Population variation and phylogeny in the endangered *Chamaesyce skottsbergii* (Euphorbiaceae) based on RAPD and ITS analyses

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Received 4 January 2005; accepted 13 April 2005

**Key words:** *Chamaesyce skottsbergii*, conservation genetics, endangered species, ITS sequence, RAPD

**Abstract**

*Chamaesyce skottsbergii* var. *skottsbergii* is federally listed as an endangered taxon, and is found in small and isolated populations restricted to calcareous soils in dry shrubland habitats on the Hawaiian islands of Oahu and Molokai. Concern over the genetic relationship among these disjunct populations arose as a result of threats to the habitat of the Oahu population. The populations were examined using random amplified polymorphic DNA (RAPD) markers and sequence analysis of the internal transcribed spacer (ITS) region of the rDNA cistron. *Chamaesyce skottsbergii* var. *vaccinioides*, a closely related variety found in several small populations on Molokai, was used for baseline comparison of the genetic divergence among populations. RAPD analysis demonstrated that variation within and among populations is the highest for any Hawaiian species examined. Polymorphism was greater than 95% within populations and was 99.4% at the species level. Similarly, measures of genetic similarity indicate that differentiation among these populations is higher than is known for some species. Both RAPD and ITS sequence analysis indicate that populations of *C. skottsbergii* var. *skottsbergii* on Oahu and Molokai are genetically distinct, and the extent of this genetic differentiation supports the recognition of these populations as distinct varieties. The Molokai population is in fact much more closely related to var. *vaccinioides* than to var. *skottsbergii* on Oahu, and thus should be recognized by the previously used variety name, *C. skottsbergii* var. *audens*. Further conservation measures for each of the varieties are addressed.

**Introduction**

Correct taxonomic classification is a vital component to implementing conservation management practices and may eliminate erroneous decisions that could otherwise occur (Frankham et al. 2002). However, classifications based on morphological characters are often problematic in species complexes that have evolved through adaptive radiation. In particular, changes in several lineages of the Hawaiian flora have resulted in morphologies that represent a completely new direction of evolution in these groups (Ziegler 2002). Moreover, convergence toward a similar morphology by more distantly related congeners has also been demonstrated in this flora (Morden et al. 2003). One consequence of these tremendous changes is a morphological relationship among congeners that is difficult to discern based on traditional characters and is only realized when coupled with molecular genetic data (Givnish et al. 1995; Howarth et al. 1997; Ballard and Sytsma 2000).

The 15 Hawaiian species of *Chamaesyce* (Euphorbiaceae) range from shrubs of only a few decimeters to trees greater than 10 m tall with a bole girth of greater than 20 cm (Koutnik 1999). *Chamaesyce skottsbergii* is a subshrub with stems that are deciduous and often die back during
the summer drought. This species is restricted to dry shrublands with calcareous or basalt soils on the coastal strand or shrublands of Oahu and Molokai. Sherff (1937) originally described this species (as *Euphorbia skottsbergii*) with four varieties. Varieties *typica* (= var. *skottsbergii*) and *kalaeloa* are both from the Ewa Plains of Oahu, and differ in stem habit (prostrate versus upright), stem diameter (thick versus thin), and leaf size (larger versus smaller) (Koutnik 1987). Varieties *audens* and *vaccinioides* are from Molokai, and are also distinguished by their stem habit (decumbent versus upright), leaf shape (ovate or obovate versus elliptic) and leaf margin (serrate versus entire).

Varietal names of Sherff (1937) were generally accepted until publication of a monograph of the Hawaiian species of *Chamaesyce* by Koutnik (1987). He concluded that the two varieties on Oahu (type locations separated by only a few miles) were the same taxon and that morphological distinctions were a consequence of habitat differences. He further found that leaf dentation, although more pronounced in Molokai populations of var. *audens*, is also present in juvenile plants of var. *skottsbergii* and var. *kalaeloa*, and considered this character alone too inconsistent to be used for taxonomic separation of varieties. Furthermore, leaf shape in var. *audens* was similar to that of Oahu varieties. Thus, differences between them were attributed largely to phenotypic plasticity rather than genetic differentiation. As such, these three varieties (var. *audens*, var. *kalaeloa*, and var. *skottsbergii*) were combined as var. *skottsbergii*. Hence, only two varieties of *C. skottsbergii* are currently recognized: var. *skottsbergii* from Molokai and Oahu and var. *vaccinioides* from Molokai.

*Chamaesyce skottsbergii* var. *skottsbergii* is a federally endangered taxon restricted to a single population at Kalaeloa (Barbers Point), Oahu and two populations in northwestern Molokai. The population at Kalaeloa is on property leased by the U.S. Navy and operated as Barbers Point Naval Air Station until 1999. This region supported at least six populations with approximately 4000 individuals in 1979 (Char 1981), but subsequent urbanization and failed plant relocation efforts reduced this to a single population soon thereafter. The remaining population was restricted to a 23 acre parcel and fluctuated in size from fewer than 100–900 individuals depending upon annual precipitation (Whistler 1998, 2003). The soils for this population were found to be contaminated by lead, and plans were developed to remove the soil surface (including all plants) to eliminate lead from the site. Because other populations of *C. skottsbergii* var. *skottsbergii* were present on Molokai, it was determined that this would not be detrimental to the survival of the variety. The Molokai populations contain several hundred individuals each, but are very localized in the coastal zones.

This study was undertaken prior to decontamination efforts to examine the population variation of *C. skottsbergii* var. *skottsbergii* specifically to determine if the Kalaeloa population is genetically distinct from the Molokai populations and warrants separate protection. Habitat and ecological differences among the populations are sufficient to suggest they represent different "evolutionarily significant units" (ESUs) sensu Templeton (Crandall et al. 2000), although genetic confirmation would provide quantitative measures to support their protection. *Chamaesyce skottsbergii* var. *vaccinioides* (considered a "species of concern" by the U.S. Fish & Wildlife Service) was also examined to establish a baseline level of variation between the two varieties of *C. skottsbergii*. Random amplified polymorphic DNA (RAPD) markers were used to assess variation within and among populations, and sequences of the intergenic transcribed spacer (ITS) region were used to place the varieties of *C. skottsbergii* in a phylogenetic framework.

**Materials and methods**

**Sample collections and DNA extraction**

Leaf tissue of *C. skottsbergii* var. *skottsbergii* was collected from two populations in the northwestern region of Molokai and from Kalaeloa, Oahu. Leaf tissue of *C. skottsbergii* var. *vaccinioides* was collected from three closely associated subpopulations on Molokai. Population locations and the number of individuals collected are provided in Table 1. Leaves were placed into sealed plastic bags and chilled until DNA was extracted. Total DNA was extracted from 0.5–1.0 g of fresh leaf material using the CTAB method of Doyle and Doyle (1987) with minor modification (Morden
Table 1. Accessions of Chamaesyce used for genetic analysis (RAPD and ITS sequencing) and diagnostic characters for varieties of C. skottsbergii

<table>
<thead>
<tr>
<th>Species/variety</th>
<th>Island</th>
<th>Population</th>
<th>Voucher</th>
<th>HPDLa</th>
<th>Nb</th>
<th>Diagnostic characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. degeneri (Sherff)</td>
<td>Oahu</td>
<td>Kaena Pt.</td>
<td>Morden 1577</td>
<td>2108</td>
<td>1</td>
<td>Stems prostrate; leaves ovate to obovate, margins serrate; coastal strand, coralline to sandy soils.</td>
</tr>
<tr>
<td>Croizat &amp; Degener</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. herbstii Wagner</td>
<td>Oahu</td>
<td>Waianae Mtns</td>
<td>Kavelo sn.</td>
<td>2787</td>
<td>1</td>
<td>Stems prostrate; leaves ovate to obovate, margins serrate; coastal strand, coralline to sandy soils.</td>
</tr>
<tr>
<td>C. olowaluana (Sherff)</td>
<td>Hawaii</td>
<td>Pohakolea</td>
<td>Morden 1311</td>
<td>403</td>
<td>1</td>
<td>Stems prostrate; leaves ovate to obovate, margins serrate; coastal strand, coralline to sandy soils.</td>
</tr>
<tr>
<td>Croizat &amp; Degener</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. remyi (Gray ex Boiss.)</td>
<td>Kauai</td>
<td>Wahiawa Stream</td>
<td>Morden 1365</td>
<td>482</td>
<td>1</td>
<td>Stems prostrate; leaves ovate to obovate, margins serrate; coastal strand, coralline to sandy soils.</td>
</tr>
<tr>
<td>Croizat &amp; Degener</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. skottsbergii (Sherff)</td>
<td>Molokai</td>
<td>Moomomi</td>
<td>Morden 1909</td>
<td>3535–3562</td>
<td>28</td>
<td>Stems prostrate or erect to 1 m; leaves elliptic to ovate, margins serrate in seedlings; coastal shrubland, calcareous soils.</td>
</tr>
<tr>
<td>Croizat &amp; Degener</td>
<td>Puu Koai</td>
<td></td>
<td>Morden 1909</td>
<td>3535–3562</td>
<td>28</td>
<td>Stems prolate or erect to 1 m; leaves elliptic to ovate, margins serrate in seedlings; coastal shrubland, calcareous soils.</td>
</tr>
<tr>
<td>var. skottsbergii</td>
<td>Oahu</td>
<td>Kalaeloa</td>
<td>Morden 1909</td>
<td>3535–3562</td>
<td>28</td>
<td>Stems prostrate or erect to 1 m; leaves elliptic to ovate, margins serrate in seedlings; coastal shrubland, calcareous soils.</td>
</tr>
<tr>
<td>var. vaccinioides (Sherff)</td>
<td>Molokai</td>
<td>Makakupaia Road</td>
<td>Morden 1957</td>
<td>3563–3565</td>
<td>3</td>
<td>Stems erect to 3 m; leaves elliptic, margins entire; low elevation shrubland, basalt soils.</td>
</tr>
<tr>
<td>Koutnik</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kamilolola</td>
<td>Morden 1947</td>
<td>3566–3586</td>
<td>21</td>
<td>Stems erect to 3 m; leaves elliptic, margins entire; low elevation shrubland, basalt soils.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Makoleleau</td>
<td>Morden 1977</td>
<td>3587–3590</td>
<td>4</td>
<td>Stems erect to 3 m; leaves elliptic, margins entire; low elevation shrubland, basalt soils.</td>
</tr>
</tbody>
</table>

aAccession in the Hawaiian plant DNA library (Morden et al. 1996; Randell and Morden 1999).

bNumber of plants sampled in population.

et al. 1996). All DNA samples were purified by cesium chloride density-gradient ultracentrifugation (Sambrook et al. 1989). Water-saturated butanol was used to remove the ethidium bromide, and the DNA was precipitated with isopropanol to remove the cesium and washed one time with 70% ethanol. DNA was accessioned into the Hawaiian Plant DNA Library (Morden et al. 1996; Randell and Morden 1999).

RAPD PCR and data analysis

Approximately 25 ng of DNA in 25 µl reactions was amplified via the polymerase chain reaction (PCR) under the following conditions: 0.2 µM random 10-mer oligonucleotide primers, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 1X Taq polymerase PCR Buffer, 1.5 mM MgCl2, 0.1% bovine serum albumin, and ca. 1 unit Taq polymerase (Promega, Madison, Wisconsin, USA). DNA extracts of single individuals from four populations (one from each location of var. skottsbergii and one sample of var. vaccinioides) were screened with 30 separate primers (kits OPA-OPC; QIAGEN Operon, Alameda, CA, USA) using RAPD analysis of the PCR to evaluate each primer for use on all individuals. Amplifications were performed in an MJ Research PTC-200 DNA thermocycler under the following conditions: 94 °C for 2 min followed by 45 cycles of 94 °C for 45 s, 35 °C for 45 s, 72 °C for 2 min with 0.5 °C/s ramp rate from denaturation to annealing and annealing to elongation, and a final incubation at 72 °C for 4 min. Amplification products were mixed with loading dye (20 mM EDTA, 10% glycerol, 1% sarcosyl with bromophenol blue and xylene cyanol) and separated in 1.5% agarose in 0.5X TBE (tris-borate-EDTA) buffer with 125 ng ethidium bromide per liter. Primers yielding consistent number and intensity of markers were then used for amplification of all individuals. Gel images were recorded with the Kodak EDAS 290 Photographic System. Size of genetic markers was estimated using the Kodak 1D Image Analysis Software (Eastman Kodak Company, New Haven, CT) by comparison to fragments in a 1 kb
ladder (Promega, Madison, WI, USA) or to a pBS plasmid (Stratagene, La Jolla, CA, USA) digested with restriction enzymes to produce fragments in a size range of 0.448–2.96 kb. Molecular markers were identified by the primer used to generate them and their approximate size (kb). Gel scoring was performed independently by the authors to produce unbiased and unambiguous analysis of RAPD amplifications.

Assumptions associated with RAPD marker analysis were made as described by Lynch and Milligan (1994). A RAPD marker was determined to be polymorphic when found in less than 95% of the individuals sampled (i.e. absent in three or more individuals). Absence of a marker within a population, although present in others, was assumed to indicate that all individuals of the population were null/null homozygotes rather than there being a loss of the locus. Expected heterozygosity was calculated for each population ($H_s$) and species ($H$) for each locus as follows:

$$H = 1 - (p^2 + q^2)$$

where $p$ is the frequency of the dominant allele and $q$ is the frequency of the null allele. Allele frequencies were estimated from the number of null/null homozygotes present in the population (Hartl and Clark 1989; also see Morden and Loeffler 1999). Genetic relationships within and among populations were estimated using the similarity coefficients of Nei and Li (1979). Pairwise similarity was averaged for individuals within and among populations. UPGMA cluster analysis from similarity coefficients and principal coordinates analysis (PCO) using the Gower general similarity coefficients were calculated using MVSP 3.0 (Multi-Variate Statistical Package; Kovach Computing Services 1987–1998). Summary statistics of average similarity measures (means, standard errors, and $t$-test) were calculated using Minitab (1996).

**ITS sequence analysis**

Four species of Hawaiian Chamaesyce (C. degeneri, C. herbstii, C. olowaluana, and C. remyi) were included in the phylogenetic analysis for outgroup comparison (Table 1). Ongoing research (T. J. Motley and C. W. Morden, unpublished) in the evolution of Chamaesyce has shown the Hawaiian species to be monophyletic, and that C. degeneri is the sister species to C. skottsbergii. Primers for PCR amplification of the ITS region are from Wendel et al. (1995). Amplifications consisted of 50 µl reactions with 1X buffer, 2 mM MgCl₂, 0.1% bovine serum albumin, 0.128 mM dNTP, 1 µM of each amplifying primer, 2 units of Taq polymerase (Promega, Madison, WI, USA), and ca. 50–100 ng DNA. Cocktails were exposed to the following amplification conditions on an MJ Research PTC 200 (MJ Research Inc., San Francisco, CA, USA): 2 min at 95 °C; 30 cycles of 1 min at 93 °C, 1 min at 50 °C, and 2 min at 72 °C; 3 min at 72 °C. PCR products (3 µl) were visualized on 1.5% agarose gels to assure proper amplification and the remaining PCR product was purified in Ultrafree-MC filters (Millipore Corporation, Bedford, MA, USA) using the manufacturers specifications; the concentration of purified products was determined using UV spectroscopy (Sambrook et al. 1989). Double stranded PCR products were sequenced in both directions using Big Dye version 3.0 (ABI, Foster City, CA, USA) in one-half the reaction volumes recommended by the manufacturer. Four sequencing reactions for each product were completed: two with the amplification primers and two with additional primers (reverse complements) for sequencing from the middle of the product toward each end (Wendel et al. 1995). Sequenced products were purified with Centri-Sep spin columns (Princeton Separations, Adelphia, NJ, USA) following manufacturer specifications, and examined on an MJ Research BaseStation 51 DNA Fragment Analyzer. Resulting sequence files were examined using Cartographer v.1.0 software (MJ Research Inc., San Francisco, CA, USA), and fragment sequences were assembled using Sequencher v.3.0 (Gene Codes Corporation, Ann Arbor, MI, USA).

Complete DNA sequences for each species and variety were manually aligned to adjust for the four indels of 1–2 bp. Neighbor joining (NJ) and parsimony analyses were performed using PAUP 4.0b8 (Swofford 1996). Parsimony searches were conducted with all characters weighted equally and using the Branch and Bound option. Bootstrap analyses (Felsenstein 1985) were done with 1000 replicates for both NJ and parsimony searches.
Results

RAPD analyses

Ten primers were examined for all individuals. From these, 340 different genetic loci were scored (range of 20-46 loci per primer with an average of 34). There was extensive variation found among the individuals within each of the populations (Table 2). Only two markers were found among all individuals in all varieties for a species level polymorphism (P) of 99.4%. Levels of polymorphism in each of the populations were comparatively high. P averaged 96.0% among the three populations of *var. skottsbergii* (range of 95.1 to 97.3%) and 95.5% within *var. vaccinioides*. Levels of estimated heterozygosity are also similar among the populations, with the Kalaeloa population the lowest at 0.095 and the Moomomi population the highest at 0.114.

Populations were compared for genetic similarities based on the Nei and Li (1979) genetic identity (I) where a value of 1.0 indicates complete genetic identity (Table 3). Examination of variation among populations of *var. skottsbergii* on Molokai and *var. vaccinioides* indicated there was some evidence of structure among the populations. Genetic similarity showed that individuals within the Moomomi and W. Molokai populations of *var. skottsbergii* were all generally more closely related to plants within their respective population (0.530 and 0.519, respectively) than they were to those from the other population (0.506). Similarly, subdivision among populations of *var. vaccinioides* was minimal and the smaller populations (Makakupua and Makoleau) were largely nested within the larger one (Kamiloloa). Because of these close relationships among intra-island populations of a variety, populations of *var. skottsbergii* and *var. vaccinioides* will each be discussed as single populations hereafter.

Genetic similarity within populations was much greater than among populations (Table 3). Similarity within populations is highest among individuals of the *var. skottsbergii* from Kalaeloa (Oahu), and followed by *var. vaccinioides* and *var. skottsbergii* from Molokai. Contrary to expectation, similarity between populations of *var. skottsbergii* from Molokai and Oahu is low (0.226). In fact, the similarity between either of these populations and *var. vaccinioides* is higher (0.349 and 0.258, respectively) than is the similarity between the populations of the same variety from separate islands.

Analysis of the genetic differentiation among populations also indicates that, although it is distinct, there is a closer relationship between the Molokai population of *var. skottsbergii* and *var. vaccinioides* than between the Molokai and Oahu populations of *var. skottsbergii*. Differentiation among the Oahu and Molokai populations of *var. skottsbergii* is nearly double ($G_{ST} = 0.076$) that of the two *var. skottsbergii* populations on Molokai ($G_{ST} = 0.040$; Table 4). By comparison, $G_{ST}$ of *var. skottsbergii* from Molokai and *var. vaccinioides* is less than that differentiating *var. skottsbergii* from Oahu and *var. vaccinioides* ($G_{ST} = 0.054$ and 0.087, respectively), again suggesting a closer genetic affinity of the Molokai populations of *var. skottsbergii* to *var. vaccinioides* than to the Oahu population of the same variety.

Relationships among individuals within and among populations were visualized using cluster analysis. Three distinctive clusters were evident corresponding to the population and island from which they were collected (Figure 1). As evidenced

<table>
<thead>
<tr>
<th>Variety/population</th>
<th>N°</th>
<th>Markers</th>
<th>P³</th>
<th>Hs ⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>var. skottsbergii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalaeloa, Oahu</td>
<td>28</td>
<td>185</td>
<td>95.1%</td>
<td>0.095</td>
</tr>
<tr>
<td>Puu Koai, Molokai</td>
<td>12</td>
<td>192</td>
<td>95.6%</td>
<td>0.112</td>
</tr>
<tr>
<td>Moomomi, Molokai</td>
<td>12</td>
<td>187</td>
<td>97.3%</td>
<td>0.114</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variety/population</th>
<th>N°</th>
<th>Markers</th>
<th>P³</th>
<th>Hs ⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>var. vaccinioides</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>176</td>
<td>95.5%</td>
<td>0.100</td>
</tr>
</tbody>
</table>

*Number of plants sampled per population.
*Number of RAPD markers identified in population or taxon.
*Percent polymorphism.
*Estimated heterozygosity.

Table 2. Variation in populations of *C. skottsbergii*

Table 3. Levels of genetic similarity within and among populations of *C. skottsbergii* based on Nei and Li (1979) coefficient

<table>
<thead>
<tr>
<th>Genetic similarity</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>var. skottsbergii</em> (Oahu)</td>
<td>0.587</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. <em>var. skottsbergii</em> (Molokai)</td>
<td>0.226</td>
<td>0.517</td>
<td></td>
</tr>
<tr>
<td>3. <em>var. vaccinioides</em> (Molokai)</td>
<td>0.258</td>
<td>0.349</td>
<td>0.570</td>
</tr>
</tbody>
</table>
Table 4. Estimated population (H2) and total (H4) heterozygosity values and genetic differentiation (GST) among pairwise comparisons of populations of C. skottsbergii and all populations combined

<table>
<thead>
<tr>
<th>Taxa</th>
<th>H2</th>
<th>H4</th>
<th>GST</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. skottsbergii</td>
<td>0.104</td>
<td>0.129</td>
<td>0.090</td>
</tr>
<tr>
<td>var. skottsbergii</td>
<td>0.113</td>
<td>0.120</td>
<td>0.040</td>
</tr>
<tr>
<td>(W. Molokai and Moomoni)</td>
<td>0.107</td>
<td>0.128</td>
<td>0.076</td>
</tr>
<tr>
<td>var. skottsbergii</td>
<td>0.097</td>
<td>0.121</td>
<td>0.087</td>
</tr>
<tr>
<td>(Molokai and Kalaeloa)</td>
<td>0.110</td>
<td>0.123</td>
<td>0.054</td>
</tr>
</tbody>
</table>

from tests of genetic similarity, cluster analysis shows that var. vaccinioides and var. skottsbergii from Molokai are more closely related to each other than either is to var. skottsbergii from Oahu. Some of the sub-structuring of the Molokai populations of var. skottsbergii can be visualized here, but the Moomoni plants are largely nested within the West Molokai cluster of plants.

**ITS analysis**

The aligned sequence used in phylogenetic analysis was 724 bp long and consisted of the first intergenic transcribed spacer (ITS1), the 5.8S rRNA gene, and the second intergenic transcribed spacer (ITS2). A comparison of the sequences revealed a single base deletion common to C. skottsbergii var. skottsbergii (Oahu) and C. degeneri and a two base insertion in C. herbstii. There were 32 variable sites in the data matrix, 18 of which were phylogenetically informative. Tree structure based on NJ analysis was fully resolved (Figure 2). Parsimony analysis (not shown) yielded two equally parsimonious trees with a length of 60 steps, a consistency index of 0.9167, and a retention index of 0.8148. Tree 1 was completely resolved and identical in structure to the NJ tree whereas tree 2 displayed a trichotomy involving the two collections of var. skottsbergii from Molokai and var. vaccinioides.

Both NJ and parsimony analyses indicate that Oahu C. skottsbergii var. skottsbergii is distinct from Molokai populations of C. skottsbergii. This separation in the NJ tree is strongly supported by bootstrap analysis (93%) and in the parsimony analysis by three step changes as well as bootstrap support (82%). The sister taxon, C. degeneri, is separated from the remainder of the C. skottsbergii clade with a bootstrap value of 82% (83% in parsimony analysis).

**Discussion**

**Taxonomic implications of genetic variation**

Correct classification of endangered taxa is necessary to establish successful management strategies for the protection and perpetuation of threatened populations. In this study, it was important to also examine C. skottsbergii var. vaccinioides in conjunction with the different island populations of C. skottsbergii var. skottsbergii because it has been recognized as a separate variety in all classifications of this species (Sheriff 1937; Koutnik 1987), and it represented the baseline genetic differentiation that must exist for varieties to be considered separate. Some differentiation was expected between populations from Oahu and Molokai based on previous studies of other Hawaiian taxa (Caraway 1997; Kwon and Morden 2002; Loeffler and Morden 2003), but the degree to which the populations might be differentiated and how this might impact the species classification was under question.

We clearly demonstrated with both RAPD and ITS sequence analysis that the population of C. skottsbergii var. skottsbergii from Oahu was genetically distinct from the populations of this taxon on Molokai. Given that the Molokai populations were also genetically more similar to C. skottsbergii var. vaccinioides than to the Oahu population of var. skottsbergii, it is necessary to recognize the distinction between these populations in their classification. As such, the Molokai populations of var. skottsbergii should be recognized as C. skottsbergii var. audens (Sheriff) Degener & I. Degener as originally designated by Sheriff (1937). Sheriff (1937) also described C. multiflorus var. capuleiensis f. pekelonis from the northern coast of Molokai, a variety and form that was also combined into var. skottsbergii by Koutnik (1987). The two populations that were sampled in this study are widely separated along the northern coast (Moomoni) and the western shore of Molokai, a range that encompasses the distribution of Sheriff's varieties. Although limited
genetic partitioning was evident in the cluster analysis and PCO, no clear genetic distinctions were evident among these plants in this study. As such, it is recommended that *C. multiflorum* var. *kapuleiensis* f. *pekelonis* remain synonymous with *C. skottsbergii* var. *audens*.

The Oahu population at Kalaeloa was originally described as *C. skottsbergii* var. *kalaeloana*...

*Figure 1.* UPGMA cluster analysis of RAPD data for all *C. skottsbergii* individuals sampled. Population sources of plants are described in Table 1. Scale bar represents coefficient of genetic similarity (Nei and Li, 1979). Numbers are HPDL accessions (see Table 1).
by Sherff (1937), but was combined with var. skottsbergii by Koutnik (1987). The type population of var. skottsbergii from the Ewa Plains of Oahu has since gone extinct. It is unknown what genetic differences may have existed between this population and that of var. kalaholoana a few miles to the west. Koutnik (1987) attributed morphological plasticity and habitat differences between these sites as the factors leading to the distinction of these varieties, but we have no means at the present time to further test the degree of genetic differentiation that may have existed. Given their close proximity to one another and the noted habitat differences, it is the recommendation here that all Oahu plants continue to be referred to as C. skottsbergii var. skottsbergii.

The level of differentiation based on ITS sequences between C. skottsbergii and its sister species, C. degeneri, was about equal to the differentiation between C. skottsbergii var. skottsbergii and the two Molokai varieties. Although morphological characters are not recognized to support such a separation, this could indicate that C. skottsbergii var. skottsbergii is genetically distinct enough from var. audens and var. vaciniooides to warrant recognition as a separate species. The amount of variation among populations of C. degeneri is unknown. This species is native to all the main Hawaiian Islands with the exception of Niilau and Kahooolawe, and the variation across this range in relation to C. skottsbergii (as well as other morphological and anatomical distinctions between C. skottsbergii varieties) should be explored further before such a change is considered.

Population variability and conservation measures

Genetic variation within the three varieties of C. skottsbergii is the highest of any population or species examined in the Hawaiian flora, let alone for taxa that are federally listed as endangered (Morden and Loefller 1999; Caraway et al. 2001; Kwon and Morden 2002; Harbin 2003; Loefller and Morden 2003). Polymorphism within the populations of C. skottsbergii is 95% or greater, and the polymorphism for the entire species is over 99% (reflecting only two genetic markers present in all individuals of all populations). This is far higher than the level of polymorphism among other island species examined (Friar et al. 1996; Caraway 1997; Morden and Loefller 1999; Caraway et al. 2001; Kwon and Morden 2002; Harbin 2003; Loefller and Morden 2003). High diversity within the three varieties is also manifested by the very low genetic similarities within and among populations. Similarity within populations ranged from 0.517 to 0.587, and similarity among the three varieties ranged from 0.226 to 0.349 (based on Nei and Li 1979). In contrast, similarity within populations of Labordia species (also based on Nei and Li 1979) was greater than 0.85, and ranged from 0.200 to 0.635 among
species including populations on different islands (Motley 1996). Similarly, populations of *Touchardia latifolia* from different islands range from 0.716 to 0.949 (Loeffler and Morden 2003). (Similarities for other Hawaiian species are also available, but are based on different statistical models and thus are not readily comparable.)

Localized endemic taxa such as found in the *C. skottsbergii* populations typically have lower levels of polymorphism (Hamrick and Godt 1990; Hamrick et al. 1991), and this is particularly true of taxa restricted to oceanic islands (DeJode and Wendel 1992; Frankham 1997). However, this is not the case for *C. skottsbergii* populations examined here. It is not uncommon for rare taxa to have high levels of variation within populations or species (Gitzendanner and Soltis 2000), yet it was surprising in this study that the levels exceeded that of any plant group we had previously studied in the Hawaiian flora. One possible explanation is that the monoeocious flowers in *Chamaesyce* promote an outcrossing breeding system that favors the maintenance of higher levels of variation within a population. However, other taxa that we have investigated, such as *Dubautia* sp. (Caraway 1997; Caraway et al. 2001), *Labordia* (Motley 1996), and *Touchardia latifolia* (Loeffler and Morden 2003), are either self-incompatible (Carr et al. 1986), dioecious (Motley and Carr 1998) or monoeocious yet have far lower levels of variation present. A contributing factor may also be that this diversity reflects the variation found in the seed bank at these sites. Habitats for the *C. skottsbergii* varieties are either typically very dry with well-drained soils or are subjected to frequent ocean spray that may further mimic desiccation, both factors that could contribute to prolonged dormancy. Hawaiian species of *Chamaesyce* have been found to exhibit physiological dormancy (Baskin et al. 2004), and long-term dormancy could lead to a build up of genetic variation within the seed bank that could be maintained even when surface plant numbers dwindle due to climatic factors or other pressures to the population.

Although the varieties of *C. skottsbergii* are either endangered or are "species of concern", it is evident that they are not genetically depauperate. The remaining populations are small and isolated, and gene flow among them is likely non-existent or at least extremely curtailed. It is apparent that their cause of endangerment due to habitat loss from expanding development rather than from anything intrinsic to the populations themselves. Each of these varieties is a distinctive evolutionarily significant unit based on both genetic and ecological differentiation (Crandall et al. 2000), and as such each should be protected. Management priorities for these populations need not involve genetic supplementation at this time. Such a practice could be particularly damaging for *C. skottsbergii* var. *skottsbergii* as it now consists of only a single population and is genetically distinct from var. *audens* and var. *vaccinioides*. Genetic supplementation under these circumstances could lead to outbreeding depression (Fenster and Dudash 1996). Although it has been suggested that error in management strategies should be made on the side of increased genetic diversity and potential for evolutionary change (Templeton 1997), mixing of gene pools is not recommended among populations with historically low levels of gene flow (Ellstrand and Elam 1993). This population has long been separated from those on Molokai, and it is more likely to have developed coadaptive gene complexes distinct from those in populations on the neighboring island.

Adoption of *ex situ* conservation measures to conserve genetic and ecological diversity of these varieties should be made a priority for this species in the likely event that their habitats are lost. This is especially critical for *C. skottsbergii* var. *skottsbergii* as this single population has recently been threatened by continued development. Optimally, additional sites should also be sought to establish safety net populations. Genetic monitoring of *ex situ* collections should be maintained as there would be no associated seedbank present as a reserve of genetic diversity. (Although the soils from the Kaleaoa population were ultimately removed for lead decontamination following this study, we did identify the need for preservation of this variety and plants are being grown *ex situ* or have been transplanted to similar habitats close by. Many plants were lost in the process of soil removal, but soils in areas with the highest densities of the plants were not disturbed.) Collections for var. *audens* and var. *vaccinioides* should also be maintained *ex situ*. However, multiple populations of these varieties are extant, and their protection should be a higher priority than the establishment of new populations.
Acknowledgements

We thank James Kwon for bringing this project to our attention and for assisting with collection of material, Winona Char for our discussions on Chamaesyce systematics, Alison Sherwood for assistance with analyses, and Joanne Birch, Susan Mazer, Tim Motley, Alison Sherwood, and an anonymous reviewer for helpful comments on the manuscript. Research was supported by funding from the U.S. Fish & Wildlife Service and USGS Cooperative Parks Studies Unit, University of Hawaii.

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