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GENETIC DIVERSITY IN *SWALLENIA ALEXANDRAE* (POACEAE, CHLORIDOIDEAE), A NARROW ENDEMIC FROM THE EUREKA DUNES (INYO COUNTY, CALIFORNIA)

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**ABSTRACT**

*Swallenia alexandrae*, Eureka Valley dune grass (Poaceae, Chloridoideae), is a narrow endemic known only from three localities in the Eureka Valley and adjoining Saline Range in the northern portion of Death Valley National Park (Inyo County, California). This monotypic genus is listed as endangered by the US Fish and Wildlife Service and as rare by the California Department of Fish and Game. Genetic diversity within and among populations of *S. alexandrae* was studied with randomly amplified polymorphic DNA. A single polymorphic band was detected in approximately one quarter of all individuals sampled. This result indicates very low genetic diversity in *S. alexandrae*. There is no evidence of private alleles and very little partitioning of genetic diversity between populations. These results are discussed in relation to conservation concerns for *S. alexandrae*.

Key words: Chloridoideae, conservation, endangered species, genetic diversity, narrow endemic, psammophyte, randomly amplified polymorphic DNA (RAPDs), Salsola spp.

**INTRODUCTION**

*Swallenia alexandrae* (Swallen) Soderstr. & H.F.Decker is endemic to three dunes in the Eureka Valley and adjacent Saline Range in the northern portion of Death Valley National Park (Inyo County, California) (Fig. 1). *Swallenia* is a monotypic genus whose plants produce robust, erect to decumbent culms with markedly pungent leaves. The plants are relatively long-lived perennials that form hummocks in deep sands from the bases of the dunes to close to the highest points (Fig. 2; Henry 1979; Pavlik and Barbour 1988). *Swallenia alexandrae* is a true psammophyte, a plant restricted to growth on sand (Seddon 1974).

The three dunes—the main Eureka Dune, Saline Spur Dune, and Marble Dune—are disjunct and occur within an area of ca. 200 km², although only a fraction of that area represents appropriate habitat for *S. alexandrae* (Cipra 2011). By far the largest population is found on the main Eureka Dune that covers 13 km² and rises ca. 200 m above the valley floor, making it the tallest dune in California (Henry 1979; Pavlik and Barbour 1988; Pavlik 1989). Pavlik and Barbour (1988) and Pavlik (1989) have speculated that the Eureka Dunes are old relative to other regional dunes and were formed during the early Holocene or late Pleistocene.

The first collection of *S. alexandrae* was made by Annie Alexander and Louise Kellogg in 1949 (Henry 1979). Their specimen was sent to Jason Swallen at the US National Herbarium where the grass was identified as a new genus and named *Ectosperma alexandrae* (1950). Soderstrom and Decker (1963) recognized that the genus name *Ectosperma* had been used previously for an alga and renamed the grass *Swallenia* in honor of Dr. Swallen. Taxonomists have been uncertain as to the affinities of this monotypic genus. Swallen (1950) placed *Ectosperma* in Festucae; however, in their renaming, Soderstrom and Decker (1963) noted that Stebbins and Crampton (1961) placed it in Aeluropoideae (Monanthochloinae). In *Genera Graminum*, Clayton and Renvoize (1986) included *Swallenia* in Monanthochloinae (along with Old World genus, *Aeluropus* Trin., and five New World genera, *Allolepis* Soderstr. & H.F.Decker, *Distichlis* Raf., *Jouvea* Fourn., *Monanthochloë* Engelm., and *Reederochloa* Soderstr. & H.F.Decker), as a subtribe of Eragrostideae (Chloridoideae). Recent work has placed *Monanthochloë* and *Reederochloa* in synonymy with *Distichlis* and *Distichlis* now comprises Monanthochloinae. Monanthochloinae, *Aeluropus*, *Allolepis*, *Jouvea*, and *Swallenia* are now placed in tribe Cynodonteae (Bell 2007; Columbus et al. 2007; Peterson et al. 2010; GPWG II 2011).

Based upon its status as a narrow endemic and threats to the dune environment from off-road vehicles (ORVs), *S. alexandrae* was listed as endangered by the U.S. Fish and Wildlife Service (USFWS) (1978) and as rare by the California Department of Fish and Game (CDFG) (California Native Plant Society [CNPS] 2012). Two additional species are endemic to the Eureka Dunes (*Oenothera californica* (S. Watson) S.Watson subsp. *eurekensis* (Munz & J.C.Roos) W.M.Klein and *Astragalus lentiginosus* Douglas var. *micans* Barneby) (Baldwin et al. 2012). In 1994, the California Desert Protection Act transferred responsibility for the Eureka Dunes from the Bureau of Land Management (BLM) to Death Valley National Park (National Park Service [NPS]).

Monitoring of the dune environment and of *S. alexandrae* (Henry 1979; Bagley 1986; Pavlik and Barbour 1988; Margoles 2008; Slaton 2008; Cipra 2011) has revealed that population sizes of this grass can vary widely depending on seasonal rainfall. Seed predation by ants and small rodents is high for *S. alexandrae*, and seedling establishment is low; however, once plants become established, they are relatively long lived (Pavlik and Barbour 1988). Insect and lagomorph herbivory can be a threat to *S. alexandrae*; however, the leaves and culms are highly sclerenchymatous, and the dune habitat is not hospitable to large herbivores. Historically, the major threat to the survival of *S. alexandrae* (and the other dune endemics) has been destruction of seedlings and plants and habitat degradation by ORVs (DeDecker 1979). In addition to human
impacts, the USFWS 5-Year Review (Croft 2007) noted an invasion of the Eureka Dunes by *Salsola* spp. (Russian thistle, tumbleweed). National Park botanists Michèle Slaton (2008) and Jane Cipra (2011) have expressed concern over a significant constriction in populations of *S. alexandrae* since the 1970s and the concurrent increase in *Salsola* spp.

Conservation of rare species can be guided by knowledge of genetic diversity (Godt and Hamrick 2001). If a rare allele is only found in a particular population, efforts can be made to preserve that population. In clonal or inbred species, levels of genetic diversity can be used to estimate the effective population size. A number of techniques have been developed to evaluate genetic diversity; for example, allozymes (Hamrick and Godt 1989), randomly amplified polymorphic DNA (RAPDs; Williams et al. 1990), inter-simple sequence repeats (ISSRs; Zietkiewicz et al. 1994), amplified fragment length polymorphisms (AFLPs; Vos et al. 1995), and microsatellites (Slatkin 1995). Use of these techniques varies among studies based on the technical and financial resources of researchers and laboratories (reviewed in Nybom 2004). RAPDs have been used extensively in studies of plant genetic diversity because they are relatively inexpensive and do not require the development of specialized primers. The present study used RAPDs to estimate the genetic diversity within and among the known populations of *S. alexandrae*.

**MATERIALS AND METHODS**

**Sampling Strategy and Experimental Design**

The three known populations of *S. alexandrae* vary greatly in size, with the main Eureka Dune population the largest (13 km²), Marble Dune intermediate, and the Saline Spur...
Because of the possibility of the formation of subpopulations (Wahlund principle, Hartl and Clark 1997), collections were made from three regions of the main Eureka Dune (northwest, northeast, and southwest), from the north and south ends of the Marble Dune, and one from the Saline Spur Dune for a total of six collection sites (Fig. 1). (The south end of Marble Dune has sometimes been referred to as a distinct, fourth population but has been determined to be continuous with the north-end population (Cipra 2011)). At each of the six sites, 30 individuals were haphazardly sampled (not formally randomized). A minimum of 10 yards separated any two sampled plants. Leaf material was removed, placed in liquid nitrogen, and held frozen prior to extraction of DNA. A single voucher was made for each collection site; collection information is given in the appendix.

Sampling of 30 specimens from six collection sites produced a total of 180 individual specimens. For screening with RAPD primers, two specimens from each collection site were assigned to a cohort. A cohort consisted of 12 individual specimens arranged in the following order, ABCDEFABCDEF, where each letter represents a collection site. All cohorts were screened against all primers at least one time; four primers (B-08, AG-15, AJ-13, AQ-04) were screened twice with all cohorts.

**Processing of Specimens and PCR**

Extraction of DNA followed the hot-CTAB method of Doyle and Doyle (1987). Specimen DNA was diluted to about 5 ng/μl. For optimization of the polymerase chain reaction (PCR), I tested concentrations from 1.5 to 5 mM Mg²⁺ in the reaction buffer and annealing temperatures from 36 °C to 42 °C. Optimal conditions were judged to be 3.0 mM Mg²⁺ in the reaction buffer and an annealing temperature of 40 °C. All PCR reactions were performed on the same Stratagene Robocycler 96 (La Jolla, California) using the following protocol: an initial denaturation step (94 °C for 2 min), followed by 40 cycles of denaturing (94 °C for 1 min), annealing (40 °C for 1 min), and extension (72 °C for 1.5 min). A master mixture was prepared for each cohort plus a negative control. Each individual reaction tube contained 2 μL reaction buffer (3.0 mM Mg²⁺); 0.8 pmole primer; 600 mmoles dNTPs; 0.5 units Taq polymerase; ca. 20 ng of template DNA, and dH₂O to bring the volume to 20 μL.

The following Operon (Eurofins, Ebersberg, Germany) 10-mer primers or primer sets (20 primers/set) were tested against one or more cohorts: entire sets—A, B, F, G, S, AG, AJ, AQ. Primer sets were selected for a G-C content that matched that of Chloridoideae (J. T. Columbus, pers. comm.). Additional primers AD-13, R-16, U-09, and Z-17 were tested because they...
had produced informative bands in a study of a putative close relative, *Distichlis spicata* (Eppley et al. 1998).

**Separation of PCR Products and Data Analysis**

PCR products were separated on 2% agarose gels run at 80 V for 14–16 hrs with 0.5 M TBE buffer. Three lanes of 1 Kb DNA ladder were run on each gel to facilitate estimation of band sizes. Ethidium bromide was added to the gels to visualize banding patterns. Gels were recorded either with Polaroid (Waltham, MA) photographs or by image capture on a Stratagene Eagle Eye digital system (La Jolla, CA). Only clear, distinct, reproducible bands between 450 and 2000 base pairs were scored.

Informative bands produced by the four primers that were replicated with all samples were scored as presence/absence data and analyzed using GenAlEx 6.5 (Peakall and Smouse 2012).

**RESULTS AND DISCUSSION**

Of the 164 primers tested, four were assessed to have possible variation. One of these four, AG-15 (5'-TTTGCCCCGT-3'), produced a single polymorphic band (along with six monomorphic bands) (Fig. 3). The other three produced no additional polymorphic bands but did show monomorphic bands: B-08 (8 bands), AJ-13 (7 bands), and AQ-04 (10 bands). The polymorphic band was present in approximately half of the samples from each of three populations, two of the populations from the main Eureka Dune and the population on the Marble S population. RAPD markers are dominant, making it difficult to detect alleles that are present at very low frequencies (Tingey and del Tufo 1993). Generally, alleles that occur with frequencies of 0.1 or lower will be missed and scored as monomorphic unless sample sizes increase (Godt and Hamrick 2001). If the populations are assumed to be in Hardy-Weinberg equilibrium, with a sampling size of 30 individuals per population, homozygous recessives that are present at a frequency of 3% or higher should be detected (J. L. Hamrick, pers. comm.).

Band data from these four primers was used to assess the percentage of polymorphism, heterozygosity (Table 1), and Nei’s distance (Table 2) using GenAlEx 6.5. Mean heterozygosity (0.006) and percentage of polymorphism (1.56%) are very low in *S. alexandrae*. Since additional polymorphic bands were not detected, this leads to the conclusion that genetic diversity is very low. No rare alleles were detected and only minor substructuring of populations of *S. alexandrae*. Low values of genetic diversity are indications that the species may have experienced a genetic bottleneck (Barrett and Kohn 1991) and that the effective population size is much smaller than a raw census of the populations would indicate (Ellstrand and Elam 1993; Newman and Pilson 1997; Cipra 2011). An alternative explanation for the observed low genetic diversity would be a founder effect: the current populations of *S. alexandrae* were established by a very few, closely related individuals (Waller et al. 1987; Barrett and Kohn 1991). Small effective population size makes *S. alexandrae* vulnerable to inbreeding and genetic drift, which could reduce the already low genetic diversity (Ellstrand and Elam 1993).

Taxonomy of *S. alexandrae* is consistent with the existence of a genetic bottleneck or founder effect. Recent phylogenetic estimates based upon molecular data (Bell 2007; Columbus et al. 2007; Peterson et al. 2010; GPWG II 2011) support inclusion of *Swallenia* in a group consisting of *Blepharidachne* Hack., *Dasyochloa* Willd. ex Rydb., *Erioneuron* Nash, *Munroa* Torr., and *Scleropogon* Phil. All these genera are small and are
joined by long branches (Bell 2007) suggesting that they may be relicts of previously larger groups (Kruckeberg and Rabinowitz 1985). Figure 4 summarizes the results of GPWG II (2011). The phylogenetic position of *S. alexandrae* is also supported by chromosome counts (*2n* = 20, base chromosome numbers in Chloridoideae are 9 and 10 [Anderson 1964; Roodt and Spies 2003]), and *C₄* (Kranz) leaf anatomy (Gómez-Sánchez et al. 2001).

**Conservation Concerns**

In 1978, *S. alexandrae* was listed as endangered under the Endangered Species Act (1973) because of concerns over habitat destruction by ORVs. At that time the Eureka Dunes were managed by BLM that closed the dunes to ORVs in 1976; however, BLM lacked resources for enforcement (DeDecker 1979). With the assistance of Mary DeDecker, USFWS published a recovery plan for the Eureka Dunes that emphasized removal of ORVs from the dunes (1982). The California Desert Protection Act of 1994 changed the status of Death Valley from National Monument to National Park and increased the size of the park to include the Eureka Valley. In 2007, USFWS recommended delisting of *S. alexandrae* based upon successful removal of ORVs from the Eureka Dunes (Croft 2007); however, as yet no action has been taken.

Henry (1979) monitored seedlings and mature plants of *S. alexandrae* in several transects at the north end of the main Eureka Dune from 1974 to 1978. Pavlik and Barbour (1988) followed up on the Henry study with an investigation of mature plant survivorship, seedling establishment, seed bank dynamics, and predation. In addition to *S. alexandrae*, two other Eureka Dune endemics, *Oenothera californica* subsp. *eurekensis* and *Astragalus lentiginosus var. micans*, were monitored by Pavlik and Barbour (1988). Pavlik and Barbour (1988) observed that *Swallenia* seeds were taken by ants, kangaroo rats, and deer mice; herbivory of seedlings and mature plants was the result of blacktailed jackrabbits.
Seedling density was positively correlated with summer rainfall and, especially, with unusually wet conditions that historically occur up to 90 years apart (Pavlik and Barbour 1988). Using data from five nearby weather stations, Pavlik (1979) extrapolated average annual rainfall in the Eureka Valley to be 11.5 cm. As observed at the other nearby weather stations, rainfall (and occasional snowfall) in the Eureka Valley is highly variable, and it is not unusual to have years with no measurable precipitation. Besides the volume, timing of rainfall is extremely critical, with July rainfall having the greatest impact on *S. alexandrae* establishment (Pavlik and Barbour 1988).

NPS botanists Michèle Slaton (2008) and Jane Cipra (2011) have summarized previous monitoring efforts and have established formal, georeferenced grids at the main Eureka Dune, Marble Dune, and Saline Spur Dune in order to survey the entire population of *S. alexandrae* as well as other dune endemics. These surveys, along with a comparison of photos taken in 1985 and repeated in 2011, show a dramatic decrease in *S. alexandrae* coverage at the main Eureka Dune and Marble Dune (Cipra 2011: appendices). From 1976 to 2008, Slaton (2008) calculated that while *S. alexandrae* decreased by 97% at the north end of the main Eureka Dune, plant litter, *Achmatherrum hymenoides* Roem. & Schult. (Indian rice grass), and *Salsola* spp. increased significantly. The increases in plant litter, Indian rice grass, and *Salsola* spp. indicate degradation of the dune habitat and a movement of desert scrub vegetation upslope onto the dune.

In addition, in 2007 no seedlings of *S. alexandrae* were observed on the main Eureka Dune and Marble Dune; at the Saline Spur Dune in 2008, 3% of observed plants were seedlings (Slaton 2008). Observations by Henry (1979) and Pavlik and Barbour (1988) suggest that germination and establishment of *S. alexandrae* occurs irregularly in response to heavy rainfall in July and August.

*Salsola* spp. arrived in California around 1890 and has rapidly spread throughout the state, especially in disturbed habitats below 2500 m (Hrusa and Gaskin 2008). Several aspects of *Salsola* spp. reproductive biology give it the potential to compete for resources in a dune environment: a single plant can produce up to 200,000 seeds, germination takes place within minutes following rainfall, and germination can be close to 100% (Noell 1994; Stallings et al. 1995); in addition, constant movement of the dunes provides the disturbance required for *Salsola* spp. establishment (Barrows et al. 2009). Hummocks of *S. alexandrae* can accumulate fragments of *Salsola* spp. and provide a “nurse plant” environment for the rapid growth of *Salsola* seedlings (Fig. 5) (Fowler 1986; pers. obs.). Several species of *Salsola* are found in California, and hybridization is known to occur, making identification to species difficult (Hrusa and Gaskin 2008).

The first appearance of *Salsola* spp. in the Eureka Valley is not known, but their presence in the valley was noted by Pavlik (1979) and by Mary DeDecker in 1983 (M. Slaton, pers. comm.). However, *Salsola* spp. are not mentioned in early monitoring reports by Henry (1979) and Pavlik and Barbour (1988), suggesting that it was not widespread at the time of those studies. It is possible that ORVs were a vector for introduction of *Salsola* spp. to the dunes (Barrows et al. 2009). The Bristlecone Chapter of CNPS attempted hand weeding in order to remove *Salsola* spp. from the main Eureka Dune (Noell 1994). The USFWS 5-year review (Croft 2007) for *S. alexandrae* noted the invasion of *Salsola* spp. on and around the Eureka Dunes. However, since a competitive relationship between *Salsola* spp. and *S. alexandrae* has not been demonstrated, USFWS does not consider *Salsola* spp. to be a substantiated threat.

A biological control agent for *Salsola* spp., *Aceria salsolae* DeLillo & Sobhian, a blister mite, is being investigated by USDA and other agencies (Sobhian et al. 1999; Smith 2005; Smith et al. 2008). The mite acts by killing the shoot meristem and greatly reducing flower production. The safety and host specificity of *A. salsolae* has been tested under quarantine conditions in the US and in field tests conducted in Italy. *Aceria salsolae* has demonstrated efficacy in significantly reducing vegetative growth and reproduction of *Salsola* spp. as well as high specificity for the genus. Permission to release *A. salsolae* in Death Valley NP has been applied for with USDA Animal and Plant Health Inspection Service—Plant Protection and Quarantine (APHIS-PPQ) but, to date, permission has been denied.

*Swallenia alexandrae* has very low genetic diversity (present study), narrow and highly specific habitat requirements within a very small total range (Henry 1979; Slaton 2008; Cipra 2011), irregular recruitment dependent on summer rainfall (Pavlik and Barbour 1988), and a demonstrated reduction of populations over the past 30 years (Slaton 2008; Cipra 2011). The timing of population constriction for *S. alexandrae* coincides with a dramatic increase in *Salsola* spp., an increase in plant litter, and movement of native scrub vegetation onto the Eureka Dunes. Although a competitive relationship between *S. alexandrae* and *Salsola* spp. has not been demonstrated formally, Pavlik (quoted in Noell 1994) noted that both require water for active growth during the summer season. *Swallenia alexandrae* is adapted to the variable moisture regime of the Eureka Dunes; however, it is not adapted to the presence of *Salsola* spp. which, as fast-growing annuals, require water during the same season as *S. alexandrae* (Pavlik quoted in Noell 1994; Barrows et al. 2009).

Conservation of the Eureka Dune environment has been successful in terms of limiting ORV incursions (Pavlik 2003). However, given evidence of a rapid increase in *Salsola* spp. and a concurrent reduction in *S. alexandrae* populations since the 1980s, additional monitoring and study of interaction between these taxa is necessary. The NPS has collected baseline data on the populations of Eureka Dune endemics, and this can provide valuable comparisons for future studies (Slaton 2008; Cipra 2011). It will be especially valuable to monitor the establishment and frequency of establishment of *S. alexandrae*. Because *S. alexandrae* exhibits a combination of very low genetic diversity and infrequent establishment, *ex situ* conservation of seeds may be appropriate (Kruckeberg and Rabinowitz 1985; Waller et al. 1987; Moran et al. 1989; Newman and Pilson 1997). Care should be taken to collect seeds from all populations in order to conserve existing ecotypes (Matroq and Villablancana 2001; Hufford and Mazer 2003), and any revegetation efforts must include appropriate placement of seeds and seedlings (Ellstrand and Elam 1993). It may be possible to place seedlings in parts of the dunes not yet colonized by *Salsola* spp.

*Swallenia alexandrae* is a narrow endemic with very low genetic diversity; because of uncertainty about the impact of...
Fig. 5. A *Swallenia alexandrae* hummock colonized by *Salsola* spp. on the Marble Dune. Photo with permission from the National Park Service.
Salsola spp. on populations of S. alexandrae and observed constrictions of its populations, it is not prudent to delist or downlist this species at this time.

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