Intra- and interspecific phylogenetic relationships of the rare serpentine endemic taxon *Caulanthus amplexicaulis var. barbarae* and related taxa in the "Streptanthoid Complex" of genera (*Streptanthus*, *Caulanthus*, *Guillenia*) were examined using nuclear ribosomal internal transcribed spacer (ITS) and chloroplast *trnL* intron sequences. Phylogenetic hypotheses generated from 81 variable ITS nucleotide sites and six variable trnL nucleotide sites indicate that *Streptanthus* and *Caulanthus* are nonmonophyletic groups. *Caulanthus amplexicaulis var. barbarae* and its more widespread nonserpentine sister taxon *Caulanthus amplexicaulis var. amplexicaulis* formed a distinct monophyletic group. Among the taxa in our study, *C. amplexicaulis* was most closely related to *Streptanthus tortuosus*. The ITS sequences supported monophyly of subgenus *Euclesia*, which includes the bulk of the serpentine endemics in the Streptanthoid Complex. The serpentine taxa were nonmonophyletic, occurring in at least three distinct clades, suggesting that tolerance to serpentine may be gained or lost through relatively few genetic changes. Intraspecific ITS1 and ITS2 sequence divergence within *C. amplexicaulis* (1.3–1.8%) was higher than in comparable species (0.0–0.3%); implications of this genetic differentiation for the conservation status of *C. amplexicaulis var. barbarae* are discussed. Evidence is presented that supports a "biotype depletion" model for the origin of this rare endemic taxon.

**Key words:** chloroplast DNA; edaphic endemism; genetic diversity; natural selection; ribosomal ITS; speciation.

Serpentine soils are derived from a class of ultramafic rocks that includes serpentinite, dunite, periodite, and t alc schists. These soils are characterized by low levels of the essential plant nutrients nitrogen, phosphorus, potassium, and calcium, as well as high levels of iron, magnesium, and manganese, and toxic levels of chromium, cobalt, and nickel (Walker, 1948; Proctor and Woodell, 1975; Brooks, 1987; Proctor, 1999). Serpentine occurrences are often characterized by an abrupt change in vegetation, with greatly reduced plant densities, biomass, and diversity, as well as distinctly different species compositions. Most plant species are excluded from serpentine soils; however, a small minority of taxa has evolved physiological and developmental mechanisms that allow them to survive—and even thrive. The mechanisms of adaptation to serpentine are largely unknown, although ability to grow at low calcium levels appears to be important (Walker, 1948; Kruckeberg, 1954). Using "paired population" experiments, Kruckeberg (1951, 1954) demonstrated that serpentine races of *Streptanthus glandulosus* Hook., *Phacelia californica* Cham., and *Salvia columbariae* Benth. had much higher levels of tolerance to serpentine soils than did nonserpentine races of these taxa, indicating the existence of distinct genetic determinants of serpentine tolerance.

Serpentine-derived soils are a significant impetus for plant speciation and endemism worldwide (Brooks, 1987; Proctor, 1999). Because of strong selective pressures associated with adaptation to serpentine, hybrids between serpentine-tolerant plants and their nonserpentine neighbors may exhibit reduced fitness in either environment. It is not surprising that adaptation to serpentine soils is often concomitant with reproductive isolation from parapatric nonserpentine populations, as determined by hand-pollination experiments (Kruckeberg, 1954, 1957, 1986). Serpentine soils contribute more rare and endemic plants to the rich flora of California than any other edaphic substrate (Skinner and Pavlik, 1994). One explanation for restriction of these taxa to serpentine soils is that the rigors of the serpentine environment exclude nonadapted species, thus providing serpentine-tolerant species a refuge from competition.

The Santa Barbara Jewelflower, *Caulanthus amplexicaulis* var. *barbarae* (J. Howell) Munz (Brassicaceae), is a rare her baceous annual plant restricted to an archipelago of serpentine exposures in the San Rafael Mountains, near the southern terminus of the outer Coast Ranges of California, USA (Howell, 1962). Its sister taxon, *C. amplexicaulis var. amplexicaulis* S. Watson, has a more widespread distribution in the Transverse Ranges of southern California. Although the Transverse Ranges contain a complex and diverse mixture of geologic substrates, *C. amplexicaulis var. amplexicaulis* is largely restricted to granitic soils. Two small populations (near Thorne Meadow in Ventura County, California) are found on shale outcrops. At a distance of ~75 km, these are the closest var. *amplexicaulis* populations to the var. *barbarae* on serpentine. Although ecologically and geographically isolated, *C. amplexicaulis var. barbarae* and *C. amplexicaulis var. amplexicaulis* are morphologically quite similar and remain fully interfertile in artificial intervarietal crosses (Kruckeberg, 1984; A. E. Pepper and L. E. Norwood, unpublished data), with 95% viable F₁ pollen, and F₁ and F₂ seed viability that was statistically indistinguishable from intravarietal crosses. The existence of
genetically compatible taxa with such distinct edaphic requirements presents a unique opportunity for intensive study of the genetic basis of tolerance to serpentine soils.

*Caulanthus* (±13 spp.) and related genera *Streptanthus* (±40 spp.), *Streptanthella* (1 sp.), and *Guillenia* (3 spp.) are sometimes referred to as the “Serpentanthid Complex” of genera due to their close taxonomic affinity. To provide a solid foundation for evolutionary and ecological-genetic studies of *C. amplexicaulis* var. *barbarae*, we sought to determine the genetic and the phylogenetic relationships of this taxon to the nonserpentine taxa *C. amplexicaulis* var. *amplexicaulis* and to other serpentine and nonserpentine taxa in the Serpentanthid Complex. The overwhelming majority of these species are annual plants with a chromosome complement of *n* = 14, 2*n* = 28 (Rollins, 1993). The ±57 species in the complex are restricted to the central and western United States and northern Mexico (Rollins, 1993). Of the ±40 species in *Streptanthus*, ±16 have affinities for serpentine soils, providing the largest contribution of any genus to the serpentine floras of the California Floristic Province (CFP). Not surprisingly, *Streptanthus* and related species are considered to be important models for the evolution of serpentine plant taxa (Raven and Axelrod, 1978; Kruckeburg, 1984, 1986). Based on traditional taxonomic treatments, most of the serpentine endemic taxa of *Streptanthus* have been placed in the subgenus *Euclea* (Morrison, 1941). Within *Euclea*, the *S. glandulosus* species complex has been particularly well characterized using traditional taxonomic methods (Kruckeburg, 1957, 1958) and molecular analyses of systematic relationships and population structure (Mayer and Soltis, 1994, 1999; Mayer, Soltis, and Soltis, 1994).

Within *Caulanthus*, however, *C. amplexicaulis* var. *barbarae* is the only taxon with known affinity for serpentine—although some cogeners show tolerance to other substrates with unusual chemistries, such as gyspsum, alkali, limestone, and gabbro soils (Kruckeburg, 1984; Beauchamp, 1986; Rollins, 1993). Because *C. amplexicaulis* var. *barbarae* is found on one of the most southerly and isolated serpentine exposures of the CFP, it is biogeographically well suited for studies of the evolution of a serpentine-adapted endemic species. In the present study, we used nuclear ribosomal internal transcribed spacer (ITS) sequences and chloroplast *trnL* intronic sequences to generate hypotheses for the intraspecific genetic differentiation within *C. amplexicaulis* and its phylogenetic relationships to other serpentine and nonserpentine taxa of the Streptanthid Complex. Further, we attempted to integrate a phylogenetic perspective with what is known about the comparative edaphic biology of several of the streptanthid taxa to develop a hypothetical model for the evolution of *C. amplexicaulis* var. *barbarae*.

**MATERIALS AND METHODS**

Plant materials—Taxonomy used in this study follows that of R. Buck and co-workers as presented in Hickman (1993). The taxa examined are listed in Table 1, and locations are shown in Fig. 1. Populations of *C. amplexicaulis* var. *barbarae* are clustered in a set of serpentine occurrences in the San Rafael Mountains of Santa Barbara County, California (inset in Fig. 1). We sampled all five known populations of this taxon (designated CAB1 through CAB5). However, some populations probably remain undocumented in this rugged mountainous region. CAB1 was obtained from a population growing on the tailings of an historic chromium mine (containing serpantinite, chromite, and other ultramafic rocks). CAB2 and CAB3 were obtained from natural serpentine outcrops. CAB4 and CAB5 were obtained from distinct populations on a recently burned alluvial slope containing soils derived from a mix of weathered serpentine and other parental rock types. Accessions of *C. amplexicaulis* var. *amplexicaulis* were obtained from a granite talus slope on Portal Ridge, Los Angeles County, California (CA01) and from shale occurrences near Thorne Meadow, Ventura County, California (CA02, CA03). Samples from 11 additional specific and intraspecific taxa in the allied streptanthid genera (*Caulanthus, Streptanthus, Guillenia*), including the serpentine taxa *S. albidas* ssp. *paramenosus* (E. Greene) Kruckeb., *S. batracopus* Morrison, *S. glandulosus* ssp. *pulchellus* (E. Greene) Kruckeb., and *S. niger* E. Greene, were obtained from the habitats and locations indicated in Table 1 and Fig. 1. Voucher specimens were not taken from small populations of rare, threatened, or endangered taxa; these populations have been adequately documented (Howell, 1962; Dieringer, 1991; Mayer and Soltis, 1994; Mayer, Soltis, and Soltis, 1994; Skinner and Pavlik, 1994). Representative voucher specimens previously collected from these populations, or from nearby populations of the same species, are listed in Table 1. Information on the voucher specimens of nonsensitive taxa is also listed in Table 1.

Genomic DNA isolation—DNA was extracted from leaves of single individuals by a simple, micropreparation method. Fresh leaf tissue (<1 cm²) was placed in a 1.5-mL microcentrifuge tube and hand-macerated using a teflon pestle (VWR, West Chester, Pennsylvania, USA). After the addition of 0.5 mL of extraction buffer (200 mmol/L Tris pH 7.5, 250 mmol/L NaCl, 25 mmol/L EDTA pH 8, 0.5% SDS), tissues were further hand-macerated for 10 sec. After either brief centrifugation or filtration through Miracloth (Calbiochem, San Diego, California, USA) to remove intact solids, nucleic acids were precipitated with the addition of 0.5 mL of isopropyl alcohol. After centrifugation for 5 min at 16,000 × g, the pellet was resuspended in 0.5 mL 50 mmol/L Tris pH 7.5, 10 mmol/L EDTA, then briefly centrifuged to remove undissolved solids. Following addition of NaOAc pH 5.2 to a final concentration of 0.3 mol/L, nucleic acids were again precipitated with 0.5 mL of isopropyl alcohol. After further centrifugation, the nucleic acid pellet was resuspended in 0.1 mL of 10 mmol/L Tris pH 7.5, 1 mmol/L EDTA.

DNA sequence analysis—Ribosomal ITS fragments were amplified using primers “ITS4” and “ITS5” (White et al., 1990). Primers “CP-C” and “CP-D” (Taberlet et al., 1991) were used to amplify an ~0.5-kb intron between the chloroplast *trnL* (UAA) 5`- and 3`- exons. Polymerase chain reaction (PCR) was performed using established conditions (Konieczny and Ausubel, 1993) in 50-µL reactions containing 10–20 ng of genomic DNA. In order to remove residual dNTPs and oligonucleotide primers prior to sequencing, PCR products were purified by precipitation with 26% PEG 8000, 6.5 mmol/L MgCl₂, 0.6 mol/L NaOAc pH 7.0 (Rosenthal, Coutelle, and Craxton, 1993). An aliquot of 20–50 ng of purified double-stranded PCR product was used as template for 35 cycles of sequencing using BigDye terminator chemistry (Applied Biosystems, Foster City, California, USA). The primers used for direct sequencing were IT54, IT55, IT51 (5`-ATTCGGGCTCTGCAGTGTAG-3`), and IT12 (5`-CAAGACTGCTGGTCCA-3`). Primers CP-C and CP-D were used to sequence the *trnL* (UAA) intron. Cycle sequencing products were purified by Bio-Gel P-30 size exclusion chromatography (Bio-Rad, Richmond, California, USA) and analyzed using an ABI 377 semiautomated sequencer (Applied Biosystems). Double-stranded DNA sequence contigs from each taxon were assembled and vetted using Sequencer 3.0 (Gene Codes, Ann Arbor, Michigan, USA). Finished sequences from the various taxa were aligned using Sequencer 3.0, and alignments across insertion/deletion differences (indels) were vetted manually. Nuclear ITS and chloroplast *trnL* sequences reported in this study were submitted to GenBank and assigned the accession numbers listed in Table 1.

In one case, an ITS sequence dimorphism within an individual accession was verified using a cleaved amplified polymorphic sequence (CAPS) strategy of Konieczny and Ausubel (1993). Ten microliters of the original ITS PCR reaction was digested for 8 hr using 10 units of the restriction enzyme *HaeII,* which only cleaves one of the two dimorphic alleles (recognition site = 5`-G GCC-3`); the dimorphic nucleotide is underlined). *HaeII* digestion products were then electrophoresed on a 2% agarose gel.
Data analysis—Phylogeny reconstructions were performed using PAUP* 4.0.b16a (Swofford, 1999). For parsimony analysis, indels of one nucleotide or longer were defined as missing data, based on arguments discussed by Wojciechowski et al. (1993); these indels were later superimposed onto trees in order to exploit their possible phylogenetic utility. Ambiguous nucleotides (e.g., divergent paralogues) were defined as polymorphisms. Branch and bound searches using unweighted (Fitch) parsimony and accelerated transformation (ACCTRAN) of character state optimization were performed to identify the most parsimonious trees. Branches of zero length were collapsed. For neighbor-joining analysis (Saitou and Nei, 1987), Kimura two-parameter distances were employed, the ITS sequences from (Kimura, 1980) and a minimum evolution objective function were employed, and ambiguous and “missing” data (e.g., indels and divergent paralogues) were ignored. The ITS sequences from Sinapis alba L. (Brassicaceae) (Yang et al., 1999) and chloroplast trnL intron sequences from Draba tomentosa (Brassicaceae) (Yang et al., 1999) were used as outgroups for the appropriate phylogenetic analyses. Relative support for various clades was determined by bootstrap analysis (Felsenstein, 1985) employing 200 replicates. MacClade 3.05 (Maddison and Maddison, 1993) was used as an aid to visually search for phylogenetically incongruent “blocks” of sequence containing two or more variable sites within a given taxa or clade that might constitute evidence of prior hybridization events followed by recombination between divergent paralogues (Baldwin et al., 1995). These searches were performed using the “tree window display,” with the “character trace” option active. MacClade 3.05 was also used for presentation of phylogenetic trait maps. A global tree-based relative rate test was performed by the likehood method of Felsenstein (1988, 1993).

RESULTS

ITS sequence divergence—The ITS regions of 24 accessions in the genera Streptanthus, Caulanthus, and Guillenia were amplified by PCR and directly sequenced yielding ±674 nucleotides of DNA sequence information. The ITS1 sequences were 263–269 nucleotides in length, and ITS2 sequences were 187–192 nucleotides in length. Sequence comparisons of the entire ±674 nucleotide ITS region (which included 23 nucleotides of the 18S ribosomal RNA gene, all of the 5.8S ribosomal RNA gene, and 27 nucleotides of the 23S ribosomal RNA gene) revealed 81 (12.0%) variable sites, including 76 sites exhibiting single nucleotide polymorphisms (25 transversion polymorphisms and 47 transition polymorphisms) and four indels. Of these, 55 single nucleotide substitutions and four indels were phylogenetically informative. At nucleotide 136 of the 5.8S rRNA gene, Streptanthus tortuosus accession ST2 showed distinctly heterozygous profiles in the chromatographs of both DNA strands, indicating a G/A paralog dimorphism. This dimorphism was verified using a CAPS strategy (Konieczny and Ausbel, 1993) by digestion of the original PCR product with HaeII, which resulted in partial (~50%) cleavage at the affected site, while ST9 (and other positive controls) showed complete digestion (not shown). Similarly, Streptanthus glandulosus ssp. pulchellus accession SG1 showed distinct G/A heterozygous profiles at nucleotide 139 of ITS2. However, the latter dimorphism could not be confirmed by the CAPS method due to the absence of an affected restriction enzyme recognition site. No other ambiguous nucleotides were observed.

Phylogenetic analysis based on ITS sequences—Parsimony analysis using the branch and bound method yielded ten equally parsimonious trees of 154 steps. Alternative most-parsimonious trees differed only with respect to the relative placement of Streptanthus campestris and in the arrangement of terminal (intraspecific) taxa. The most parsimonious trees had a consistency index of 0.822 (0.710 excluding uninformative characters), a retention index of 0.860, and a homoplasy index of 0.184 (0.290 excluding uninformative characters). For comparison, neighbor-joining analysis was performed using Kimura two-parameter distances. Neighbor-joining yielded a tree that differed from a 50% majority-rule consensus parsimony tree only in the topological arrangement of a few terminal, intraspecific taxa. One of ten most parsimonious trees, which had identical topology to the neighbor-joining tree, is depicted in Fig. 2. This tree, along with 50% majority rule and strict consensus parsimony trees, indicated that Streptanthus and Caulanthus were each nonmonophyletic.

The three representatives of the subgenus Euceslia, S. niger, S. albidus ssp. paramoenum, and S. glandulosus ssp. pulchellus, formed a well-supported monophyletic clade (bootstrap value = 100). This observation is in agreement with the results of Mayer and Solits (1994, 1999) from chloroplast restriction site and ITS sequence analysis of Streptanthus glandulosus species complex and related taxa. A possible sister clade to this group (bootstrap value = 67) included species from Streptanthus, Caulanthus, and Guillenia. Within this postulated sister clade, several topological relationships were poorly supported, possibly indicating an episode of rapid divergence with “lineage sorting” (Wendel and Doyle, 1998). The morphologically distinctive “desert candle” C. inflatus fell to a basal position within this postulated sister clade. Caulanthus amplexicaulis var. barbarea, C. amplexicaulis var. amplexicaulis, and C. tortuosus formed a distinct subclade supported by three synapomorphic nucleotide substitutions, indicating that the largely nonserpentine S. tortuosus species complex is a likely sister group to C. amplexicaulis. Caulanthus amplexicaulis var. barbareae and var. amplexicaulis formed a monophyletic subclade (bootstrap value = 93) supported by two synapomorphic indels, although they showed substantial divergence within this subclade. Other likely members of the postulated “non-Euceslian” clade include the diminutive “Tamalpais Jewelflower” S. brachypus, a highly restricted serpentine endemic, and C. campestris, a nonserpentine taxon with a wide distribution in the Transverse and Peninsular Ranges of southern California. A poorly supported subclade (bootstrap value = 31), that was nonetheless present in all most-parsimonious trees, included C. heterophyllus, a fire-following annual species from southern California, S. bracteatus, a biennial limestone endemic from central Texas, and the common desert annual Guillenia lasiophyllum.

Visual examination with the aid of MacClade 3.05 was used to search for phylogenetically incongruent “blocks” (two or more variable sites) of sequence, potentially indicative of recombination between divergent paralogues; no such blocks were observed. However, within C. amplexicaulis var. barbareae there was a block containing three variable nucleotides at positions 238 and 240 of ITS1 and position 28 of ITS2, which were not shared with C. amplexicaulis var. amplexicaulis. One of these nucleotides, a T at position 238 of ITS1, was shared with representatives of the Euceslia clade. We considered this block to be potentially significant since reticulation might explain the apparent branch asymmetry within the C. amplexicaulis clade (Fig. 2). However the nucleotide characters at positions 240 of ITS1 and 28 of ITS2 were not shared by any of the streptanthoid taxa in this study or those reported by Mayer and Solits (1999). Furthermore, the intervening 212 nucleotides (with 11 variable positions), as well as the immediately surrounding nucleotides (also with several variable
### Table 1. Taxa examined in this study.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Acronym</th>
<th>Voucher</th>
<th>Substrate/habitat</th>
<th>Location</th>
<th>GenBank accessions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caulanthus amplexicaulis var. amplexicaulis S. Watson</td>
<td>CAA1</td>
<td>A.E. Pepper 141&lt;sup&gt;c&lt;/sup&gt;</td>
<td>granite scree</td>
<td>Portal Ridge, 1.5 km NW of Lake Hughes, Los Angeles County, California, USA</td>
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<td>CAA2</td>
<td>A.E. Pepper 142&lt;sup&gt;c&lt;/sup&gt;</td>
<td>shale scree</td>
<td>N end of Grade Valley, 5.5 km N of Thorn Meadows, Ventura County, California USA</td>
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<td>CAA3</td>
<td>A.E. Pepper 143&lt;sup&gt;c&lt;/sup&gt;</td>
<td>shale scree</td>
<td>N end of Grade Valley, 5.5 km N of Thorn Meadows, Ventura County, California, USA</td>
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<td>var. barbarae (J. Howell) Munz</td>
<td>CAB1</td>
<td>Dennis Breedlove 466 (CAS)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>mine tailings (serpentine)</td>
<td>White Rock Cyn. Mine, 1.3 km N of Ranger Peak, Santa Barbara County, California, USA</td>
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<td>CAB2</td>
<td>Dennis Breedlove 466 (CAS)&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>0.5 km SW of Cachuma Saddle, Santa Barbara County, California, USA</td>
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<td>CAB3</td>
<td>Dennis Breedlove 466 (CAS)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>serpentine cliff</td>
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<td>CAB5</td>
<td>A.E. Pepper 140 (TAMU 029141)</td>
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<td>Caulanthus heterophyllus var. heterophyllus (Nutt.) Payson</td>
<td>CHH1</td>
<td>A.E. Pepper 92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>burn</td>
<td>Los Peñasquitos Canyon, 100 m NW of the corner of Calle Cristobal and Caminito Propico, Mira Mesa, San Diego County, California, USA</td>
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<td>var. pseudosimulans R. Buck</td>
<td>CHP1</td>
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<td>burn</td>
<td>1 km NW of Highland Springs, San Bernardino County, California, USA</td>
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<td>Caulanthus inflatus S. Watson</td>
<td>CI1</td>
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<td>7 km SW of Blackwater Well, San Bernardino County, California, USA</td>
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<tr>
<td>Guillenia lasiophylla (Hook. &amp; Arn.) E. Greene</td>
<td>GL1</td>
<td>A.E. Pepper 96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>alkali sand</td>
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<td>GL2</td>
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<td>SAP1</td>
<td>M.S. Mayer 580&lt;sup&gt;c&lt;/sup&gt;</td>
<td>serpentine barren</td>
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<td>Streptanthus batrachopus J. Morrison</td>
<td>SBA1</td>
<td>A.E. Pepper 70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>serpentine outcrop</td>
<td>Barth’s Retreat, Mt. Tamalpais, Marin County, California, USA</td>
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<td>Streptanthus bracteatus A. Gray</td>
<td>SB1</td>
<td>A. E. Pepper 149&lt;sup&gt;c&lt;/sup&gt;</td>
<td>limestone ledge</td>
<td>Bull Creek District Park, Austin, Travis County, Texas, USA</td>
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<td>Streptanthus campestris S. Watson</td>
<td>SC4</td>
<td>A.E. Pepper 119&lt;sup&gt;c&lt;/sup&gt;</td>
<td>granitic soil</td>
<td>Heaps Peak, 3.5 km W of Skyforest, San Bernardino County, California, USA</td>
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Table 1. Continued.

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<th>Taxon</th>
<th>Acronym</th>
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<th>Substrate/habitat</th>
<th>Location</th>
<th>GenBank accessions</th>
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<td><em>Streptanthus glandulosus</em></td>
<td>SGP1</td>
<td>M.S. Meyer 538*</td>
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<td><em>Streptanthus niger</em> E. Greene</td>
<td>SN1</td>
<td>M.S. Meyer 536*</td>
<td>serpentine grassland</td>
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<td><em>Streptanthus tortuosus</em> Kellogg</td>
<td>ST2</td>
<td>A.E. Pepper 2*</td>
<td>basaltic flow</td>
<td>North Table Mountain, Cherokee Road, 8.3 km N of Oroville, Butte County, California, USA</td>
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<td>ST9</td>
<td>A.E. Pepper 9*</td>
<td>granitic soil</td>
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<td>ST47</td>
<td>A.E. Pepper 47*</td>
<td>basaltic plug</td>
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<td>ST49</td>
<td>A.E. Pepper 49*</td>
<td>mixed talus of basalt and schist</td>
<td>Pacific Crest Trail, 3.0 km N of the Klamath River, Siskiyou County, California, USA</td>
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</table>

a Abbreviations used for voucher identification: TAMU, Texas A&M University; CAS, California Academy of Sciences.

b The prefix GBAN- has been added to all GenBank accession numbers to link the online version of *American Journal of Botany* to GenBank, but is not part of the actual accession number.

c To be deposited at TAMU.

d Representative voucher specimen taken from a different population than that examined in this study.

e Representative voucher specimen taken from a population at the same location as that examined in this study.

positions), were phylogenetically consistent with the remainder of the *C. amplexicaulis* ITS sequence and did not share any variable characters with the Euclisia clade. It is therefore likely that all three of the nucleotide characters in question are derived within the *C. amplexicaulis* var. *barbarae* clade rather than the result of reticulation followed by recombination.

**Chloroplast trnL sequence analysis**—Prior to our analysis of the ITS sequence data, we had the expectation that our study would encompass several distinct genera, including Guillenia as a likely outgroup. For this reason, chloroplast *trnL* intron sequences were included as a set of phylogenetic characters that would be useful for comparisons at the intergeneric level. The *trnL* introns were amplified from 19 accessions, yielding ±379 nucleotides of unambiguous double-stranded DNA sequence information. However, *trnL* intron sequences had a very limited number of variable sites within the taxa examined. There were only five nucleotide substitutions, two of which were phylogenetically informative, and one autapomorphic indel. The lack of informative sites yielded parsimony and neighbor-joining trees that were fraught with zero-branch length polymorphisms (not shown). However, a unique and informative *trnL* haplotype arising from a single nucleotide substitution was synapomorphic within the *C. amplexicaulis* group. Another unique haplotype was synapomorphic within the Euclisia serpentine endemics examined in this study. Given the low level of divergence seen in the *trnL* data set, the probability that either of these apparent synapomorphies is actually homoplastic is quite remote. Thus, trees generated by *trnL* sequence analysis were fully consistent with ITS trees, with unique haplotypes supporting some of the key ITS-derived topological arrangements (Fig. 2).

**DISCUSSION**

**Nonmonophyly of the individual “streptanthoid” genera**—The allied streptanthoid taxa have been subject to numerous taxonomic revisions, including recently (Hickman, 1993). The application of DNA-based characters will play a significant role in the clarification of taxonomic and phylogenetic relationships within this group. In our limited analysis, the genera of *Caulanthus* and *Streptanthus* appear to be non-monophyletic (for example, a minimum of five additional steps is required to produce a tree that places Guillenia outside of the clades represented by *Caulanthus* and *Streptanthus*). Although much of the structure of our tree lacks bootstrap support, this general finding holds true even if several weakly supported branches in our tree are statistical artifacts. The present study contributes to our understanding of the phylogenetic relationships within this group, through cladistic (parsimony) and distance analyses. However, further work in this direction should be undertaken through comprehensive examination of molecular and morphological evidence from all potential members of the streptanthoid group, including *Streptanthella*, the remainder of the Guillenia species, and the *Streptanthus* species of the southwest (Arizona, New Mexico, and west Texas) and southern plains (Texas, Oklahoma, and Arkansas) regions of the United States and of northern Mexico (Rollins, 1993).

**Phylogenetic perspectives on the evolution of serpentine tolerance**—Although the Brassicaceae family only constitutes ~4% of the native taxa of California, it contributes nearly 13% of the serpentine taxa (Kruckeberg, 1984; Hickman, 1993). Well represented in the California serpentine floras are the gen-
era of *Streptanthus* (±16 taxa) and *Arabis* (five taxa). Other brassicaceous genera of the CFP with at least one serpentine species include *Cardamine, Caulanthus, Guilemina, Erysimum,* and *Thalspi* (Hickman, 1993; Rollins, 1993). However, tolerance to serpentine environments is not universal within the Brassicaceae in general, or within *Arabis* and the Streptanthoid taxa in particular. Among numerous examples, Kruckeberg (1951, 1954) used experimental studies to determine that certain *Brassica* species were intolerant to serpentine soil, as were several races of *S. glandulosus* from nonserpentine locations. In the species complex *S. tortuosus* Kellogg, an apparent sister group to *C. amplexicaulis* (Fig. 2), the bulk of the taxa are annuals that are intolerant to serpentine (Kruckeberg, 1984). One uncommon biennial or perennial subspecies, *S. tortuosus* ssp. *suffrutescens* (E. Greene) Jepson (in part *S. tortuosus* ssp. *orbatus* Jepson), found on serpentine in central California (Kruckeberg, 1984; Hickman, 1993), needs further study. In the geologically complex Trinity-Klamath-Siskiyou region of extreme northern California and southern Oregon, we observed that populations of *S. tortuosus* were excluded on a fine scale from patches of serpentine soil. Considered together, these results indicate that tolerance to serpentine soils is determined by genetic differentiation among various lineages of the Brassicaceae and within the streptanthoid group itself.

For phylogenetic trait analysis (Fig. 3), we somewhat simplistically designated the taxa in our study as either “serpentine” or “nonserpentine.” In the absence of comprehensive side-by-side serpentine tolerance tests (e.g., Kruckeberg, 1954), these assignments were based on the habitats in which the specimens were collected and were in all cases in agreement with the available literature (including Kruckeberg, 1984; Hickman, 1993; Reeves, 1993; Mayer and Soltis, 1994;
August 2001] PEPPER AND NORWOOD—EVOLUTION OF A RARE SERPENTINE ENDEMIC PLANT

Fig. 2. A phylogram of Caulanthus amplexicaulis and related taxa based on one of ten most-parsimonious ITS trees and identical to an ITS tree generated by neighbor-joining. Numbers above branches indicate the nucleotide substitutions supporting the branch (based on ACCTRAN character state optimization). Percentages are bootstrap values from 200 bootstrap replications (bootstrap values of branches below 50% are not given). Outgroup for rooting ITS sequences was Sinapus alba (SINA). Closed and open rectangles indicate informative and uninformative ITS insertion/deletion differences (indels), respectively. Closed and open ovals indicate informative and uninformative nucleotide substitutions, respectively, within the chloroplast trnL intron (chloroplast trnL changes relative to SINA outgroup not shown).

When superimposed onto a semistrict consensus parsimony tree, serpentine habit (Fig. 3) and endemism were clearly nonmonophyletic in nature. These results support an earlier finding of nonmonophyly of serpentinism in a study of chloroplast restriction site polymorphism-based phylogenies in S. glandulosus and related taxa that included the nonserpentine taxa S. tortuosus and S. hispidus (Mayer and Soltis, 1994). In our study, the serpentine taxa emerged in at least three distinct phylogenetic clades. Given that the serpentine substrate is relatively rare (occupying only 1% of the CFP land mass; Raven and Axelrod, 1978; Kruckeberg, 1984), it would be prudent to assume that the ancestral or "primitive" state of the Streptanthoid Complex is intolerance to serpentine. With this assumption, one must surmise that "serpantinism" probably arose independently on several occasions. Given that all 16 species of the subgenus Euclesia have at least one intraspecific taxa on serpentine and that most of these species are in fact restricted to serpentine (Kruckeberg, 1951), serpentine tolerance may very well be the ancestral state of the Euclesia.

Alternatively, it is possible that serpentine tolerance arose in one of the taxa of the streptanthoid group and was subsequently spread by wide hybridization to other taxa, such as C. amplexicaulis var. barbarae. However, in our analysis we observed no positive evidence for reticulate evolution. There was complete congruence between the ITS data set and our limited chloroplast trnL data set. Further, in the putative non-Euclesian clade, there was only one dimorphic nucleotide—a possible indicator of recent hybridization—in S. tortuosus accession ST2. In this case, the character state of the novel or "exotic" paralog did not match that of any of the other taxa in our study (this nucleotide site was not included in the data set reported by Mayer and Soltis, 1999). Finally, we searched for phylogenetically incongruous blocks of divergent ITS se-
Fig. 3. Phylogenetic trait map displaying taxa with serpentine (black line) and nonserpentine (gray line) habits superimposed onto the semistrict consensus parsimony tree generated by ITS sequences and supported by trnL intron sequences. Only taxa included in ITS and trnL phylogenetic analyses are shown here (Streptanthus albidus, S. glandulosus, and S. tortuosus show intraspecific variation for serpentine tolerance).

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sequence, indicative of past recombination between divergent paralogs. No compelling evidence of recombination between divergent ITS paralogs was observed. Therefore, reticulation appears to have played a limited role in the evolution of C. amplexicaulis and closely related taxa.

Given that reticulation does not adequately explain the pattern of serpentine tolerance in the streptanthoid taxa, then the trait was apparently independently gained (or lost) multiple times. It is tempting to hypothesize the existence of an evolutionary “predisposition” within the Brassicaceae in general, and in Arabis and the streptanthoid group in particular, that facilitates adaptation to serpentine environments when and where these are encountered. Various models for the evolution of tolerance to heavy metals (Antonovics, Bradshaw, and Turner, 1971) and serpentine soils (Kruckeberg, 1986) stipulate the necessity of preexisting genetic variation as a requisite for microevolutionary adaptation through strong selection processes (this is variously termed “preadaptation” or “genetic potential” by Kruckeberg [1986] and others). A predisposition to the evolution of serpentine tolerance might simply be the presence of the requisite genetic variation in a given population. However, the mechanisms by which such genetic variation is maintained in specific lineages over many generations on nonserpentine soils—without apparent selection—remain to be elucidated (for example, C. amplexicaulis var. barbara does not appear to have a close phylogenetic relationship with any of the other serpentine taxa).

Additional possible predisposing factors need only provide a slight survival advantage on transitional soils at the margins of serpentine outcrops, as suggested by Kruckeberg (1954). Such contact zones could provide a temporary “stage” upon which microevolutionary forces could act to exert changes in allele frequencies that would eventually confer greatly enhanced tolerance to serpentine soils. Because this model incorporates a priori selective forces and biological processes, it is more satisfactory than drastic “catastrophic selection” models (Lewis, 1962; Raven, 1964; Antonovics, Bradshaw, and Turner, 1971; Kruckeberg, 1986) for explaining that the acquisition of serpentine tolerance is largely confined to a limited and recurring set of plant families (Kruckeberg, 1984). As an example, the Brassicaceae and Caryophyllaceae families, which are both conspicuous among serpentine floras (Proctor, 1999), are predominantly nonmycorrhizal, and would therefore not suffer a fitness cost with any loss of mycorrhizal associations due to geochemical limitations on microbial growth.

Furthermore, serpentine habitats are often open, rocky, and strongly exposed to evaporation and light. An evolutionary predisposition to evolving serpentine tolerance might take the form of adaptation to similar physical habitats that might arise on any number of geochemical substrates. In Hickman (1993), fully 50% of the specific and intraspecific brassicaceous taxa of California have the words “barren,” “cliff,” “crevice,” “gravel(ly),” “outcrop,” “rock(y),” or “talus” in their habitat description; the percentage is highest in Arabis (84%) and Streptanthus (64%). Successful adaptation to the rocky, exposed environments in geologically active and topographically complex western North America may have placed some elements of the Brassicaceae in an excellent position to take advantage of the refugia from competition afforded by serpentine habitats.

The uptake of nickel and its sequestration in plant tissues has also been postulated to enhance tolerance to serpentine...
soils in certain plant taxa (Brooks, 1987). About half of the known nickel-accumulating species occur in the Brassicaceae. Reeves, Brooks, and MacFarlane (1981) found that some herbarium specimens of C. amplexicaulis var. amplexicaulis, S. tortuosus, and C. inflatus from nonserpentine habitats had nickel levels in their tissues (up to 95, 14, and 47 μg/g dry tissue, respectively) that were comparable to the levels observed in serpentine-tolerant Streptanthus species sampled from serpentine sites (where underlying soil nickel concentrations were 10- to 100-fold higher). These data suggest that from serpentine sites (where underlying soil nickel concentrations were 10- to 100-fold higher). These data suggest that from serpentine sites 

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The apparent differences in intraspecific sequence divergence among these otherwise comparable species could be due to undersampling or could easily fit within a simple Poisson distribution of nucleotide differences for any given average divergence rate and length of time. Alternatively, they may reflect a limited or nonexistent gene flow between C. amplexicaulis var. amplexicaulis and C. tortuosus, which could result from genetic bottlenecks—perhaps because of microevolution or ``homogenization'' of ITS1 and ITS2 regions (1.2±1.8%). In contrast, four S. tortuosus accessions sampled over a transect nearly 300 km long, from the Sierra Nevada foothills near Sacramento, California, to the Siskiyou Mountains on the Oregon border, showed at most two nucleotide substitutions (including the dimorphic accession ST2). Further, the morphologically distinctive subspecies C. heterophyllus ssp. heterophyllus and C. heterophyllus ssp. pseudosimulans, obtained from locations 127 km apart, had only two divergent nucleotides. Finally, G. lasiophyllum populations sampled from Sonoran Desert (Yaqui Well) and Mojave Desert (Blackwater Well) stations separated by 260 km and two major (3000 m) mountain ranges had identical ITS sequences.

Intraspecific ITS sequence divergence in C. amplexicaulis and related taxa—G. lasiophyllum, C. heterophyllus, C. amplexicaulis, and S. tortuosus have similar life histories, floral morphology, and seed dispersal mechanisms. The pollination biology of these species needs further study, but all were self-
fertile when hand-pollinated (Preston, 1991; A. E. Pepper and L. E. Norwood, unpublished data). Species within this group are thus well suited for comparative analyses, including comparisons of intraspecific DNA sequence variation. As indicated in Table 2, C. amplexicaulis var. barbarae and C. amplexicaulis var. amplexicaulis populations sampled 75–105 km apart were divergent at six to eight nucleotide positions in the ITS1 and ITS2 regions (1.2–1.8%).
road vehicle activity, mining, and other pressures. Given the possibility of substantial genetic differentiation from its sister taxon *C. amplexicaulis var. amplexicaulis*—as evidenced by the data presented here—it should be granted a conservation status appropriate to an ecologically and phylogenetically distinct evolutionary unit.

A model for the evolution of *C. amplexicaulis* var. *barbarae*—Using a global tree-based maximum likelihood test (Felsenstein, 1988, 1993), a clock-like model for ITS divergence within the streptanthoid taxa could not be excluded \[ -2 \left( \log L_{\text{clock}} - \log L_{\text{site}} \right) = -34.1, P = 0.10 \]. However, local branch-length asymmetry along with a paucity of fossil records and biogeographic events for calibrating molecular clocks conspire to make any estimates of the time of divergence of *C. amplexicaulis* var. *barbarae* and *C. amplexicaulis var. amplexicaulis* highly speculative. However, if we presume that the actual ITS divergence rate within the streptanthoid group is within the range previously reported for herbaceous annual plants—from 1.45 \( \times \) 10\(^{-9}\) to 3.62 \( \times \) 10\(^{-9}\) substitutions-site\(^{-1}\) yr\(^{-1}\) in the Cucurbitaceae (Jobst, King, and Hembleben, 1998) up to 7.83 \( \times \) 10\(^{-9}\) substitutions-site\(^{-1}\) yr\(^{-1}\) in the genus Robinsonia (Sang, Crawford, and Stuessy, 1995)—we can estimate that *C. amplexicaulis var. barbarae* and *var. amplexicaulis* diverged at or after the time that areas of the San Rafael Mountains were exposed, during the middle Pliocene to early Quaternary (1–3.5 mya) (Raven and Axelrod, 1978). A lack of reproductive barriers between the two extant taxa suggests that the duration of any parapatric coexistence of serpentine and nonserpentine populations must have been brief.

Furthermore, *C. amplexicaulis* and related taxa have only minimally winged seeds (<1 mm), and no mechanisms for long-distance dispersal. The nearest extant population of *C. amplexicaulis* to the San Rafael Mountains serpentine sites is at Thorne Meadows—some 75 km distant. Considered together, these findings suggest that a “biotype depletion” scenario for the origin of endemic plant species (Stebbins, 1942) may be applicable. According to this model, Pliocene populations of *C. amplexicaulis* would have had a more continuous distribution than today. As serpentine became exposed, some combination of “genetic potential” (sensu Kruckenberg, 1987) and other predisposing factors present in *C. amplexicaulis* facilitated the evolution of adapted ecotypes on serpentine sites. An emerging Mediterranean climate, with decreasing summer rainfall (Raven and Axelrod, 1978), would have subsequently led to the confinement of *C. amplexicaulis* to a few “more local environments” (Kruckenberg, 1984) where competition was limited. Indeed, present-day *C. amplexicaulis var. amplexicaulis* populations at lower elevations of the Transverse Ranges and on the fringes of the Mojave Desert are found on loose, nearly barren shale or granite “scree” slopes—environments that could also be considered refugia from competition. At higher elevations (>2000 m) where moisture is more abundant, such as Frazier Mountain and Mount Pinos in Ventura County, or the San Bernardino and San Gabriel Mountains, *C. amplexicaulis var. amplexicaulis* is often found in environments more generally suitable for plant growth, such as the “yellow pine” forest floor. With geographic isolation resulting from biotype depletion, as is suggested by our intraspecific ITS sequence divergence data, reproductive isolation would not have been favored by selection. As a result, *C. amplexicaulis var. barbarae* and *C. amplexicaulis var. amplexicaulis* remained interfertile—as appears to be the “default” situation among several widely divergent taxa within the streptanthoid group, including *C. amplexicaulis, C. inflatus, C. heterophyllus,* and *G. lasiophyllus,* which are interfertile in hand-pollination experiments (A. E. Pepper and L. E. Norwood, unpublished data).

Further experiments, using defined growth conditions, will determine the phenotypic and genetic components of serpentine tolerance in the two *C. amplexicaulis* varieties. Since the obvious morphological differences between the two taxa are minimal (i.e., anthocyanin content of the perianth), adaptive differences are likely to be physiological or biochemical in nature or may involve root development. Determination of these adaptive genetic differences will permit the further development of a comprehensive model for the evolution of these two divergent taxa. *Caulanthus amplexicaulis var. barbarae* and other members of the streptanthoid group provide fascinating and experimentally tractable subjects for the study of serpentine tolerance and endemism. While the exact sequence of events in the evolution of *C. amplexicaulis var. barbarae*—or any other taxon in nature—will never be precisely known, the development of testable ecological and evolutionary hypotheses, combined with the application of powerful new tools for the study of extant genetic variation, will almost certainly lead to an enhanced understanding of the processes of natural selection and the formation of new plant species.

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