Section 6 (Texas Traditional) Report Review

Attachment to letter dated (mm/dd/yyyy): 03/22/2010

TPWD signature date on report: Y

Project Title: An Assessment Of The Genetic Status Of The Bracted Twistflower, Streptanthus Bracteatus (Brassicaceae), An Imperiled Species Of The Balcones Canyonlands.

Final or Interim Report? Final

Grant #: TX E-89-R

Reviewer Station: Austin ESFO

Lead station was contacted and concurs with the following comments:

☐ Yes ☐ No ☒ Not applicable (reviewer is from lead station)

Interim Report (check one):	**Final Report (check one):**
☐ Is acceptable as is (no comments)	☒ Is acceptable as is (no comments)
☐ Is acceptable as is, but comments below need to be addressed in the next report	☐ Is acceptable, but needs minor revision (see comments below)
☐ Needs revision (see comments below)	☐ Needs major revision (see comments below)

Comments:

These results will be extremely useful for conservation of Bracted Twistflower. The report should be distributed to owners and managers of the 14 remaining populations, and to researchers and volunteers who conduct annual surveys.

FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-89-R

Endangered and Threatened Species Conservation

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

Prepared by:

Alan Pepper

TEXAS
PARKS &
WILDLIFE

Carter Smith
Executive Director

Clayton Wolf
Division Director, Wildlife

22 March 2010

FINAL REPORT

STATE: Texas **GRANT NUMBER:** TX E-89-R

GRANT TITLE: Endangered and Threatened Species Conservation

REPORTING PERIOD: 6 Sep 07 to 28 Feb 10

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

OBJECTIVE(S):

To provide information on the genetic status of existing populations of *Streptanthus bracteatus* for the successful establishment of persistent populations as a preventive measure against listing under the Endangered Species Act.

Segment Objectives:

1. isolate tissue samples from leaves of > 30 individuals from each population that is being considered as either a seed source or as a target for augmentation,
2. use DNA-based microsatellite marker analysis to determine the population-genetic status of potential sources of seed for the establishment of persistent populations,
3. obtain obtain preliminarystatistically robust estimates of 1) the level of inbreeding in extant populations, and in potential seed sources for reintroduction and augmentation, and 2) the extent of historical gene flow between extant populations in order to assess the risk of introduction of maladaptive alleles into a population,
4. 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.

Summary Of Progress: See Attachment A

Significant Deviations: None.

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

Cost: available upon completion of grant.

Prepared by: Craig Farquhar **Date:** 22 Mar 2010

Approved by: **Date:** 22 Mar 2010
C. Craig Farquhar

FINAL REPORT

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

Section 6 Grant E-89

(Contract #186090)

Principal Investigator:
Alan E. Pepper
Department of Biology
Texas A&M University

Principal Investigator:

Alan E. Pepper
Associate Professor, Department of Biology
Texas A&M University
College Station, TX 77843
Phone: 979-731-1051
Fax: 979-862-4790
E-mail: apepper@bio.tamu.edu

Report date:

February 28, 2010

Key project participants:

Norma Fowler, Ph.D. Co-Principal Investigator, University of Texas, Austin
Dana Price, Ph.D. Co-Principal Investigator, TPWD (now with U.S. Army Corps of Engineers)
Chris Best, USFWS
Ryan Williams, Technician, Texas A&M University
Maria Dixon, undergraduate student, Texas A&M University
Ross Bee, volunteer

Other project contributors (partial list):

William Carr, The Nature Conservancy
Flo Oxley, Lady Bird Johnson Wildflower Center/U.T. Austin
Lucas Cooksey, Wildlife Biologist, U.S. Army
Nico Hauwert, geologist, University of Texas
Mary Ruth Holder, volunteer
JayNe Neal, Park Naturalist, City of San Antonio
Liz Ramsey, graduate student, University of Texas
Mark Sanders, Biologist, City of Austin
Tim Schumann, Private Lands Biologist, USFWS

CONTENTS:

SUMMARY

The Bracted Twistflower, *Streptanthus bracteatus* (Brassicaceae) is a rare annual plant that is endemic to regions of central Texas that are undergoing rapid population growth. Narrow and sporadic distribution. There are only a handful of remaining populations (±15), with only eight of these are 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:

- Based on pairwise *Fsts* and Nei's genetic distance, we found significant genetic differentiation *within* the species. Thus, care must be taken in the selection of materials for genetic translocation (for population augmentation or re-establishment efforts aimed at conservation of this species) in order to avoid loss of adaptive local genotypes.

- We identified major clusters of populations in the Medina County and in Austin area that comprise the core genetic diversity for the species. We consider the establishment of multiple, protected 'safe sites' in each of these areas to be critical to the long-term genetic viability of the species (by preventing inbreeding depression and maintaining evolutionary potential). Our genetic studies inform the ranking of the populations as potential long-term safe sites.

- Our studies showed that some, but not all, of the populations showed levels of inbreeding that are high for predominantly outcrossing plant (Dieringer, 1991). The inbreeding was most prevalent in smaller, more isolated populations, such as Eisenhower Park in San Antonio. In other populations, inbreeding was not detected, indicating that these populations are genetically healthy, and that pollinators are active and present in sufficient numbers to ensure high levels of outcrossing. This information is being used in the selection of sources for seed collection and banking efforts.

NEED FOR THE STUDY

Streptanthus bracteatus (Brassicaceae) is a rare annual wildflower endemic to the eastern and southern Edwards Plateau of central Texas, nearby to the cities of Austin and San Antonio, which have experience explosive growth in the last several decades. It appears to have very strict habitat requirements. All known populations occur within 1 km of the Balcones Fault Zone, and are perched above a thick impermeable layer of limestone or dolomite. By the definition of Krukeberg (1999) , is considered a geological or 'edaphic' endemic. It has a G2S2 NatureServe ("imperiled") rank, but is not listed under the US Endangered Species Act. The species is threatened by land development, excessive herbivory by deer (*Odocoileus virginianus*), recreational activities (on both public and private land), road construction, and 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, only 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 Farm-to-Market Road 1283 right-of-way in Medina Co.

Public ownership appears not to assure conservation, as the Mount Bonnell population is 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 (1,420 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 *Streptanthus* 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. None of the populations on private land have any legal protection. We were unable to relocate two of the previously known populations on private land in the 2006, 2007, 2008 and 2009 field seasons. Based on GPS coordinates, one of the populations has likely been destroyed due to construction on a private ranch in Medina County.

Establishment of stable, healthy populations in 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. 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. This project will provide that is information critical to meeting the goals of the Reintroduction Plan now being written. 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 assess the genetic status of individual populations, and to obtain information to be used 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

The field collections were performed at 14 sites in central Texas, comprising all known extant populations of *S. bracteatus* at the time of collections (TABLE 1). Also shown in TABLE 1 are likely threats to the long-term survival of each population (based on our observations made during the field collections). Collections and/or observations were made during the peak March-June field seasons from 2006 to 2009. In TABLE 1, "Small pop." indicates highly vulnerable populations in which we observed less than 20 individuals in good rainfall years, and as few as zero individuals in poor rainfall years.

TABLE 1: Study locations

Population designation	Location	Owner/manager	Construct.	Recreation	Herbivory	Small pop.
Garner State Park	Uvalde Co., TX	Texas Parks and Wildlife		●		●
County Road 274	Medina Co., TX	Private	●			●
Private Road 2632	Medina Co., TX	Private	●			
Farm-to-Market Rd. 1283	Medina Co., TX	TxDOT	●	●		●
County Road 2700	Medina Co., TX	Private	●		●	
Camp Bullis	Bexer Co., TX	U.S. Army	●			●
Eisenhower City Park	Bexer Co., TX	San Antonio Parks and Rec.			●	●
Barton Creek	Travis Co., TX	Austin Parks and Rec.		●	●	
Ulrich Water Treat. Plant	Travis Co., TX	City of Austin				
Mount Bonnell	Travis Co., TX	Austin Parks and Rec.	●	●	●	
Fall Trail	Travis Co., TX	Private	●		●	●
Bright Leaf Preserve	Travis Co., TX	Austin Comm. Foundation			●	●
Cat Mountain	Travis Co., TX	Private	●		●	
Bull Creek	Travis Co., TX	Private	●	●	●	

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 (*Ne*) and inbreeding coefficient (*Fis*) and gene flow between populations *(Nm*).

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 augmentation, reintroduction and stewardship of this species.

METHODS

DNA sampling

Surveys to identify locations of plants were conducted periodically from December through June of 2006-2009, 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 eletrophoresis (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, eight microsatellite loci were amplified using HEX or FAM fluorescently-labeled primers (TABLE 2), 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 2: Microsatellite markers

Marker	Label	Forward (labeled) primer	Reverse primer	Repeat
Ca59	5′-HEX	GTCTTTTCTAAACATACACAGATG	GCATAATTTAATTTAGAGTCTCATCC	$(CT)_n$ dinuc.
Ca85	5′-HEX	CTGGACTAACCAAAGCTGCCAAG	GTCACAGTTGACAAATCCACTGTCC	$(CT)_n$ dinuc.
Ca87	5′-HEX	GCCAATCCAATCCTTTCCTTCC	GTGTCCCCAGAAAAAGCGCG	$(GAA)_n$ trinuc.
Ca139	5′-HEX	CCTTAGACGGATCTTCTTTAGAG	CTCGATCCCCTTTTCTTTGCAG	$(GA)_n$ dinuc.
Ca185	5′-FAM	CGCAAAGTGAGAGCCGATAGG	CGGACTACCGGAGATTTTTTGC	$(GA)_n$ dinuc.
Ca227	5′-FAM	GAAGGTTATTCACAGGACTCTTTC	GTAGTGAAGCATCGAGGAAGAAG	$(GAA)_n$ trinuc.
Ca229	5′-FAM	CTCGAAATGCTGCAAGATGCG	GTTATAACCAATGCGCGATGCAC	$(GAA)_n$ trinuc.
Ca241	5′-FAM	ATTACCTGCCTACATTTTTCCATG	CATATGTGACTGACCAGTTCGAG	$(GA)_n$ dinuc.

Genetic data analysis

DNA fragment sizes were calculated using GeneScan ver. 31 software (Applied Biosystems Inc.) based on internal size standards (ROX 400HD). Scoring of microsatellite loci 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 (*Ne*) and inbreeding coefficient (*F*). The level of gene flow between populations (*Nm*) was be determined by tracking rare 'private' alleles (Slatkin and Barton, 1989). Partitioning of genetic diversity among the whole species, populations, and individuals was examined using the Analysis of Molecular Variance (AMOVA) (Excoffier et al., 2005) and *Fst* (Slatkin, 1995) methods.

RESULTS AND DISCUSSION

Population surveys and DNA sampling

We sampled a total of 318 individual plants from 14 geographically-designated populations in Uvalde, Medina, Bexer and Travis Co., TX (APPENDIX 1). In the course of this project we discovered a new population on public land on the Camp Bullis Military Training Reservation, near San Antonio. With assistance from Lucas Cooksey (Wildlife Biologist, U.S. Army) we surveyed the outcrop and discovered another small population (8 plants). Although this population was severely damaged by recent security fencing and road construction, they are located on a small portion of the base 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 addition, through landowner contacts and the assistance of Chris Best (USFWS) we gained access to samples from another new population on private land near lake Medina (near Private Road 2632).

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 available plants were sampled (APPENDIX 1). In particular, only four plants were observed in Garner State Park (Uvalde Co.). This is down from population counts of several dozen plants earlier in the decade. 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.

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 were seriously degraded by multiple alignments, construction activities (widening and bridge building), and off-road vehicle activity. We observed several plants eroding out into the road cut. No plants were observed at this site in 2008 or 2009. Finally, only three plants were sampled at the Bright Leaf Natural Area (Austin). No plants were observed at Bright Leaf in 2008 or 2009.

We also sampled six populations on private land — with permission and cooperation from the landowners (TABLE 1). One of these sites (CR2700) 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 small clusters may represent the remains of a larger and more contiguous population that has been fragmented by development.

DNA marker genotyping

All 318 individual samples yielded DNA suitable for polymerase chain reaction (PCR) experiments. These samples were genotyped using eight polymorphic microsatellite markers developed from the closely related taxon *Caulanthus amplexicaulis* (Burrell and Pepper 2006) for a total of 2,544 genotypic determinations. These markers are designated 'Ca' for the source taxon, *Caulanthus amplexicaulis*. In *S. bracteatus*, we obtained robust amplification, and detected an average of 10.2 alleles per locus. with a low of six alleles (Ca59) and a high of 19 alleles (Ca139). All markers appeared to represent single co-dominant loci with no detectable null alleles.

Genetic differentiation of sub-populations

Our 14 geographically designated populations showed substantial genetic differentiation, as indicated by both Wright's *Fst* (APPENDIX 2) and Nei's genetic distance (FIGURE 1). Overall genetic differentiation across all loci and all populations was high ($Fst = 0.238 \pm 0.027$). In pairwise tests (APPENDIX 1), 31 of 91 (34%) of populations showed high pairwise differentiation ($Fst > 0.15$), while 51 of 91 (56% showed moderate differentiation ($Fst > 0.05$), and only 5 of 91 (5.5%) population pairs showed low levels differentiation by the criteria of Wright (1978). These results indicate that much of the overall genetic diversity of *S. bracteatus* is partitioned *between* populations, and that most populations are genetically distinct and not undergoing appreciable inter-population gene flow (overall $Nm = 0.874 \pm 0.130$).

Principle coordinates analysis (PCA), based on a matrix of Nei's genetic distances, partitioned 39% of the total genetic variation into the first principal coordinate, and 22% into the second principal coordinate (FIGURE 1). Based on this analysis, populations fell into a tight cluster for the Medina Co. populations, and a loose cluster for the Austin populations. Several of the smaller and more geographically isolated populations (including Garner S.P., Camp Bullis, and Eisenhower Park) are scattered about the genetic 'periphery,' possibly as a result of genetic drift, founder effects, isolation, and/or lineage sorting of alleles.

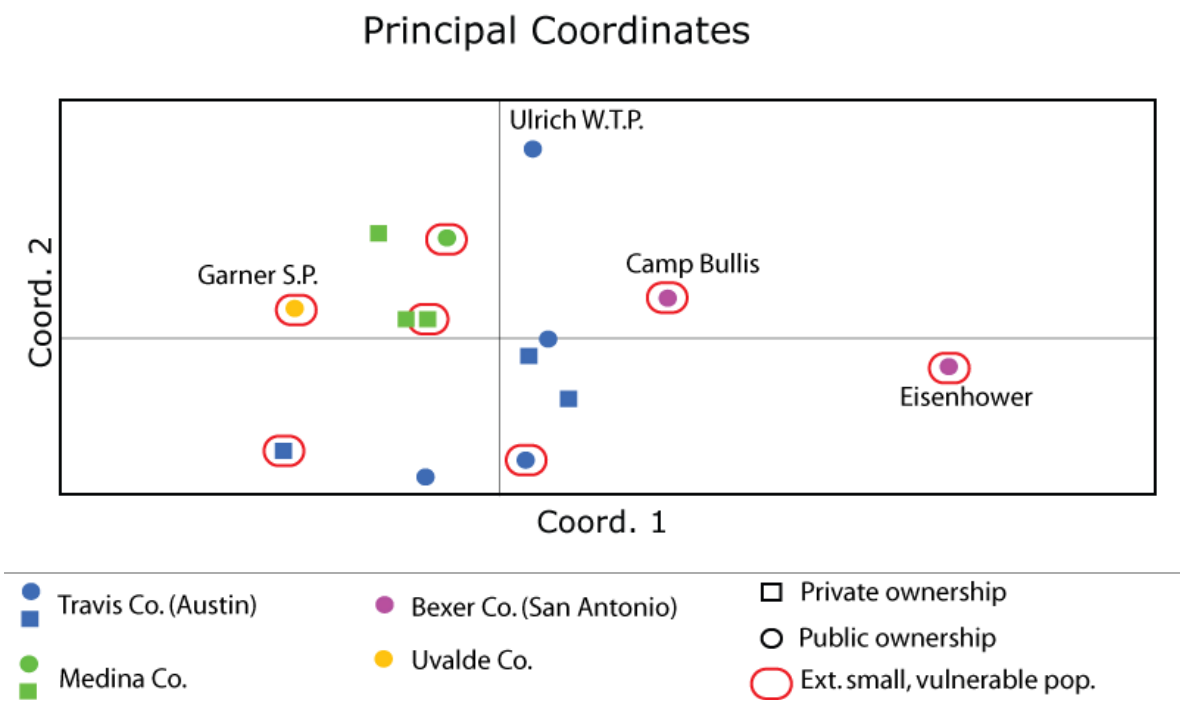

FIGURE 1: Principle Coordinates Analysis (PCA) Based on Nei's Genetic Distances.

Genetic diversity within populations

Within-population genetic diversity was estimated by several methods, including the number of alleles present in each population (*Na*) and Shanon's Information Index (*I*), which is a measure of allelic richness (FIGURE 2 and FIGURE 3). In both of these analyses, Barton Creek (public ownership) and Cat Mountain (private ownership) rank as core reservoirs of genetic diversity — both within the Austin cluster of populations and for the species as a whole. Similarly, the

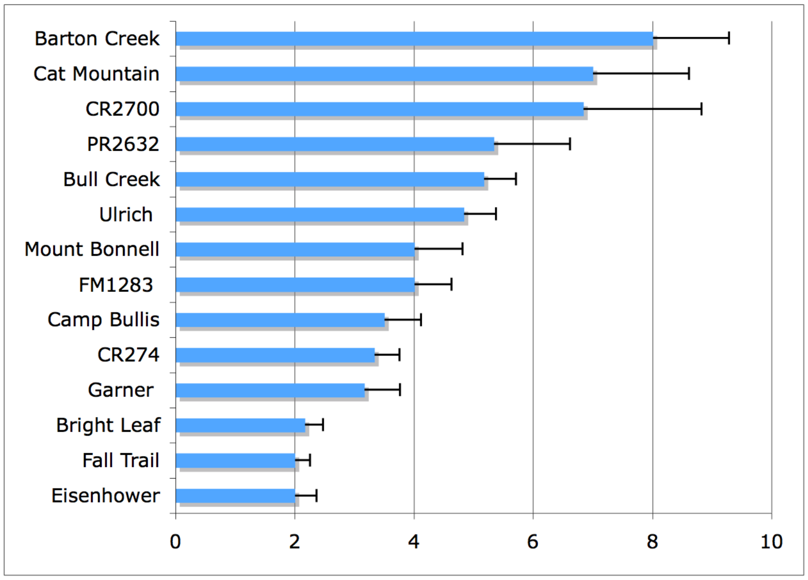

FIGURE 2: Genetic diversity within populations based on number of alleles (*Na*).

CR2700 and PR2632 are important centers of genetic diversity, both for the Medina Co. cluster of populations and for the species as a whole. The CR2700 and PR2632 populations also provide the most suitable genetic match for possible augmentation or reintroduction of the Garner population. Unfortunately, the CR2700 and PR2632 sites are distributed among a number of private owners.

Small populations including Garner State Park, Bright Leaf Preserve, Camp Bullis, Fall Trail (Private), and Eisenhower Park showed exceedingly low levels of genetic diversity. Unexpectedly, one of the larger populations, located on Mount Bonnell ($N = 25$) had relatively low genetic diversity ($Na = 4.0 \pm 0.82$, $I = 0.932 \pm 0.24$), possibly the result of genetic bottlenecks due to population decline and fragmentation resulting from construction and heavy recreational use in this very popular city park (discussed below).

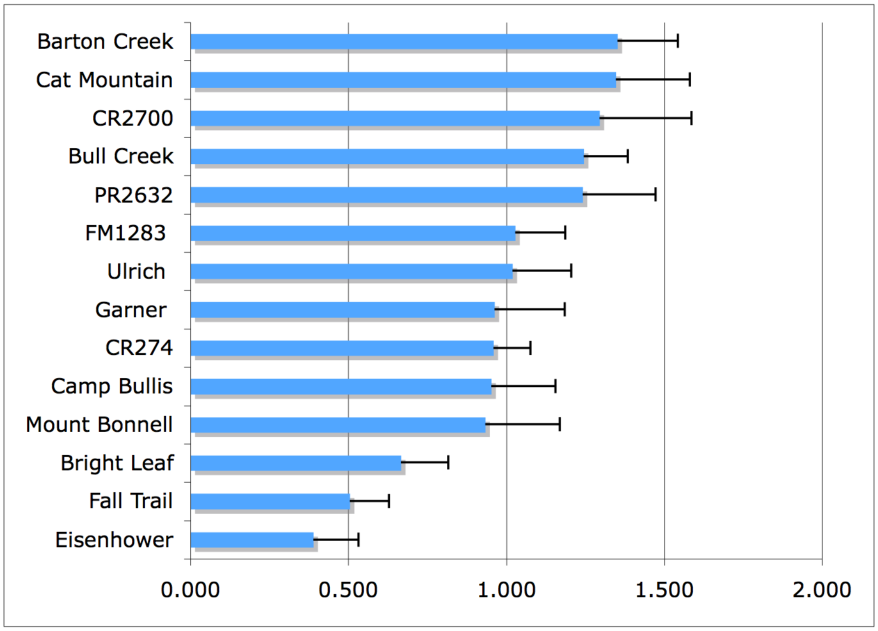

FIGURE 3: Genetic diversity within populations based on Shanon's Information Index (*I*).

Inbreeding and deviation from Hardy-Weinberg Equilibrium

Microsatellite genotypic data from the 14 sites was used to estimate the degree of inbreeding in each population. The inbreeding coefficients (F) for each population are shown in FIGURE 4. The value of F ranges from 0 (no inbreeding) to 1 (completely inbreed or 'clonal' population). The Garner and Bright Leaf collections were not included in Table 4 because of their extremely small sample sizes ($N < 5$ individuals). The small population in Eisenhower City Park (San Antonio) showed a very high level of inbreeding ($F = 0.375$) for a plant with significant outcrossing, as has been observed in *S. bracteatus* by Dieringer (1997).

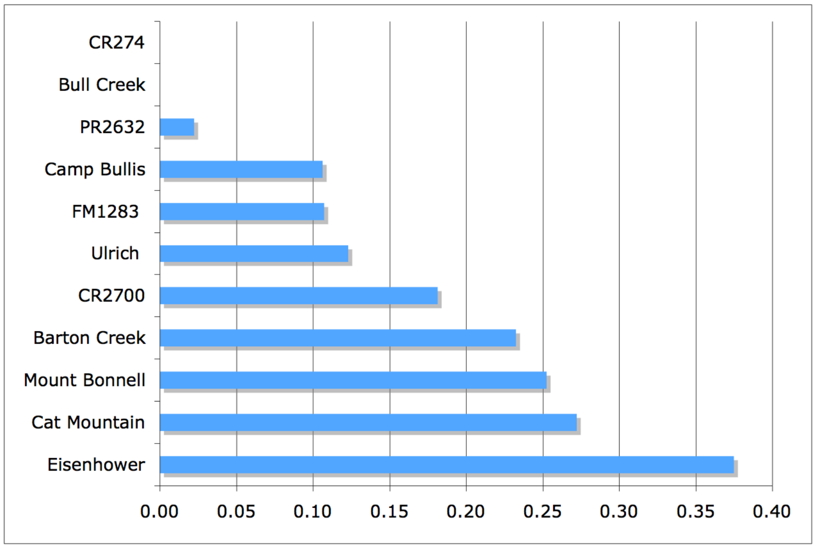

FIGURE 4: Inbreeding coefficient (*F*) in selected populations.

Unexpectedly, we observed relatively high levels of inbreeding in the (relatively) large Cat Mountain, Mount Bonnell, and Barton Creek populations. This finding was particularly unexpected for Cat Mountain and Barton Creek, as these populations showed high levels of genetic (allelic) diversity (FIGURE 2 and FIGURE 3). Based on cursory visual examination of the data, we hypothesize that these elevated levels of *F* observed in these genetically diverse populations are a manifestation of excess homozygosity resulting from population subdivision (e.i. genetic differentiation) *within* each of these larger populations. Deviations from Hardy-Weinberg equilibrium support this hypothesis, as the Cat Mountain, Barton Creek and Mount Bonnell (along with the Eisenhower population) all showed significant deviation at multiple loci (p ranged from < 0.05 to < 0.001 for individual population/locus combinations). This hypothesis is being tested by Analysis of Molecular Variance (AMOVA)(Excoffier et al., 2005), which requires examination of the GPS coordinates of individual plant collections within each of these populations.

SPECIES-WIDE CONCLUSIONS AND SPECIFIC CONSERVATION RECOMMENDATIONS

Streptanthus bracteatus is a morphologically and evolutionarily distinct species within the Brassicaceae family. Its closest extant relative is the Broadpod jewelflower *Streptanthus platycarpus*, a west Texas endemic plant (A.M. Burrell and A. Pepper, manuscript in preparation). Based on pairwise *Fsts* (APPENDIX 2) and Nei's genetic distance (FIGURE 1) we found significant genetic differentiation within the species. Thus, care must be taken in the selection of materials for genetic translocation for population augmentation or re-establishment efforts aimed at conservation of this species in order to avoid loss of adaptive local genotypes. Further, care must be taken to preserve the overall genetic diversity of the species in order to avoid eventual inbreeding depression, and maximize its potential to respond to environmental change. We have identified major, distinct clusters of genetic diversity in Medina County and in the Austin area. We consider the establishment of multiple, protected 'safe sites' in each of these areas to be critical to the long-term genetic viability of the species. Our genetic studies inform the ranking of existing populations as potential long-term safe sites (discussed below).

Further our studies showed that some, but not all of the populations are showing levels of inbreeding that are high for a predominantly outcrossing plant (Dieriger 1991). Currently, inbreeding is most prevalent in some of the smaller, more isolated populations, such as Eisenhower Park in San Antonio.

CONCLUSIONS AND SPECIFIC CONSERVATION RECOMMENDATIONS FOR POPULATIONS ON PUBLIC LAND

Garner State Park

Over three years of surveys (2007-2009) we only found four plants in Garner State Park. The winter of 2009-2010 has seen improved rainfall over 2009 (which was classified as an 'exceptional' drought in south central Texas). Our surveys will continue this year (2010) on an unfunded voluntary basis. The short-term survival of the Garner population is a serious concern. Our genetic sampling has been far too limited to provide any solid conclusions about the genetic status of this population, but our limited data seems hopeful, as there was a fair amount genetic (allelic) diversity present in just the four plants that were sampled (FIGURE 2 and FIGURE 3).

We recommend continued and intensive surveys for the presence of plant at Garner State Park, and the immediate implementation of appropriate measures to mitigate damage from informal 'social' trails and associated erosion. Should population augmentation or reintroduction be considered, our data indicated that seed sources from Medina County (CR2700 and PR2632) would provide the best genetic as well as geographic match.

Eisenhower City Park and Camp Bullis Military Training Reservation

Because of their close geographic proximity (< 100 m), these populations will be discussed together. Both represent highly distinct populations based on both genetic distance (FIGURE 1) and pairwise *Fst* values (APPENDIX 2). Although both populations are very small in size, Eisenhower has a much lower allelic diversity than Camp Bullis (FIGURE 2 and FIGURE 3), as well as a much higher level of inbreeding (FIGURE 4). From these data, we surmise that the Eisenhower population is a descendent 'offshoot' of the Camp Bullis population that arose by a drastic founder effect, perhaps only involving one or a few seed propagules. Alternatively, the Eisenhower and Camp Bullis populations resulted from fragmentation of a larger original population by construction activities at the boundary of the preserve (FIGURE 5).

FIGURE 4: Google Earth image (January 2008) showing the locations of the Eisenhower and Camp Bullis populations, along with recent security construction (fence, roadway, cleared area for visibility) along the Camp Bullis boundary.

Based on our genetic findings, we strongly recommend that the Eisenhower and Camp Bullis populations be managed cooperately (by the City of San Antonio and the Department of Defense) as a single entity. Together, these populations represent a distinct evolutionarily significant unit (ESU) and an important component of the evolutionary legacy of this species. Further, we strongly recommend that the Department of Defense initiate further surveys (with the cooperation of the City of San Antonio and other interested parties) on appropriate sections of Camp Bullis for additional *Streptanthus* plants and populations.

Ulrich Water Treatment Plant

The Ulrich population is located on a limestone bluff overlooking Lake Austin on city-owned property. Because of fencing and other security for the nearby water treatment plant (WTP4) this is, without doubt, the best protected site for the entire species. This is fortunate, because Ulrich is a genetically distinct population within the Austin area (FIGURE 1, APPENDIX 2) and may thus represent a distinctive remnant of the original genetic diversity of the species. For planning purposes, this location should be considered as an essential ‘safe site’ for the species, and should be managed as an independent, distinct population. In particular, genetic translocation of propagules into this population should be avoided under any ordinary circumstances.

Barton Creek Preserve

The Barton Creek preserve contains the largest known population of *S. bracteatus*, both in terms of geographic area and numbers of individuals. Our data indicates that it is a core reservoir for the genetic diversity of species. For these reasons, it should be considered as a critical ‘safe site’ for the species. However, our data also suggest that the genetic diversity of the Barton Creek population may be subdivided into several distinct sub-populations (based on inbreeing coefficient F and deviation from Hardy-Weinberg equilibrium). Further, we observed that a subset of these sub-populations were being seriously impacted by recreational use (informal trails) and by excessive herbivory. Thus, the genetic diversity of the population is threatened by impacts on small sub-populations. We strongly recommend that vigorous management actions are taken within the Barton Creek preserve to minimize these impacts (these might include additional deer exclosures, public education, and appropriate fencing and trail closures).

Mount Bonnell Park

The very small Mount Bonnell park (5.4 acres) provides vistas of the city of Austin, Lake Austin and surrounding areas, and has been a popular tourist attraction since the mid-19th century. The park is still very popular and hosts large numbers of visitors in good weather. The impacts of large numbers of visitors in Streptanthus and its habitat are clearly visible. Most of the remaining plants are found either along cliff edges or under deep vegetation cover (where they receive low levels of light and grow less vigorously than plants in the open). Our genetic data indicate that Mount Bonnell has unexpectedly high levels of inbreeding ($F = 0.252$) with only moderate allelic diversity (FIGURE 2 and FIGURE 3). Considering these genetic observations, and the small size of the site, we surmise that the observed excess in homozygotes in the Mount Bonnell population is due to local habitat and population fragmentation. Given its historical and current land use, under no reasonable or foreseeable circumstances could Mount Bonnell be considered a 'safe' or 'protected' site for *S. bracteatus*.

Bright Leaf Preserve

Bright Leaf Preserve is a large (216 acre), nearly pristine natural area located near the center of the Austin cluster of *Streptanthus* populations. The geology of much of the area is an identical match to the known habitat requirements of *S. bracteatus*. The preserve was acquired by philanthropist Georgia Lucas through 34 separate real estate transactions, then donated to Texas Parks and Wildlife. Her will stipulated the land be preserved in a wild state. In 2006, the preserve was turned over to the Austin Community Foundation, and is being managed under the conditions of Georgia Lucas' will. The preserve is an obvious location for a core 'safe site' for *S. bracteatus*. However, only a small cluster of plants persists on the property (< 4 individuals in good rainfall years). Much like Garner State Park, we recommend continued and intensive surveys for the presence of plant. Should population augmentation or reintroduction be considered for Bright Leaf, our data indicated that seed sources from nearby Mount Bonnell and Cat Mountain would provide the best genetic match.

CONCLUSIONS AND SPECIFIC CONSERVATION RECOMMENDATIONS FOR POPULATIONS NOT ON PUBLIC LAND

Given that there are only a handful of 'protected,' or 'safe sites' on public land, and that some of these are small highly vulnerable populations, it is essential for the long and medium-term survival of this species to promote its protection on private lands. In particular, the Cat Mountain and Bull Creek sites in the Austin area encompass a significant portion of the genetic diversity of the species (TABLE 2 and TABLE 3). Cat Mountain is large enough that it may contain several genetic sub-populations, while the

Bull Creek site (adjacent to, but not included in Bull Creek city park) is comprised of a single healthy population with low inbreeding ($F = 0.0$) at Hardy-Weinberg equilibrium for all loci. With appropriate protection, each of these could serve as a critical 'safe site' for the species. Therefore, steps should be taken to engage the current landowners in the conservation process, perhaps through the establishment of conservation easements.

Several populations on private lands in Medina Co. (e.g. CR2700 and PR2632) also constitute a critical reservoir for the genetic diversity of the species. In addition, it is also possible, perhaps even likely, that additional, undiscovered viable populations remain on private lands in the Medina County area. This area near Lake Medina has been the focus of extensive subdivision and construction of vacation and retirement homes. Many people vacation in, or retire to this area because of its natural environment. Based on our personal conversations land owners during the field collection phase of the project, we found that many are already actively involved in the conservation of this species, and most appreciated the value of this showy spring wildflower as part of the natural heritage of the Texas hill country. Several landowners expressed an interest in learning more about conservation easements or other options for participating in the conservation of this, and other imperiled plant and animal species in the region. We recommend continued contacts and cooperative interactions with private landowners in the area in order to ensure survival of this vital component of the genetic legacy of the species. These contacts and interactions may also lead to opportunities for additional surveys on private land, and for seed collection (for conservation seed banking) on sites that are being prepared for roadway and homesite construction.

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 or 2009. 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 previously-mapped population areas. We hope to address these deficiencies by further collections in the Spring of 2010. These additional surveys will be performed on an unfunded, voluntary basis.

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 plants will drop onto the roadway culvert, the long-term scenario for the survival of this population is unlikely without intervention.

LITERATURE CITED

Burrell AM and Pepper AE (2006) Primers for ten polymorphic microsatellites from *Caulanthus amplexicaulis* var. *barbarae*, and cross-amplification in other species within the Streptanthoid Complex (Brassicaceae). *Molecular Ecology Notes*, 6: 770-772.

Dieringer G (1991) Pollination ecology of *Streptanthus bracteatus* (Brassicaceae): a rare central Texas endemic. *Southwestern Naturalist* 36: 341-343.

Excoffier L, Laval G and Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

Lambert DM and Millar CD (1995) DNA science and conservation. *Pacific Conservation Biology* 2: 21-38.

Lulkart G and England PR (1999) Statistical analysis of microsatellite data. *Trends in Ecology and Evolution* 14: 253-255.

Peakall R and Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Pepper AE and Norwood LE (2001) Evolution of Caulanthus amplexicaulus var barbarae (Brassicaceae), a rare serpentine endemic plant: a molecular phylogenetic perspective. *American Journal of Botany* 88: 1479-1489.

Remy PJ, El-Mousadik A and Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* 12: 844-855.

Shannon and Weaver (1949) The mathematical theory of Communication. University of Illinois Press, Urbana, IL, USA

Slatkin M and Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. *Evolution* 43: 1349-1368.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.

Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. *Biological Conservation* 87: 173-180.

Terry M, Pepper AE and Manhart JR (2006) Development and characterization of microsatellite loci in endangered *Astrophytum asterias* (Cactaceae) *Molecular Ecology Notes* 6: 865-866.

Wright S (1978) *Evolution and the Genetics of Populations. Volume 4. Variability within and among Natural Populations*. University of Chicago Press, Chicago

Yeh FC, Yang RC, Boyle TBJ, Ye ZH, and Mao JX (1997) POPGENE, the User-friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta.

Zippin DZ (1997) Herbivory and the population biology of a rare annual plant, the bracted twistflower (*Streptanthus bracteatus*). Ph.D. dissertation, University of Texas at Austin. Advisor: Norma Fowler.

APPENDIX 1: Number of individual plants sampled at each population site.

Population designation	Location	Owner/manager	Indiv. sampled
Garner State Park	Uvalde Co., TX	Texas Parks and Wildlife	**4**
County Road 274	Medina Co., TX	Private	**10**
Private Road 2632	Medina Co., TX	Private	**14**
Farm-to-Market Rd. 1283	Medina Co., TX	TxDOT	**15**
County Road 2700	Medina Co., TX	Private	**39**
Camp Bullis	Bexer Co., TX	U.S. Army	**8**
Eisenhower City Park	Bexer Co., TX	San Antonio Parks and Rec.	**13**
Barton Creek	Travis Co., TX	Austin Parks and Rec.	**94**
Ulrich Water Treat. Plant	Travis Co., TX	City of Austin	**23**
Mount Bonnell	Travis Co., TX	Austin Parks and Rec.	**26**
Fall Trail	Travis Co., TX	Private	**7**
Bright Leaf Preserve	Travis Co., TX	Austin Comm. Foundation	**3**
Cat Mountain	Travis Co., TX	Private	**40**
Bull Creek	Travis Co., TX	Private	**22**

APPENDIX 2: Pair-wise genetic differentiation of populations (*Fst*)

Pop. 1	Pop. 2	*Fst*	Differentiation
Eisenhower	Fall Trail	0.460	**High**
Eisenhower	Garner	0.416	**High**
Eisenhower	Bright Leaf	0.368	**High**
Fall Trail	Bright Leaf	0.337	**High**
Eisenhower	CR2700	0.330	**High**
Eisenhower	Ulrich	0.309	**High**
Eisenhower	FM1283	0.296	**High**
Eisenhower	CR274	0.294	**High**
Eisenhower	PR2632	0.294	**High**
Eisenhower	Mount Bonnell	0.272	**High**
Ulrich	Bright Leaf	0.257	**High**
Eisenhower	Bull Creek	0.247	**High**
Camp Bullis	Fall Trail	0.246	**High**
Camp Bullis	Bright Leaf	0.245	**High**
Bright Leaf	Garner	0.242	**High**
Eisenhower	Barton Creek	0.234	**High**
Camp Bullis	Garner	0.227	**High**
Ulrich	Fall Trail	0.225	**High**
Eisenhower	Cat Mountain	0.210	**High**
Mount Bonnell	Bright Leaf	0.208	**High**
FM1283	Bright Leaf	0.205	**High**
Ulrich	Garner	0.190	**High**
CR274	Bright Leaf	0.190	**High**
CR2700	Bright Leaf	0.190	**High**
Eisenhower	Camp Bullis	0.176	**High**
Fall Trail	Cat Mountain	0.176	**High**
CR274	Fall Trail	0.172	**High**
Mount Bonnell	Garner	0.165	**High**
Ulrich	Mount Bonnell	0.163	**High**
Cat Mountain	Garner	0.158	**High**
Barton Creek	Bright Leaf	0.156	**High**
Bull Creek	Fall Trail	0.155	**High**
CR274	Ulrich	0.153	**High**
Fall Trail	Garner	0.152	**High**
Bull Creek	Garner	0.150	**High**
Barton Creek	Garner	0.148	**Moderate**
Barton Creek	Fall Trail	0.143	**Moderate**
Bull Creek	Bright Leaf	0.141	**Moderate**
Camp Bullis	CR274	0.141	**Moderate**
FM1283	Garner	0.136	**Moderate**
Camp Bullis	Mount Bonnell	0.132	**Moderate**
FM1283	Fall Trail	0.130	**Moderate**
Bright Leaf	PR2632	0.130	**Moderate**
CR274	Mount Bonnell	0.129	**Moderate**
Camp Bullis	CR2700	0.128	**Moderate**

CR274	Garner	0.123	**Moderate**
Camp Bullis	PR2632	0.121	**Moderate**
Camp Bullis	Ulrich	0.120	**Moderate**
Mount Bonnell	Fall Trail	0.119	**Moderate**
Camp Bullis	Bull Creek	0.118	**Moderate**
Fall Trail	PR2632	0.116	**Moderate**
Camp Bullis	FM1283	0.112	**Moderate**
Camp Bullis	Cat Mountain	0.110	**Moderate**
Ulrich	Cat Mountain	0.109	**Moderate**
CR2700	Fall Trail	0.108	**Moderate**
PR2632	Garner	0.106	**Moderate**
CR2700	Mount Bonnell	0.102	**Moderate**
Mount Bonnell	Cat Mountain	0.101	**Moderate**
FM1283	Ulrich	0.100	**Moderate**
CR2700	Cat Mountain	0.097	**Moderate**
Bright Leaf	Cat Mountain	0.096	**Moderate**
Ulrich	Bull Creek	0.094	**Moderate**
CR274	Bull Creek	0.093	**Moderate**
CR274	Barton Creek	0.092	**Moderate**
Ulrich	Barton Creek	0.091	**Moderate**
Mount Bonnell	PR2632	0.091	**Moderate**
Ulrich	PR2632	0.089	**Moderate**
CR2700	Garner	0.087	**Moderate**
FM1283	Mount Bonnell	0.087	**Moderate**
Camp Bullis	Barton Creek	0.086	**Moderate**
Barton Creek	Mount Bonnell	0.084	**Moderate**
CR2700	Ulrich	0.083	**Moderate**
CR274	Cat Mountain	0.082	**Moderate**
FM1283	Cat Mountain	0.082	**Moderate**
Bull Creek	Mount Bonnell	0.078	**Moderate**
CR2700	Barton Creek	0.077	**Moderate**
FM1283	Bull Creek	0.076	**Moderate**
CR2700	Bull Creek	0.074	**Moderate**
FM1283	Barton Creek	0.070	**Moderate**
CR2700	CR274	0.065	**Moderate**
CR274	FM1283	0.064	**Moderate**
Barton Creek	PR2632	0.060	**Moderate**
Cat Mountain	PR2632	0.060	**Moderate**
Bull Creek	Barton Creek	0.057	**Moderate**
CR274	PR2632	0.057	**Moderate**
Bull Creek	Cat Mountain	0.054	**Moderate**
Barton Creek	Cat Mountain	0.049	**Low**
FM1283	PR2632	0.047	**Low**
Bull Creek	PR2632	0.045	**Low**
CR2700	FM1283	0.032	**Low**
CR2700	PR2632	0.031	**Low**