FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-82-R

Endangered and Threatened Species Conservation

A preliminary assessment of the genetic status of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), an imperiled species of the Balcones canyonlands

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FINAL REPORT

STATE: Texas **GRANT NUMBER:** TX E-82-R

GRANT TITLE: A preliminary assessment of the genetic status of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), an imperiled species of the Balcones canyonlands

REPORTING PERIOD: 1 Aug 06 to 30 Nov 08

OBJECTIVE(S):

To provide baseline information on the genetic status of existing populations of Bracted Twistflower, *Streptanthus bracteatus,* in Texas.

Significant Deviation:

Please see Attachment A.

Summary Of Progress:

Please see Attachment A.

Location: Bexar, Medina, Travis, and Uvalde counties in Texas.

Cost: Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.

Prepared by: Craig Farquhar **Date:** 2 Dec 2008

Approved by:  **Date:** **2 Dec 08**

C. Craig Farquhar

ATTACHMENT A

FINAL REPORT

A preliminary assessment of the genetic status of the bracted twistflower, *Streptanthus bracteatus* (Brassicaceae), an imperiled species of the Balcones canyonlands

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Project period:

September 1, 2006 through November 30, 2008

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SUMMARY

The Bracted Twistflower, *Streptanthus bracteatus* Gray (Brassicaceae) is a rare annual plant that is endemic to regions of central Texas that are undergoing rapid population growth. There are only a handful of remaining populations (±15), with only eight of these on public land. Several of the populations on public land are threatened or have undergone severe and well-documented population declines. In an effort to avert listing of this species under the ESA, a consortium of government agencies, educational institutions, NGOs, and volunteers has been organized to support, manage and augment existing populations, and to establish new populations in suitable protected habitat. As a component of this effort, we used DNA-based microsatellite markers to ascertain the population-genetic status of potential sources of seed for the establishment of persistent populations.

Key findings of the project:

1) Microsatellite markers originally developed for *Caulanthus amplexicaulis* var. *barbarae*, (a rare endemic plant of southern California) are well suited for population genetic studies of *Streptanthus bracteatus.* This set of microsatellite primers yields robust, specific PCR amplification in *S. bracteatus* populations. Further, these markers are polymorphic in *S. bracteatus,* and will thus be informative in population-genetic studies.

2) Levels of microsatellite polymorphism in *S. bracteatus* are consistent with a breeding system that includes both selfing and outcrossing.

3) Several of the small *S. bracteatus* populations are highly inbred and show low levels of genetic diversity. This information is being used to direct seed collection and banking efforts.

4) Several thriving populations with high densities of plants may represent progeny from only one or a few individuals, and may thus poorly represent the genetic diversity of the population as a whole (i.e. 'jackpot effects'). This information is being used to direct seed collection and banking efforts.

5) Several geographically separated populations (on private land) are genetically a single interbreeding population. This information is also being used to direct seed collection

and banking efforts.

NEED FOR THE STUDY

Streptanthus bracteatus Gray (Brassicaceae) is a rare annual wildflower endemic to the eastern and southern Edwards Plateau of central Texas. Nearly all populations are located nearby or within cities of Austin and San Antonio, which have experience explosive growth in the last two decades. The plant appears to have very strict habitat requirements and may be an edaphic endemic, localized to limestone or dolomite. The species is closely associated with exposures of the upper Glen Rose, Walnut, and lower Edwards geological formations on the upper (west, north) side of the Balcones Fault Zone. Stands of *S. bracteatus* occur on thin soils perched above massive low-porosity limestone or dolomite layers. The species has a G2S2 NatureServe ("imperiled") rank but is not listed under the US Endangered Species Act. The species is threatened by land development, deer herbivory, recreational activities (on public and private land), road construction and, it is hypothesized, by habitat change caused by increases in woody plant cover (Zippin 1997). In 2005, 15 populations were known in four Texas counties, although several of these populations had only a few plants when they were last visited. In 2005, six of these known populations were on public land (Bee Creek Preserve, Mount Bonnell City Park, and Barton Creek Greenbelt in Travis County; Garner State Park, Uvalde Co.; Eisenhower Park, Bexar Co. and the Highway 1283 right-of-way in Medina Co.).

However, public ownership does not assure survival, as the Mount Bonnell population appears to in severe decline due to a combination of development of adjacent private land, vegetation change, and heavy recreational use. The Highway 1283 right-of-way site has been severely degraded by recent construction (widening). The Eisenhower Park population (within the city of San Antonio) is extremely small and vulnerable. Finally, Garner State Park (1,419.8 acres) is the most popular State Park in Texas with more than 300,000 visitors per year (including 230,000 overnight campers). The population of *S. bracteatus* in Garner State Park appears to be in the midst of a disastrous decline due to extreme recreational overuse and the possible effects of climate change (this population is at the southwestern edge of the species range). Thus, the level of protection of several populations on public land is tenuous at best, and none of the populations on private land have any protection (it should be noted that we were unable to relocate several of these previously documents populations on private land during the 2006, 2007 and 2008 field seasons). Establishment of stable populations in the protected sites (including the

augmentation of existing populations that are in decline) appears to be the only viable approach to insure the long-term survival of this species and to avoid federal listing under the ESA.
A Memorandum of Agreement between the US Fish and Wildlife Service, Texas Parks and Wildlife Department, the City of Austin, Travis County, the Lower Colorado River Authority, and the Lady Bird Johnson Wildflower Center was signed in March 2004. This MOA formalizes the work of a group of volunteers who have searched for new populations and monitored existing populations in Travis County. Further, a reintroduction Plan is being drafted under the auspices of the Austin offices of the US Fish and Wildlife Service and Texas Parks and Wildlife Department, with the goal of establishing enough viable populations of *S. bracteatus* in secure sites to ensure the survival of this species.

Although seeds of the species have been successfully germinated and grown in greenhouses and gardens, at least ten efforts to establish persistent populations in apparently suitable sites have all failed. Clearly, more knowledge about the habitat requirements of this species is needed if new stable populations are to be established. Furthermore, critical baseline data on the genetic status and population divergence in this species is needed to guide decisions about seed sources used to expand existing populations and establish new ones. Some of the small, isolated populations of *S. bracteatus* that might serve as potential seed sources may already be suffering the combined effects of bottlenecks, genetic drift and inbreeding, resulting in loss of allelic diversity, reduced fitness (due to inbreeding depression) and reduced 'evolutionary potential' to respond to environmental change including urbanization, altered herbivory and pathogen pressures, changes in vegetation composition, invasive species, and climate change. In addition, there may be some degree of ecological-genetic adaptation of *S. bracteatus* populations to distinct local conditions such as soil chemistry, hydrology, topography and light conditions (i.e. 'ecotypic differentiation'). The augmentation of such a population with seeds from a genetically distinct population that is adapted to a different set of conditions would reduce the fitness of the target population (i.e. 'outcrossing depression') and diminish its potential for long-term viability.

We consider the work described in project to be the first component of an integrated and sustained research and adaptive management effort that will provide urgently needed information on the habitat requirements, ecological interactions, demographics and populations genetics of this species, in order to develop a set of effective science-based

protocols for the augmentation of existing populations, reintroduction of new populations, and long-term stewardship of the species that will avert listing of the species under the Endangered Species Act. In particular, this information will be used to identify an optimal genetic strategy for reintroduction that will maximize recovery potential and avoid long-term risk to the viability of the species. Undertaking species introductions without any *a priori* knowledge about the population-genetic status of existing populations entails significant risk to the long-term viability of the species. Here, we employed DNA-based microsatellite markers to gather information to identify optimal seed sources for introduction and population augmentation that minimize the risk of inbreeding depression, loss of evolutionary potential, and loss of fitness.

LOCATION OF THE STUDY

Streptanthus bracteatus is a highly localized endemic plant that is found on the upper side of the Balcones Fault Zone (Balcones Escarpment) at the margin of the Edwards Plateau near the cities of Austin and San Antonio (Figure 1). This zone is very rich in endemic, threatened and endangered

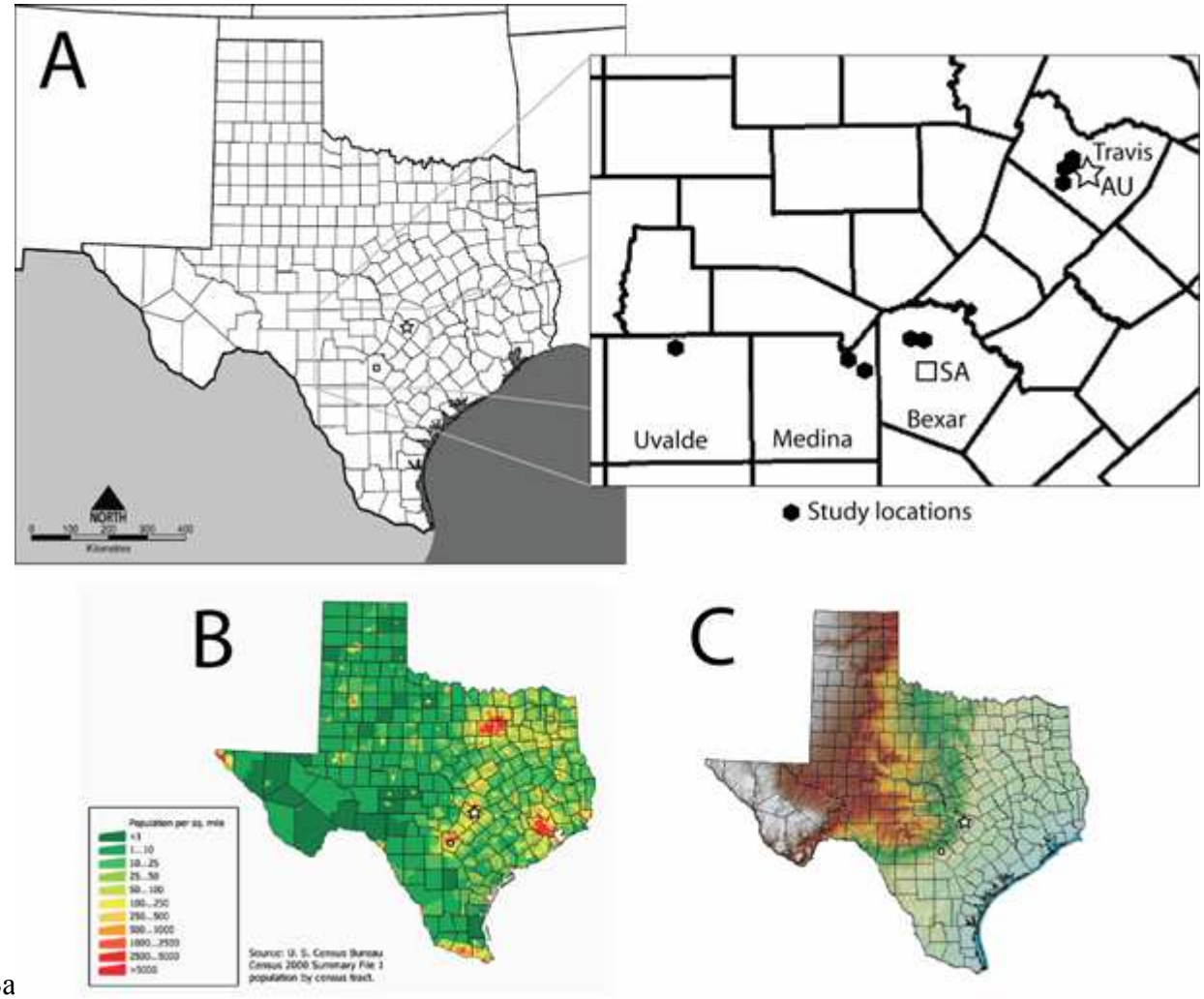

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Figure 1: Locations of S. bracteatus populations. City centers of Austin (star) and San Antonio (square) are indicated in A) counties of Texas, B) population density (2000 census), and C) physiographic map.

species from all taxonomic groups and is undergoing rapid urban development. Known populations of
S. bracteatus comprise several highly disjunct population clusters. One of these is in the rapidly growing west side of the city of Austin, Travis County, on a few remaining fragments of natural landscape. Another is on a set of bluffs and canyonlands overlooking Lake Medina in Medina, Co. Texas. A small population cluster is located on the rapidly growing northern margin of the city of San Antonio, Bexar County. Finally, a single disjunct population occurs on a bluff overlooking the Frio River in Garner State Park, Uvalde County.

OBJECTIVES

The overall objective of this project is to provide preliminary information on the genetic status of existing populations of *Streptanthus bracteatus* that is essential for the successful establishment of persistent viable populations in order to prevent its listing under the Endangered Species Act.

Specific objectives:

1) Extract genomic DNAs from individuals of all populations that are being considered as either a seed source or as a target for augmentation, including all populations on public land.

2) DNAs shall be extracted from dried leaf tissue samples and five to seven microsatellites shall be amplified then genotyped.

3) Use genotypic data from each population to assess deviation from Hardy-Weinberg expectations (an indicator of inbreeding, genetic drift, gene flow or natural selection), within-population genetic diversity, effective population sizes (N_e), fixation index (F), inbreeding coefficient (F_{IS}) and gene flow between populations (N_m).

4) Interpret and integrate genetic results, in the context of all available ecological and

demographic information on this species, in order to develop effective science-based strategies and protocols for seed collection and banking, and for population augmentation, reintroduction and long-term stewardship of this species.

METHODS

DNA sampling

Surveys to identify locations of plants were conducted periodically from December through June of 2006-2008, with the most intensive surveys occurring in April and May (period of maximum blooming). Very small tissue samples (< 0.5cm^2, < 5 mg fresh weight) were harvested from cauline leaves or (in a very few cases) young rosette leaves, then dried using silica gel dessicant. To avoid cross-contamination, a new pair of sterile forceps was used for each collection. DNAs were extracted from dried leaf tissue samples by the method described by Pepper and Norwood (2001). Extracted genomic DNAs were checked for quality and quantity by agarose gel electrophoresis (with ethidum bromide staining) and UV-visible spectroscopy (700nm-200nm).

Microsatellite marker genotyping

Isolation and characterization of microsatellite markers from *Caulanthus amplexicaulis* var. *barbarae* and their cross-species amplification in *S. bracteatus* have been described previously (Burrell and Pepper, 2006). From each genomic DNA sampled in the field, six to ten microsatellite loci were amplified using fluorescently-labeled primers (Table 3), then genotyped using the ABI3130 automated capillary DNA sequencer as described by Terry et al. (2006). To reduce costs, a combination of one HEX and one FAM labeled PCR reaction were multiplexed in each capillary.

Table 1 : Microsatellite markers selected for *S. bracteatus*

Marker	Label	Forward (labeled) primer	Reverse primer
Ca2	5′-HEX	CTCACTCATACTTAACGTTCAC	GCAGGAGACTTCATCTTCTTCAC
Ca7	5′-HEX	GTCTTTTCTAAACATACACAGATG	GCATAATTTAATTTAGAGTCTCATCC
Ca15	5′-HEX	CTGGACTAACCAAAGCTGCCAAG	GTCACAGTTGACAAATCCACTGTCC
Ca59	5′-HEX	GCCAATCCAATCCTTTCCTTCC	GTGTCCCCAGAAAAAGCGCG

Ca85	5′-HEX	CCTTAGACGGATCTTCTTTAGAG	CTCGATCCCCTTTTCTTTGCAG
Ca185	5′-FAM	CGCAAAGTGAGAGCCGATAGG	CGGACTACCGGAGATTTTTTGC
Ca227	5′-FAM	GAAGGTTATTCACAGGACTCTTTC	GTAGTGAAGCATCGAGGAAGAAG
Ca229	5′-FAM	CTCGAAATGCTGCAAGATGCG	GTTATAACCAATGCGCGATGCAC
Ca247	5′-FAM	ATTACCTGCCTACATTTTTCCATG	CATATGTGACTGACCAGTTCGAG
Ca249	5′-FAM	AAGCTCATCCAAGGAACGATTAC	CTCCTCGTCCTTTGATTCATCAG

Genetic data analyses

DNA fragment sizes were calculated using GeneScan ver. 3.1 software (Applied Biosystems Inc.) based on internal size standards (ROX 400HD). Scoring of microsatellites was performed using Genotyper ver. 2.5 software (Applied Biosystems Inc.). Genotypic data was exported to GeneAlEx ver. 6.1 (Peakall and Smouse 2006) then reformatted for export to other software applications. Popgene, a free-ware analysis package (Yeh et al. 1997), was used to calculate deviation from Hardy-Weinberg expectations (an indicator of inbreeding, genetic drift, gene flow or natural selection), within-population genetic diversity using Shanon's information index (1949), effective population sizes (N_e) and inbreeding coefficient (F_{IS}). The level of gene flow between populations (N_m) was determined by tracking rare 'private' alleles (Slatkin and Barton, 1989). Partitioning of genetic diversity among the whole species, populations, and individuals were examined using the Analysis of Molecular Variance (AMOVA) (Excoffier et al., 2005) and F_{ST} (Slatkin, 1995) methods.

RESULTS AND DISCUSSION

Population surveys and DNA sampling

During the duration of this project three new *S. bracteatus* populations were discovered; one of these was a direct result of the project. New populations were discovered on a limestone bluff overlooking the Colorado River on the grounds of the Ullrich Water Treatment Plant (City of Austin). After extensive surveys, three plants were discovered on the property of the Bright Leaf Natural Area in Austin (Austin Community Foundation). Both Bright Leaf (200 acres) and the Ullrich site are small 'islands' of natural habitat in the rapidly urbanizing west Austin region.

While collecting from a small population (13 plants in 2007) in Eisenhower Park (City of San Antonio), we noticed the geologic formation on which the plants were growing outcropped again nearby, inside the boundaries of Camp Bullis, a large (27,990 acre) U.S. Army base that is actively used for training. With assistance from Lucas Cooksey (Wildlife Biologist, U.S. Army) we surveyed the outcrop and discovered a new small population (8 plants) growing on the exact geology on which we expected them. These plants are on a small portion of the Camp Bullis that is already set aside and actively managed for conservation (the Golden-checked Warbler, Black-capped Vireo, archeological sites, karst features). Further surveys of possible habitat at Camp Bullis are planned, but because of manpower and security concerns (including unexploded ordinance) have not yet been conducted.

In the 2006-2007 field seasons, we collected tissue samples for a total of 318 individual plants in four Texas counties (Bexar, Medina, Travis, Uvalde), representing all known native occurrences of *S. bracteatus*. No new populations were discovered and no new sampling occurred in the 2008 field season. A minimum of thirty (30) individuals was sampled in all cases where 30 plants were available for sampling. In some populations, there were less than 30 plants present; in such cases, all plants were sampled (as indicated in Tables 2 and 3). In particular only two plants were observed in Garner State Park (Uvalde Co.) in 2007, and no plants were observed in 2008. This is down from population counts of several dozen plants earlier in the decade, and is of particular concern since 2007 was a very good year (e.g. rainfall) for plants in nearby Medina County. Sites of several previously observed plants at Garner (from GPS coordinates) showed severe human impacts in the form of informal 'social' trails and associated erosion. Further, we were only able to collect samples from 15 surviving plants in the TxDOT right-of-way on south side of highway 1283 near Lake Medina (Medina, County). This population and its habitat are seriously degraded by multiple alignments, construction activities (widening and bridge building), and off-road vehicle activity. As mentioned previously, only three plants were sampled at the Bright Leaf Natural Area (Austin).

We also sampled four populations on private land — with permission and cooperation from the landowners (Table 3). One of these is a complex mosaic of smaller stands of plants on and around County Road 2700 in Medina County. This area is a low-density development consisting of vacation and mobile homes on large, often semi-natural lots. Based on the underlying geology and pattern of existing plants, these clusters may

represent the remains of a larger and more contiguous population that has been fragmented by development.

Table 2: Populations on public land (or otherwise protected)

Population	Ownership/management	County (TX)	Number sampled
Barton Creek Greenbelt	City of Austin	Travis	67
Bright Leaf Preserve	Austin Comm. Foundation	Travis	3 (all plants)
Eisenhower Park	City of San Antonio	Bexar	13 (all plants)
Camp Bullis	U.S. Army	Bexar	8 (all plants)
FM1283	TxDOT	Medina	15 (all plants)
Garner State Park	TPWD	Uvalde	2 (all plants)
Mount Bonnell Park	City of Austin	Travis	47
Ullrich W.T.P.	City of Austin	Travis	34

Very small tissue samples were collected from individual plants. The damage done during tissue collection was much less than commonly (perhaps universally) observed from small insect herbivores (e.g flea beetle). New, sterile forceps were used in each collection in order to avoid DNA cross-contamination and prevent the spread of viruses, bacteria and fungi. Genomic DNA was extracted and quality-tested (for quantity, purity and amplification efficiency) from all 318 individual tissue samples. These are archived in duplicate in frozen state in four 96-well sample plates.

Table 3: Populations not protected

Population	Ownership/management	County (TX)	Number sampled
Cat Mountain	Private	Travis	42
CR274	Private	Medina	8 (all plants)
CR2700 (complex)	Private	Medina	56
Valburn Drive	Private	Travis	23 (all plants)

DNA marker development

Micrsatellite-containing DNA fragments were obtained from *Caulanthus amplexicaulis* var. *barbarae* genomic DNA by a biotinylated oligonucleotide capture method (Burrell and Pepper, 2006). *Caulanthus amplexicaulis* var. *barbarae*, is a rare endemic species of southern California and is a sister taxon to *Streptanthus bracteatus* (Johnston et al. 2005). A subset of 10 of these markers had been previously shown to support robust PCR amplification using *S. bracteatus* genomic DNA as template (Burrell and Pepper, 2006). From our total collection, a subset of 51 markers was selected for further evaluation

based on efficacy of amplification in *S. bracteatus*. From these we further selected 21 markers that were unlinked when mapped in the *Caulanthus amplexicaulis* genome. Because of genome colinearity (synteny) these markers are also likely to be unlinked (e.g. dispersed) in the *S. braceatus* genome. Non-linkage minimizes redundant data collection and maximizes the informative value of individual markers in population-genetic studies. Further, marker linkage interferes with analyses of population structure and diversity. A final set of ten (10) robust and polymorphic microsatellite markers were selected for analyses in the entire set of population samples (Table 1).

Preliminary population-genetic assessments

Rather than performing a 'shallow' but broad study using just a few markers across all populations, we instead concentrated on 'deep' genotyping using up to 10 markers on a subset of populations (see 'Significant Deviations'). A major rational for this change in strategy was the additional funding from contract Section 6 #186090 which will fund complete deep genotyping of all population samples. To date, deep genotyping has performed using up to ten primers (Ca2, Ca7, Ca59, Ca185, Ca227, Ca229, Ca81, Ca85, Ca247, Ca249) on the first plate of 96 samples (designated Plate I). This plate includes populations from Bexar County, Medina County, and from the Ullrich Water Treatment Plant in Austin, Travis County. The rational for deep genotyping this plate is the pressing need for genetic information to aid ongoing seed collection efforts in Bexar and Medina counties. In particular, efforts are underway to collect seed from the Eisenhower population for augmentation and possible establishment of new populations within Eisenhower Park or other public lands in the San Antonio area. Camp Bullis and the Medina County Populations (CR2700, CR274, and FM1283) are the most proximal locations to Eisenhower Park, and would thus be logical sources for outside seed introductions, if needed. We are working with Flo Oxley (Lady Bird Johnson Wildflower Center/U.T. Austin) and JayNe Neal, Park Naturalist, City of San Antonio, to help guide seed collection and banking for this species.

Our results (Table 4) showed that the small Eisenhower Park population deviates significantly from Hardy-Weinberg equilibrium, has a low genetic diversity (I=0.51), and is deeply inbred (F=0.88). Based on these findings we have recommended that seeds from this population not be relied upon for seed banking and reintroduction efforts. Further, the Eisenhower Population shows moderate to high population differentiation ($F_{ST} > 0.143$) from all populations except the nearby Camp Bullis population ($F_{ST} = 0.066$). Eisenhower Park was formerly part of Camp Bullis. Our genetic data, when

considered along with geological evidence, suggest that the Eisenhower and Camp Bullis populations may be remnants of a much larger ancestral population that has been fragmented by road construction, fence building and training activities at Camp Bullis (established in 1917). The Camp Bullis plants display greater genetic diversity (I=0.97) and are less inbred (F=0.35) than the Eisenhower plants. However, the number of plants observed on the Camp Bullis property is very small, the level of inbreeding is relatively high, and the level of genetic diversity is still low compared to other populations in our study (Table 4). Based on these combined results, we have made the following recommendations: 1) that additional surveys be conducted on the Camp Bullis property to locate additional plants and populations, 2) that the Bullis and Eisenhower populations be treated as a single population for conservation and recovery planning, and 3) seeds used to augment or establish populations in the north San Antonio area should be derived from a composite of seeds from the Bullis and Eisenhower populations that has been carefully selected to maximize genetic diversity. F_{STS} between the Bullis-Eisenhower population and the Medina County populations (CR2700, CR274, and FM1283) were moderate to high (Table 5). Based on this finding, we recommended that Medina County not be used at this time as a primary source of seed for population establishment and augmentation in the San Antonio area.

Table 4: Summaries of population-level genetic parameters

Population	HW Prob.	HW Sig.	*I*	H_0	H_e	*F*
Eisenhower	**0.0025**	**	**0.51**	0.038	0.303	**0.88**
Camp Bullis	0.341	ns	0.97	0.038	0.032	0.35
CR2700	0.450	ns	1.3	0.625	0.721	0.11
CR274	0.026	ns	1.28	0.813	0.713	0.00
FM1283	0.185	ns	1.11	0.533	0.644	0.13
Ullrich	**0.046**	*	1.35	0.500	0.735	0.29
All populations	0.206	ns	1.17	0.510	0.624	0.23

HW Prob.: Hardy-Weinberg chi-squared values

HW Sig.: Hardy-Weinberg significance levels, ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

I: Shanon's information index (1949)

H_0: Observed Heterozygosity

H_e: Expected heterozygosity

F: Fixation (inbreeding) index

Table 5: Pair-wise F_{ST} values

Population	Eisenhower	Bullis	CR2700	CR274	FM1283	Ullrich

Eisenhower	—	Low	Moderate	High	High	High
Camp Bullis	0.066	—	Moderate	Moderate	Moderate	Moderate
CR2700	0.143	0.123	—	Moderate	Moderate	Low
CR274	0.249	0.143	0.138	—	Low	Moderate
FM1283	0.244	0.127	0.143	0.046	—	Low
Ullrich	0.230	0.131	0.088	0.102	0.098	—

The CR2700 population set is a conglomerate of several smaller sub-populations scattered over a large area (not shown). These small sub-populations occur on the private properties of several landowners in the area. The genetic parameters for the population as a whole were close to that expected for Hardy-Weinberg equilibrium (HW probability value = 0.45), and genetic diversity was relatively high (I = 1.3) while inbreeding was low (F=0.11). However, several of the smaller sub-populations within this cluster showed much higher levels of inbreeding (F>0.44) and lower diversities (I<1.0). This was particularly true of small populations where plants were occurring in high densities in a small area. Surveys in which sites were revisited over several years revealed that at a particular GPS location there may be only one or two plants one year, and 20-30 the next. This demographic pattern, coupled with our genetic data, suggests that in some locations one or a few plants may give rise to a large number of progeny in favorable years (e.g. above average rainfall). This possible 'jackpot' effect is important since seed collection for this plant typically occurs only in years in areas where the plants are present at high density and in years in which there has been above-average rainfall. In these situations, extensive seed collection in these sub-populations would under-sample the genetic variability present in the population as a whole.

Currently a full genotypic dataset is available only for the first 96 plants in the collection, thus any extrapolation of our results to the entire species should be considered preliminary. However, some broad patterns of genetic diversity and differentiation across the entire species are discernable. Inbreeding coefficient within populations (F=0.23) is high compared to outcrossing species in the Brassicaceae such as *Physaria thamnophila* (Williams and Pepper, unpublished). These results suggest that *S. bracteatus* is both self-fertile and outcrossing. This finding is in agreement with earlier inferences that were made based on floral morphology and pollinator observations (Dieringer, 1991). Further, the allelic diversity in *S. bracteaus* (3-7 alleles per locus across the first 96 samples) appears to be relatively low compared to other species in the Brassicaceae (Williams, Burrell and Pepper, unpublished). Further, F_{ST} values between the Medina County

populations and the Ullrich population (Travis County) were much lower than expected given the geographic distance. These observations raise the hypothesis that *S. bracteatus* is a very young species, that in the recent past had a much more contiguous geographic distribution, and has undergone sudden precipitous decline. The acquisition of a complete dataset for the species (recently funded under Section 6 contract #186090) will allow the application of statistical tools that will test this hypothesis.

SPECIFIC RECOMMENDATIONS (PRELIMINARY)

1. Seeds for *ex-situ* seed banking (and eventual reintroduction or population augmentation) of *S. bracteatus* should be collected and archived as seed from individual plants, rather than bulk seed from populations as is currently the practice. This will allow better assessment of the genetic status of seed stocks and better control of the genetic composition of seeds used in recovery efforts.

2. Only a limited number of seeds should be collected from small, dense stands of plants. Although a dense stand of healthy plants may seem attractive and convenient source of seed for collectors (especially volunteers), such stands may be the progeny of a very small number of plants that have increased due to favorable local environments (such as run-off from landscape watering). Such collecting will not effectively capture the genetic diversity of the population as a whole.

3. Seeds from Eisenhower Park (San Antonio) should not be used exclusively in population augmentation and population establishment efforts in the area. Rather, a composite collection of seeds from Eisenhower and nearby Camp Bullis would constitute a first choice of biological materials for recovery efforts in the local area.

4. Additional surveys should be undertaken, when and where possible, on likely habitat within Camp Bullis (US. Army) to identify new populations and possible seed sources.

SIGNIFICANT DEVIATIONS

Task 1: *TAMU shall isolate small tissue samples (< 0.5cm2, < 5 gm fresh weight) from cauline leaves or young rosette leaves of > 30 individuals from each population that is being considered as either a seed source or as a target for augmentation. At a minimum, this shall include individuals from all five populations on public land. TAMU shall attempt to genotype 20 individuals from each population.*

Deviations in Task 1: We were unable to collect statistically adequate numbers of individuals from two important populations (in protected sites) that are being seriously considered for population augmentation. These are Garner State Park (TPWD) and Bright Natural Area (Austin Community Foundation). After extensive surveys in 2007, we found only two (2) in Garner S.P. and three (3) plants in Bright Leaf. None were found in either location in 2008. This represents a serious reduction in the sizes of both populations since the early 2000s. This is in part explained by inadequate or sporadic rainfall. The situation in Garner S.P. is exacerbated by serious erosion and disturbance by the human impacts of informal 'social' trails throughout most of the historic population areas. We hope to address these deficiencies by further surveys and collections in the Spring of 2009. This work will be funded by Section 6 contract #186090.

Further, we were only able to collect samples from 15 surviving plants in the TxDOT right-of-way on south side of highway 1283 near Lake Medina (Medina, County). This population and its habitat are seriously degraded by multiple alignments, construction activities, and off-road vehicle activity. The only remaining plants are quite literally 'hanging on' in crevices in the road-cut. As the seed from these (annual/biennial) plants will drop onto the roadway culvert, the long-term scenario for the survival of this population is unlikely without intervention. Further, with additional funding from Section 6 contract #186090, we have expanded the objectives of Task 1 to include the genotypic analysis of up to 40-60 individual plants in large 'core' populations of *S. bracteatus*, such as the Barton Creek Greenbelt, Mount Bonnell Park and County Road 2700 in Medina County.

Task 2: *DNAs shall be extracted from dried leaf tissue samples and five to seven microsatellites shall be amplified then genotyped.*

Deviations in Task 2: With additional funding from TPWD contract #186090, we are able to expand this objective to include analysis of eight to ten microsatellite loci, rather than five to seven. This will help compensate for the rather limited genetic diversity that we have seen species wide and thus make high-resolution analysis of gene flow and population structure possible.

Task 3: *Genotypic data from each population shall be used to calculate deviation from Hardy-Weinberg expectations (an indicator of inbreeding, genetic drift, gene flow or*

natural selection), within-population genetic diversity, effective population sizes (Ne) and inbreeding coefficient (FIS).

Deviations in Task 3: However, task is not yet complete because of the expanded number of samples to be analyzed (see deviations to Task 1, above) and number of microsatellite loci employed (see deviations to Task 2, above).

Task 4: *Gene flow between populations (*Nm*) shall be determined by tracking rare 'private' alleles. Partitioning of genetic diversity among the whole species, populations, and individuals shall be examined.*

Deviations in Task 4: While we have preliminary *Nm* estimates for several populations, this task is not yet complete because of the expanded numbers of microsatellite loci that had to be employed in order to identify private alleles among the populations in the study (due to generally low levels of allelic diversity).

Task 5: *The above results shall be integrated with - and interpreted in the context of - all available ecological and demographic information on this species, in order to develop effective science-based strategies and protocols for seed collection and banking, and for augmentation, reintroduction and stewardship of this species.*

Deviations in Task 4: The full implementation of this task is still very much in progress and cannot be adequately addressed in depth until a complete dataset and analyses are in hand. However, we are engaged in a vigorous collaboration with Dr. Norma Fowler and graduate student Liz Ramsey, to address the ecological conditions (such as the light requirement) for establishment/reintroduction of this species. We are providing a genetic assessment of the germplasm being used these ecological studies to ensure that any results obtained will be broadly applicable to the species as a whole. In addition, we are guiding the selection of germplasm for use in follow-up experiments.

We are also working with Norma Fowler, Liz Ramsey and geologist Nick Hauwert, to address the geological/edaphic requirements of this species. Together we have collected soil and bedrock samples from all populations in the Austin area. Further, during my tissue collection trips, I have collected from all populations in all four counties (Bexar, Travis, Medina, Uvalde) across the species range. These rock and soil samples are being analyzed by Nick Hauwert using various geochemical and geophysical methods.

Further, we are working with Flo Oxley (Lady Bird Johnson Wildflower Center/U.T. Austin) and other and JayNe Neal, Park Naturalist, City of San Antonio, and other parties to help guide seed collection, banking, and reintroduction for this species.

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