var. aureum and E. thompsoniae, the broad spread of their genetic variability and the degree of overlap between the two taxa as demonstrated in Fig. 5, suggest continuing migration and hybridization. The region of southern Utah bordering southern Nevada and northern Arizona, where E. thompsoniae and E. corymbosum var. aureum populations are found, appears to be a zone of hybridization between these two taxa, and contributions to these populations from E. corymbosum var. nilesii may explain the patterns we found in our analyses. Perhaps not coincidentally, this region is also a transition zone between the Mojave desert (which encompasses Clark County’s populations of variety nilesii) and the southwestern portion of the Colorado Plateau (the region where variety aureum and E. thompsoniae reside). Population N11 apparently lies on a contact zone between taxa from the two regions.

In contrast, the relatively cohesive nature of Clark County’s E. corymbosum var. nilesii samples (Figs. 5, 6) suggests little influence on those populations by either E. corymbosum var. aureum or E. thompsoniae. This distinctness is also apparent in the Structure analysis, with little or no evidence of introgression from either E. corymbosum var. aureum or E. thompsoniae apparent in the Clark County samples (Fig. 7). Thus, the patterns we found may be the result of the long establishment of E. corymbosum var. nilesii as a distinct taxon while genetic exchange between populations of E. corymbosum var. aureum and E. thompsoniae appears to be ongoing.

**Taxonomic Designation**—The species taxon, as the fundamental unit of evolution, is unique among taxonomic ranks. The species taxon designates a cohesive metapopulation composed of sexually reproductive organisms forming a separate lineage on its own evolutionary trajectory (Zimmerman 1959; Simpson 1961; Hennig 1966; Mayr 2000; Wiley and Mayden 2000; Ghiselin 2002; De Queiroz 2005; Rieseberg et al. 2006). The infraspecific taxon ‘variety’, on the other hand, denotes a population or group of populations presumed to have
Fig. 8. Most parsimonious tree from a parsimony analysis of 13 *Eriogonum* taxa based on sequences of the *trnS/trnF* cpDNA region. Branch numbers are bootstrap values.

the potential to eventually gain the necessary separation to achieve their own evolutionary path. But where does one draw the line? Charles Darwin’s (1875) view of varieties as “incipient species” remains conceptually accepted today, but he provided no methodology of discerning such taxa. O’Brien and Mayr (1991) suggested that populations could be recognized as subspecies if 1) their members can be identified by phylogenetically concordant phenotypic traits, 2) they are found in a unique habitat or geographic range, and 3) they demonstrate a unique natural history compared to any other subdivisions within the species. The apparently disjunct set of Clark County populations of *E. corymbosum* var. *nilesii* meets
these requirements. But phenotypic traits might not be genetically based (Haig et al. 2006), particularly in plants (which can show edaphic and other environmental effects phenotypically), and the geographic separation might merely demonstrate distance but not divergence. More is required than a phenotypically diagnosable population or set of populations within a defined geographic range and habitat in order to demonstrate potential evolutionary independence.

The seventy-five percent rule provides a quantitative, although arbitrary, method whereby 75% of the members of the population of interest must be separable from all members that make up the overlapping population (Amadon 1949; Patten and Unitt 2002). This method also suffers from the subjective approach to choosing characters used to determine any overlap. Still, although some members of the Clark County populations of E. corymbosum var. nilesii show a close relationship to E. corymbosum var. aureum in all our AFLP-based analyses, there is no overlap. If we identify the population N11 as part of E. corymbosum var. nilesii (based only on morphological similarity) then the proportion of members separable in our AFLP tests becomes 88%, and the criterion is also met.

A more effective approach to assigning infraspecific designations to populations of sexually reproductive organisms is to demonstrate multiple lines of mutually corroborative evidence that demonstrate the populations as distinctive entities evolutionarily (Haig et al. 2006). We have shown here the genetic cohesion between E. corymbosum var. nilesii samples and their divergence from all other closely related taxa tested, using AFLP and cpDNA markers. Our results corroborate Reveall’s (2005) description of E. corymbosum var. nilesii’s unique geographic range, habitat, and morphological distinctiveness in relation to other varieties. Some of the phenotypic characters Reveall examined, such as leaves that are “white-lanate to densely white-tomentose abaxially, silvery-floccose adaxially,” also suggest the adaptive divergence of E. corymbosum var. nilesii, making it particularly well suited to the harsh desert climate of the Mojave. And the suggestion that E. corymbosum var. nilesii may be an edaphic “extremophile” (Drohan et al. 2006) adds further support to its adaptive divergence. Given these multiple lines of evidence for the cohesion of Nevada’s Clark County populations and their evolutionary divergence from other populations of E. corymbosum, the description of the set of Clark County populations as variety nilesii is strongly supported.

One might argue, given the confluence of evidence for genetic cohesion between the subpopulations in Clark County and their evolutionary divergence from other taxa, that E. corymbosum var. nilesii populations form a metapopulation that deserves recognition as a species rather than a variety. Its apparent allopatri, possible edaphic endemic, and ecological value to the Mojave ecosystem all suggest its existence as a separate taxonomic entity on its own evolutionary trajectory. But its close genetic association to E. corymbosum var. aureum and one population of var. glutinosum, and its morphological similarity to the N11 population, provide a gray area that requires further study with wider sampling before one might conclude that Nevada’s Clark County populations constitute a unique species of buckwheat.

**Future Work**—The similarity found between E. corymbosum var. nilesii and one population of E. corymbosum var. glutinosum should be examined more closely. It is possible that E. corymbosum var. aureum is the result of hybridization between E. corymbosum var. nilesii migrants and E. thompsoniae, and that E. corymbosum var. nilesii is a long-established metapopulation that may have diverged from a variety like E. corymbosum var. glutinosum.

It has been suggested that E. corymbosum var. nilesii is an edaphic “extremophile” that may have established a niche in the soils derived from a Pleistocene marsh environment and now found in arid Mojave regions of Clark County, Nevada (Drohan et al. 2006). Perhaps soil endemism provides the isolation to protect this distinctive variety of E. corymbosum from introgression by other taxa, maintaining the stability of this metapopulation. Further work to verify the edaphic regime of E. corymbosum var. nilesii would be worth pursuing.

An ecological study of the structural importance of E. corymbosum var. nilesii on the landscape would be a valuable contribution since it appears to be the dominant plant species in the places where it thrives. Its cover percentages relative to other plant taxa appears to be significant, its soil holding properties may be important, and its relationships with other organisms may be extensive. Finally, an examination of ploidy levels could provide a clearer picture of the relationships within the E. corymbosum complex, and provide a window into the history of hybridization within this group.

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**Literature Cited**


Appendix 1. *Eriogonum* collection sites listed in alphabetical order by: *taxon*; coded site name; general location; latitude and longitude.
N36° 15′ 58.2″ W115° 4′ 43.5″; N09; NW Las Vegas, Clark Co, NV; N36° 14′ 59.6″ W115° 8′ 7.0″; N10; White Basin, Clark County, NV; N36° 20′ 26.7″ W114° 33′ 37.3″. *E. corymbosum* N11; N11; 22 km NW of Mesquite, AZ in Lincoln Co, NV; N36° 57′ 42.1″ W114° 13′ 5.8″. *E. corymbosum orbiculatum*; U11; 14 km E of Escalante, Garfield Co, UT; N37° 44′ 58.6″ W111° 26′ 38.0″; U16; 15 km W of Hanksville, Wayne Co, UT; N38° 21′ 56.9″ W110° 53′ 11.9″; U17; Three Forks, Wayne Co, UT; N38° 0′ 2.5″ W110° 30′ 46.4″; U30; Anches, Grand Co, UT; N38° 37′ 3.8″ W109° 37′ 5.4″; U31; 22 km S of Mexican Hat, San Juan Co, UT; N37° 3′ 21.5″ W110° 5′ 27.3″. *E. corymbosum velutinum*; U28; 17 km NE of Bluff, San Juan Co, UT; N37° 25′ 5.8″ W109° 26′ 47.1″; U29; Bluff; San Juan Co, UT; N37° 17′ 21.8″ W109° 32′ 53.6″; A02; 13 km E of Kayenta, Navajo Co, AZ; N36° 43′ 48.7″ W110° 6′ 42.0″. *E. effusum*; C01; 12 km NW of Salida, Chaffee Co, CO; N38° 37′ 12.5″ W106° 4′ 42.1″; C02; 13 km NW of Salida, Chaffee Co, CO; N38° 37′ 59.4″ W106° 4′ 45.7″. *E. hylophilum*; U20; Upper 9-Mile Canyon, Duchesne Co, UT; N39° 52′ 57.9″ W110° 13′ 51.1″; U21; Upper 9-Mile Canyon, Duchesne Co, UT; N39° 52′ 57.2″ W110° 14′ 6.0″. *E. lancifolium*; U24; 8 km E of Wellington, Carbon Co, UT; N39° 32′ 48.3″ W110° 38′ 34.6″. *E. leptoclodon*; U25; 20 km SW of Green River, Emery Co, UT; N38° 54′ 30.3″ W110° 22′ 10.0″; U15; 3 km S of Hanksville, Wayne Co, UT; N38° 20′ 40.1″ W110° 42′ 23.6″. *E. loganum*; U06; Logan, Cache Co, UT; N41° 44′ 25.2″ W111° 48′ 25.5″. *E. microthecum simpsonii*; NM1; 20 km W of Shiprock, San Juan Co, NM; N36° 49′ 4.7″ W108° 54′ 44.2″; U18; 6 km N of Kanab, Kane Co, UT; N37° 6′ 16.0″ W112° 32′ 55.6″. *E. nummulare*; U10; 17 km N of Dugway Proving Ground, Tooele Co, UT; N40° 20′ 12.7″ W112° 36′ 47.0″. *E. racemosum*; U05; N Salt Lake City, Salt Lake Co, UT; N40° 47′ 28.5″ W111° 51′ 42.7″. *E. smithii*; U26; Little Flat Top, Emery Co, UT; N38° 32′ 15′ W110° 29′ 38.5″; U27; N Texas Hill, Emery Co, UT; N38° 30′ 2.6″ W110° 24′ 47.8″. *E. thompsoniae matthewsiae*; U19; SW Zion Ntl Park, Washington Co, UT; N37° 11′ 47.4″ W112° 59′ 33.7″. *E. thompsoniae thompsoniae*; U32; 4 km W of Bloomington Hills, Washington Co, UT; N37° 3′ 16.2″ W113° 39′ 54.7″; U33; 4 km SE of Hurricane, Washington Co, UT; N37° 8′ 53.2″ W113° 15′ 20.6″; U03; La Verkin, Washington Co, UT; N37° 13′ 8.6″ W113° 15′ 0″; U04; Rockville, Washington Co, UT; N37° 9′ 42.4″ W113° 1′ 56.6″.