Population Structure and Genetic Variability of Sunset Crater Beardtongue (*Penstemon clutei*)

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Summary

Sunset Crater beardtongue (*Penstemon clutei*) is a narrowly endemic species with a known range of approximately 350 km^2, located northeast of Flagstaff, Arizona. This species has been assigned a global/state ranking of G2/S2 (imperiled) and is on the U.S. Forest Service sensitive species list for Region 3 (Southwestern Region). Primarily restricted to tephra deposits from the Sunset Crater eruption at an elevation of approximately 2135 m (7000 ft), a disjunct population of the species is also present on older cinder cones about 20 km to the northwest of Sunset Crater.

Population dynamics of *P. clutei* are largely unknown. In particular, processes and mechanisms related to dispersal, colonization, and population persistence are poorly understood. This study was designed to examine population structure and establish long-term monitoring plots, which may provide insights concerning population dynamics and relationships to disturbances such as fire, herbivory, and OHV activity.

We installed twenty-eight long-term monitoring plots in total. Twenty-two were established in late summer, 2009, in the Coconino National Forest and six were established in early fall, 2010, in Sunset Crater Volcano National Monument. Each plot was 10m x 10 m in size, with the corners permanently monumented. At each plot, general observational information was collected. We also mapped the entire plot, noting the locations of each *P. clutei* individual and all trees and shrubs, as well as coarse woody debris. For each *P. clutei* individual we assigned a size class, measured the total height, length, and width, counted the numbers of flowering and browsed stems, and recorded comments about insect or other damage to the plant.

The population structure results showed an overall trend of a fairly high relative abundance of mature plants compared to seedlings and juveniles. The range of population densities for total *P. clutei* across the forest plots was 6-213 plants per 10 m^2 plot (average of 56.1 plants per plot or 5.61 plants/m^2). Plots in the monument ranged from 7-13 plants per 10 m^2 plot (average of 10 plants per plot or 1 plant/m^2). The range of live plants was 6-187 plants per 10 m^2 plot (average of 45.59 live plants/plot) in the forest and 7-11 live plants per 10 m^2 plot in the monument (average of 8.33 live plants/plot).

A non-metric multidimensional scaling (NMS) analysis indicates that *P. clutei* grows across a wide range of diverse and variable habitats. The five Coconino NF plots from pinyon-juniper habitat clustered to the right of the graph, indicating a distinct plant community. The vectors indicate a relationship between dead trees and downed woody material and the plant community growing in the pinyon-juniper habitat. However, these relationships were not found to be significant in this study and warrant further exploration.

A correlational analysis revealed no significant relationships between the number of trees (either living or dead) and the number of *P. clutei* plants on the study plots. Nor was there a significant relationship between tree canopy cover, shrub canopy cover, grass cover or downed woody material and the number of *P. clutei*.

Preliminary results from a microsatellite study with an *a priori* determination of two distinct populations (separated by U.S. 89), suggest that there is one single population of *P. clutei* and that the two suspected populations are not significantly different or distinct from each other. These preliminary results suggest that gene flow is occurring between the

two subpopulations on either side of the highway. However, it must be emphasized that these results are based on a small number of individuals (n=30) and are *very* preliminary.

P. clutei subpopulations are often widely dispersed across the landscape and the Indian Flat and Monument subpopulations are currently most protected from OHV activity and are therefore likely most important for long-term conservation efforts. In addition to continued occasional monitoring (perhaps every 3-5 years to minimize trampling) of the long-term plots in the Coconino NF and the Monument, priority research needs include: 1) additional molecular studies that examine variability, potential for hybridization, and possible parental species to determine if *P. clutei* is a good species or a subspecies, 2) demographic studies that follow individual plants through time, and 3) the impact of two types of disturbance: those that affect tree competition and those that impact the soil, particularly OHV activity.

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Introduction

Sunset Crater beardtongue (*Penstemon clutei*) is a narrowly endemic species with a known range of approximately 350 km^2, located northeast of Flagstaff, Arizona (Appendix A). This species has been assigned a global/state ranking of G2/S2 (imperiled) and is on the U.S. Forest Service sensitive species list for Region 3 (Southwestern Region) (Arizona Game and Fish Department 2003; Natureserve 2010). Primarily restricted to tephra deposits from the Sunset Crater eruption (estimated dates of eruption vary from approximately 1040-1100 AD) at an elevation of approximately 2135 m (7000 ft), a disjunct population of the species is also present on older cinder cones about 20 km to the northwest of Sunset Crater (Appendix A). The soils on which *P. clutei* grows are susceptible to extreme environmental fluctuations.

Figure 1. *P. clutei* surrounding a snag in the Cinder Hills OHV Recreation Area, northeast of Flagstaff.

Potential threats to this species include unregulated off-road vehicle activity (Figures 1-4), herbivory (Figures 5-7), and hybridization with cultivated *Penstemon* species (Glenne 2003). Very little research has been conducted on this species to date, and basic ecological and demographic information is lacking. Such information is critical to conservation efforts, particularly if this species is to be protected from current and future threats.

Figure 2. *P. clutei* plant in Cinder Hills OHV (off-highway vehicle) Area, Coconino National Forest.

Figure 3. *P. clutei* plant growing with Apache plume (*Fallugia paradoxa*) in Cinder Hills OHV Area.

Figure 4. *P. clutei* plants growing in islands created by shrubs, trees, and downed logs within the Cinder Hills OHV Area.


Figure 5. Flowering stems were nipped off of this and other nearby plants prior to seed maturation.

Figure 6. This *P. clutei* plant was one of only a few perennial forbs growing in a large area of cinder soils. It was uprooted, apparently by a gopher, as evidenced by underground soil disturbance adjacent to and below the uprooted plant.


Figure 7. Minute holes created by seed bugs previously identified as *Kleidocerys ovalis* Barber (Hemiptera: Lygaediae) by the USDA Systematic Entomology Lab (Fule et al. 2001) are visible on these *P. clutei* seed capsules.

Phylogeny

Bateman (1980) speculated that *P. clutei* is a descendant of desert penstemon (*P. pseudospectabilis*) and that geographic isolation occurred following the Sunset Crater eruption. There has also been speculation that *P. clutei* may be intermediate between *P. pseudospectabilis* and Palmer's penstemon (*P. palmeri*) (Clokey and Keck 1939). Phylogenetic reconstruction of the genus *Penstemon* using nuclear and chloroplast sequence data and parsimony analysis produced inconsistent results (Wolfe et al. 2006). Strict consensus trees generated from ITS (internal transcribed spacer) placed *P. clutei* in a polytomy with pinto beardtongue (*P. bicolor*; 2 subspecies in AZ, CA and NV), Panamint beardtongue (*P. floridus*; 2 varieties in AZ and NV), *P. palmeri* (3 varieties that range from NM to CA north to WY and WA), and Wassuk Range beardtongue (*P. rubicundus*; NV). In contrast, strict consensus trees generated from chloroplast sequence data placed *P. clutei* as sister to scarlet bugler (*P. centranthifolius*; CA) (Wolfe et al. 2006). With both methods (ITS and chloroplast sequence), the genera within the tribe Cheloneae had high bootstrap values. However, few terminal lineages of sister taxa within the *Penstemon* clade had bootstrap values above 70%, which is the generally accepted value for moderate to strong support. The contradictory results are likely due to hybridization and/or rapid speciation among penstemons. Wolfe and her co-authors (1998) have documented hybridization among some penstemon species and have also demonstrated that pollen-mediated gene flow occurs via hummingbird vectors.

Ecological Research

Little is currently known about the ecology of *P. clutei,* and information in the scientific literature is sparse, but observations in the field have suggested a positive association with disturbance to interspecific competitors. Prolific growth was observed following the Burnt Fire in 1973 (Goodwin 1979) and the Hochderffer Fire in 1996 (Fulé et al. 2001). It has also been observed growing in large numbers in the path left by a tornado (Crisp 1996) as well as surrounding pinyon pine (*Pinus edulis*) trees that were killed by drought and bark beetles in 2002-2003 (J.D. Springer, personal observations, 2008 and 2009) (Figures 8 and 9), although it is also capable of germination and survival in the shade of live junipers (Figure 10). Based on these anecdotal observations, persistence of this species is likely due to dual processes involving reduced competition in response to tree mortality, as well as microsite effects which allow it to survive both in shady areas near live trees and near woody material on the ground.

Phillips et al. (1992) noted vigorous plants and high seedling numbers in areas of past disturbance, especially from logging operations (Figure 11). Plants were particularly prevalent near decaying logs and stumps. Large numbers of reproductively mature plants are also sometimes found in a ring surrounding recent ponderosa pine (*Pinus ponderosa*) snags (Fulé et al. 2001) (Figures 12 and 13).

Figure 8. High mortality occurred among pinyon pine trees (*Pinus edulis*) following a bark beetle outbreak and drought in 2002. *P. clutei* plants are often found surrounding these dead trees.

Figure 9. Vigorous *P. clutei* plants surround fallen pinyon pine (*Pinus edulis*).

Figure 10. *P. clutei* plants growing next to a live one-seed juniper (*Juniperus monosperma*).

Figure 11. *P. clutei* plants growing near a cut snag and more recently dead ponderosa pine trees that were most likely killed by bark beetles.

Figure 12. *P. clutei* plants growing near an old-growth ponderosa pine snag.

Figure 13. *P. clutei* plants grow in a ring surrounding a ponderosa pine snag.

Because plants had been noted to emerge in abundance following wildfire, two prescribed burning studies were established by the U.S. Forest Service, but results were inconclusive (Nagiller 1992). Ecological Restoration Institute (ERI) staff at Northern Arizona University initiated a study in 1992 to test the hypothesis that restoration of historic ecosystem conditions may enhance the reproduction and survival of this species

(Fulé et al. 2001). The results suggested that prescribed burning caused a significant (up to 75%) decline in *P. clutei* density. However, density also declined in two of the three control areas (in one area also by as much as 75%). So, while prescribed burning can kill mature plants, natural population declines also occur in the absence of disturbance.

After evaluating results from the prescribed burning experiment, ERI staff investigated the possibility that vigorous responses following fire were a result of tree mortality and the consequent removal of root competition (Fulé et al. 2001). The ERI initiated a study in 1998 to test the hypothesis that cutting root competition through trenching would increase *P. clutei* density. Two preliminary conclusions were drawn from the root trenching study: 1) trenching had a positive effect on *P. clutei* reproduction, and this trend was still evident a year later, and 2) increases in *P. clutei* were likely due to reduced root competition with trees.

The study area was revisited in 2008, ten years after root trenching and 13 and 14 years following prescribed spring and fall burning, respectively. There was no significant difference in *P. clutei* density between burned and unburned plots (Springer et al., in press). Over the course of the study, there was a general decline in *P. clutei* in both burned and control plots. However, there was still a significant difference in mean density between trenched and control plots ten years after the first remeasurement, with mean density in trenched plots significantly higher ($F=7.8$, $p<0.01$). There was also a highly significant treatment effect over time ($F= 31.4$, $p <0.0001$).

The results showed that *P. clutei* populations may persist for at least a decade following disturbances that reduce root competition, possibly due to increased available soil moisture and/or nutrients. Additional long-term monitoring is needed in order to determine the duration of the effect.

Scope and Purpose of the Research

Population dynamics of *P. clutei* are largely unknown. In particular, processes and mechanisms related to dispersal, colonization, and population persistence are poorly understood. This study was designed to examine population structure and establish long-term monitoring plots, which may provide insights concerning population dynamics and relationships to disturbances such as fire, herbivory, and OHV activity.

Determining the relative importance of factors that influence the long-term population dynamics of this species is integral to future conservation planning. Main factors are likely to include disturbance, competition, herbivory, drought, and climate change.

Preliminary molecular data will be used to identify the overall genetic diversity within and among populations and subpopulations of plants. This information will be important for conservation and restoration efforts for this species for several reasons: 1) It will help us to identify any potential genetic bottlenecks that could occur, 2) it will help in our understanding of whether plants have genetic variability based on soil type, disturbance history or other factors, and 3) it will help to identify the most genetically diverse subpopulations for future restoration and conservation efforts. The entire suite of information will ultimately aid USFS and NPS personnel in management decisions for this species across its range.

Methods

Study Area

P. clutei is a narrowly endemic species with a range centered on Sunset Crater National Monument and surrounding National Forest lands approximately 15 miles (24 km) north and east of Flagstaff, Arizona, with a possibly disjunct population several miles northwest near Indian Flat. Elevations range from approximately 5900 ft (1800 m) to 8825 ft (2690 m). The Terrestrial Ecosystems Survey (TES) of the Coconino National Forest (Miller et al. 1995) reveals a wide variety of soils in the area ranging from fairly recent lava flows to black cinders from the Sunset Crater eruption (estimated date of eruption 1040-1100 AD), to oxidized red cinders and derived soils. The most common soil types within the range of this species are typic ustorthents and vitrandic ustochrepts. Average daily temperatures range from a maximum of 84.6°F (29.9°C) in July to a minimum of 12.2°F (-11°C) in December. Average annual precipitation is 16.7 inches (42.4 cm), with about half of this falling as rain during the summer monsoon and the other half as snow in the winter (WRCC, 2010).

The surrounding vegetation is predominantly open ponderosa pine (*Pinus ponderosa*) forest and pinyon-juniper (*Pinus edulis* and *Juniperus* spp.) woodland, with a shrubby understory including Apache plume (*Fallugia paradoxa*) and rabbitbrush (*Ericameria* spp.). Grasses such as bottlebrush squirreltail (*Elymus elymoides*) and blue grama (*Bouteloua gracilis*), and forbs such as ragleaf bahia (*Bahia dissecta*) and common mullein (*Verbascum thapsus*) are sparsely scattered across a rather open landscape with large patches of bare ground.

Experimental Design

We conducted initial surveys for populations of *P. clutei* and then randomly chose plots from within groups of 5-10 populations using a random number generator, with the requirement that each plot contain at least five individual plants. Twenty-eight plots were installed in total. Twenty-two were established in late summer, 2009, in the Coconino National Forest (Appendix C) and six were established in early fall, 2010, in Sunset Crater Volcano National Monument (Appendix D). Each plot was 10m x 10 m in size, with the corners permanently monumented by driving steel rebar stakes into the ground (Figure 14). Plot location (Universal Transverse Mercator (UTM)) coordinates were recorded with hand-held global positioning system (GPS) units for future relocation and remeasurement (Appendix B). At each plot, general observational information was collected including slope, aspect, general vegetation type, soil type or parent material, evidence of disturbance, insect activity on plants, and an estimate of the amount of coarse woody debris and OHV activity on the plot.

A measurement of overstory canopy cover at the center of the plot was taken using a spherical densiometer. Photographs were also taken at each corner looking both directions along the plot boundaries, and at the midpoint of each side facing inward toward the center of the plot. Locations of plots are given in Appendices D and E. UTM coordinates for each plot are listed in Appendix B (NAD 27, Zone 12).

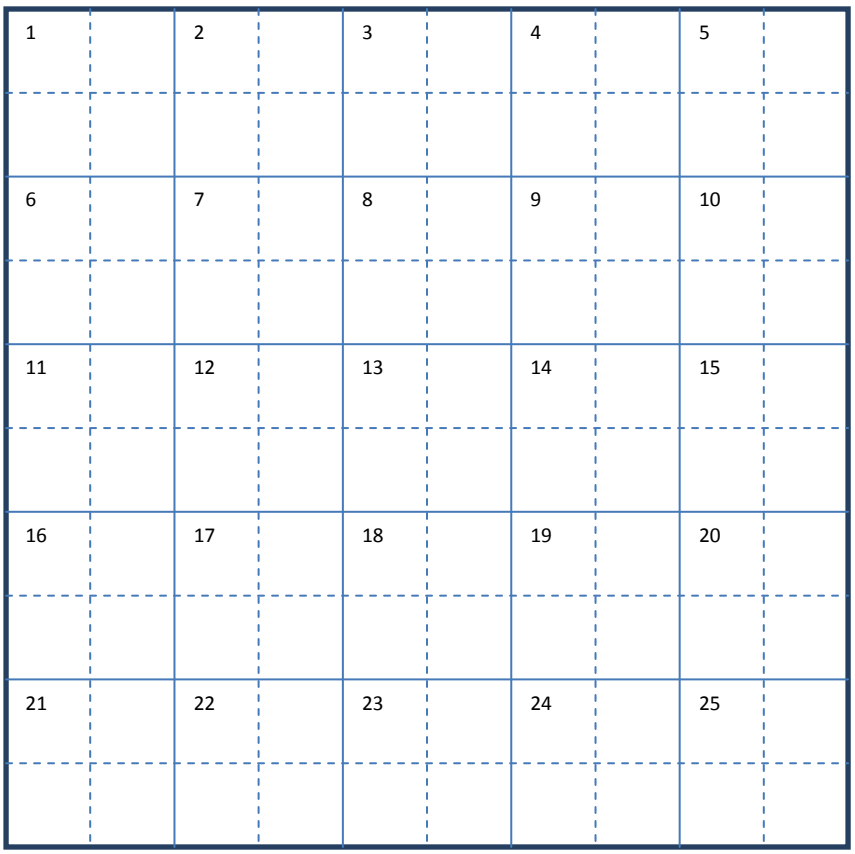

Figure 14. Plot layout, showing the 10m x 10-m plot divided into twenty-five 2m x 2-m cells (dashed lines further subdividing the cells were used for mapping).

In the field, each plot was temporarily subdivided using measuring tapes into twenty-five 2m x 2-m cells for measurement and mapping purposes. The entire plot was mapped, noting the locations of each *P. clutei* individual and all trees and shrubs, as well as coarse woody debris. For each overstory tree, we measured the diameter at breast height (dbh) and the average crown radius, and recorded the condition class. We also conducted a census of the understory community, noting the species present and estimating their overall percent cover within the plot. For each *P. clutei* individual we assigned a size class (descriptions are presented in Table 1), measured the total height, length, and width, counted the numbers of flowering and browsed stems, and recorded comments about insect or other damage to the plant. From the length and width measurements we estimated the area covered by each plant using the formula for an ellipse (π*plant length*plant width).

Data Analysis

For analytical purposes, plots were stratified into Terrestrial Ecosystem Survey (TES) mapping units (Appendices B and C), which we considered to be discreet sites. We investigated life history strategies and modes of persistence by calculating relative abundance of individuals within life stage classes. Relative abundance charts were

examined across sites for similarities and differences. Correlational analyses were conducted in order to test relationships between number of *P. clutei* plants on plots and explanatory variables including overstory tree density, and cover of tree canopy, shrubs, grass, and downed woody debris. For these analyses we used SAS JMP software (Version 8.0, SAS, Inc., 2004). Nonmetric multidimensional scaling (NMDS) was used in order to examine similarity in plant species composition across *P. clutei* plots. NMDS was conducted using PC-ORD software (version 5.31; McCune and Mefford 2006).

Genetic Variability

In a companion study to examine genetic variation across the range of the species, leaf tissue samples were collected from two individuals from each of 15 Coconino National Forest plots for a pilot microsatellite study. Leaves were stored in silica gel using methods outlined in Chase and Hills (1991). Plots used for this initial trial span the known range of *P. clutei* and are mapped in Appendix C. Tissue samples from the Monument were not collected for this initial pilot study due to permitting issues but were collected in 2010 and will be included in future studies (see below). Because of funding limitations, only an initial pilot study was conducted by the Environmental Genetics and Genomics Laboratory (EngGen) at Northern Arizona University to identify informative microsatellite loci. Methods, including thermocycler settings, follow that of Kramer and Fant (2007) and are detailed in Appendix E. Total genomic DNAs of two individuals were extracted from each of the fifteen plots (n=30 samples). A second trial was conducted with slightly modified methods in an attempt to optimize amplification. These methods are also detailed in Appendix E.

In total, the eight primer pairs developed by Kramer and Fant (2007) were screened by EngGen. All primers were obtained from a commercial vendor. Of these, three amplified variable loci (Pen 02, Pen 04 and Pen 18) were used for subsequent analyses. Preliminary analysis of microsatellite data was conducted using Structure (version 2.3.3; Pritchard, Stephens & Donnelly (2000); Falush, Stephens and Pritchard (2003) and (2007); and Hubisz, Falush, Stephens and Pritchard (2009)) and based on an a priori determination of two disjunct populations (one at Indian Flat and the other on the east side of US 89).

In addition, we collected leaves from 30 individuals in each of ten subpopulations in both the forest and the Monument for a future study of genetic variability on a larger scale (n=300). These samples are currently being stored in silica gel at cool temperature and are housed at the ERI at Northern Arizona University.

Results

Life Stage Characteristics

Average height and area of the six size classes across the forest and monument populations are listed in Table 1. Seedlings ranged from 1-8 cm in height and 3.14-226.19 cm^2 in area; juveniles ranged from 1-19 cm in height and 3.14-2827.43 cm^2 in area; young reproductive plants ranged from 4-59 cm tall and 6.28-5843.36 cm^2 in area; mature plants ranged from 5-83 cm and 31.42-64119.91 cm^2 in area; and senescing plants ranged from 1-73 cm in height and 3.14-4281.99 cm^2 in area.

Table 1. Six life stages (age classes) and average height and size of *P. clutei.* Classes were determined a priori and averages were calculated from measurements in the field. Averages are from combined populations from the Coconino National Forest and from Sunset Crater Volcano National Monument.

Class	**Life Stage**	**Description**	**Average height (cm)**	**Average area (cm^2)**
1	Seedling	First season growth as indicated by cotyledons.	2.37	34.56
2	Juvenile	One year or more of growth and pre-reproductive. Without cotyledons or flowering stems.	5.13	237.64
3	Young reproductive	1-5 flowering stems. No evidence of flowering stems from previous years.	17.33	764.52
4	Mature	6 or more flowering stems. Plants often have woody characteristics at base of plant and evidence of old leaves and flowering stems from previous years.	30.59	2462.71
5	Senescing	Plants are discolored with brown, gray or off-colored green leaves. Obviously suffering from severe stress, with no evidence of healthy vegetation.	14.99	614.09
6	Dead	No evidence of living vegetation.	n/a	n/a

Population Structure

The population structure results showed an overall trend of a fairly high relative abundance of mature plants compared to seedlings and juveniles (Figure 15). Relative abundance at the seedling stage was highest at the single cinder cone plot at Sunset Crater (27.27%). Seedling relative abundance was also high in the black cinders type (TES map unit 559) at 17.46%. There were no seedlings encountered in plots situated in TES map units 561, 510, or 562 (Figure 15). The highest relative abundance of reproductively mature plants was in the TES mapping unit 510 at 78.57% (plot 14). TES mapping unit 441 also had relatively high abundance (57.42%). The lowest relative abundance of reproductively mature plants was in the TES 562 type (11.11%) and the 512 type (18.92%) (Figure 15).

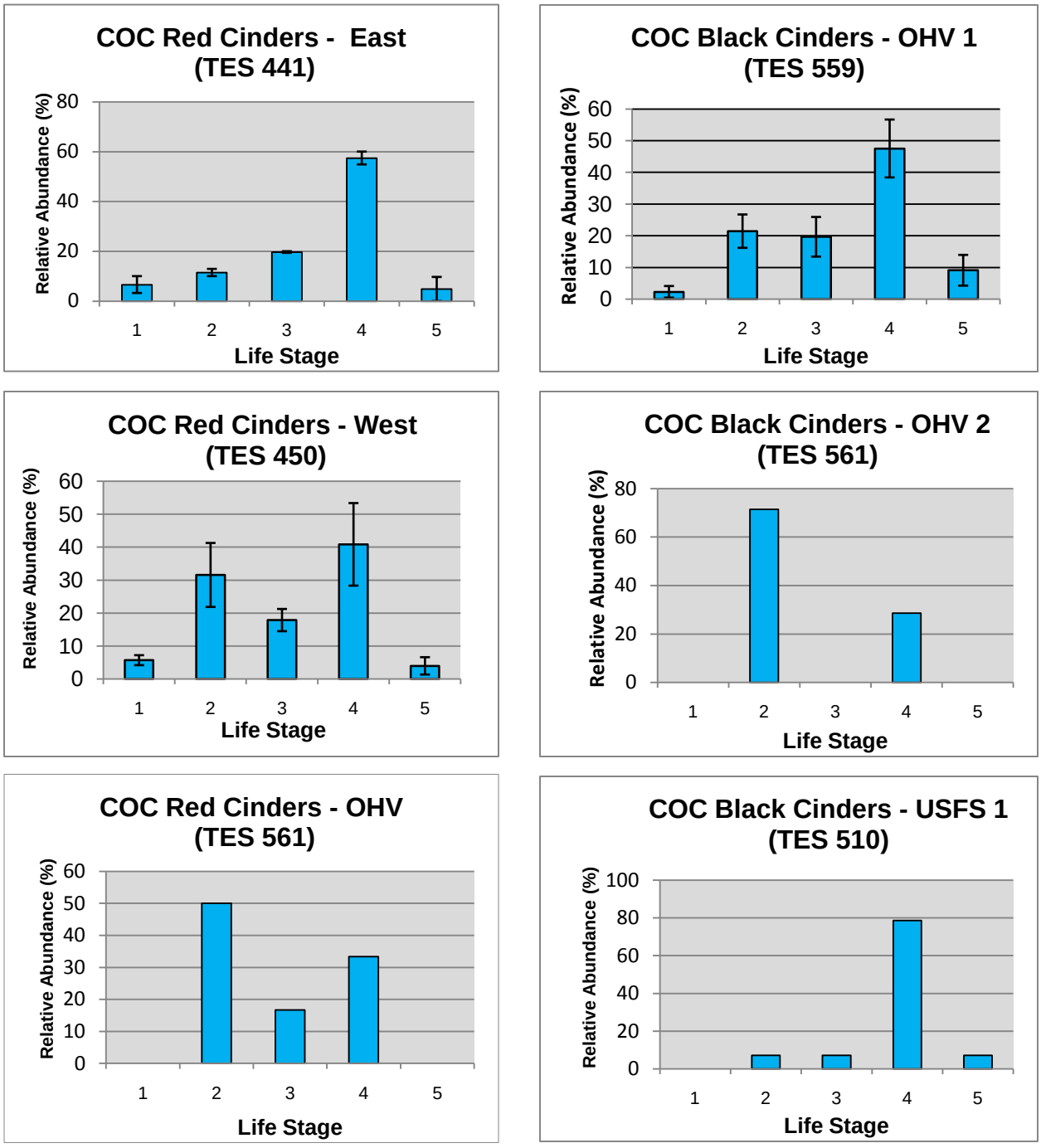

Figure 15. Abundance of different life stages at each sampled site (1=seedling, 2=juvenile, 3=young reproductive, 4=mature, 5=senescing). Error bars (+/- standard error) are shown for sites including more than one plot.

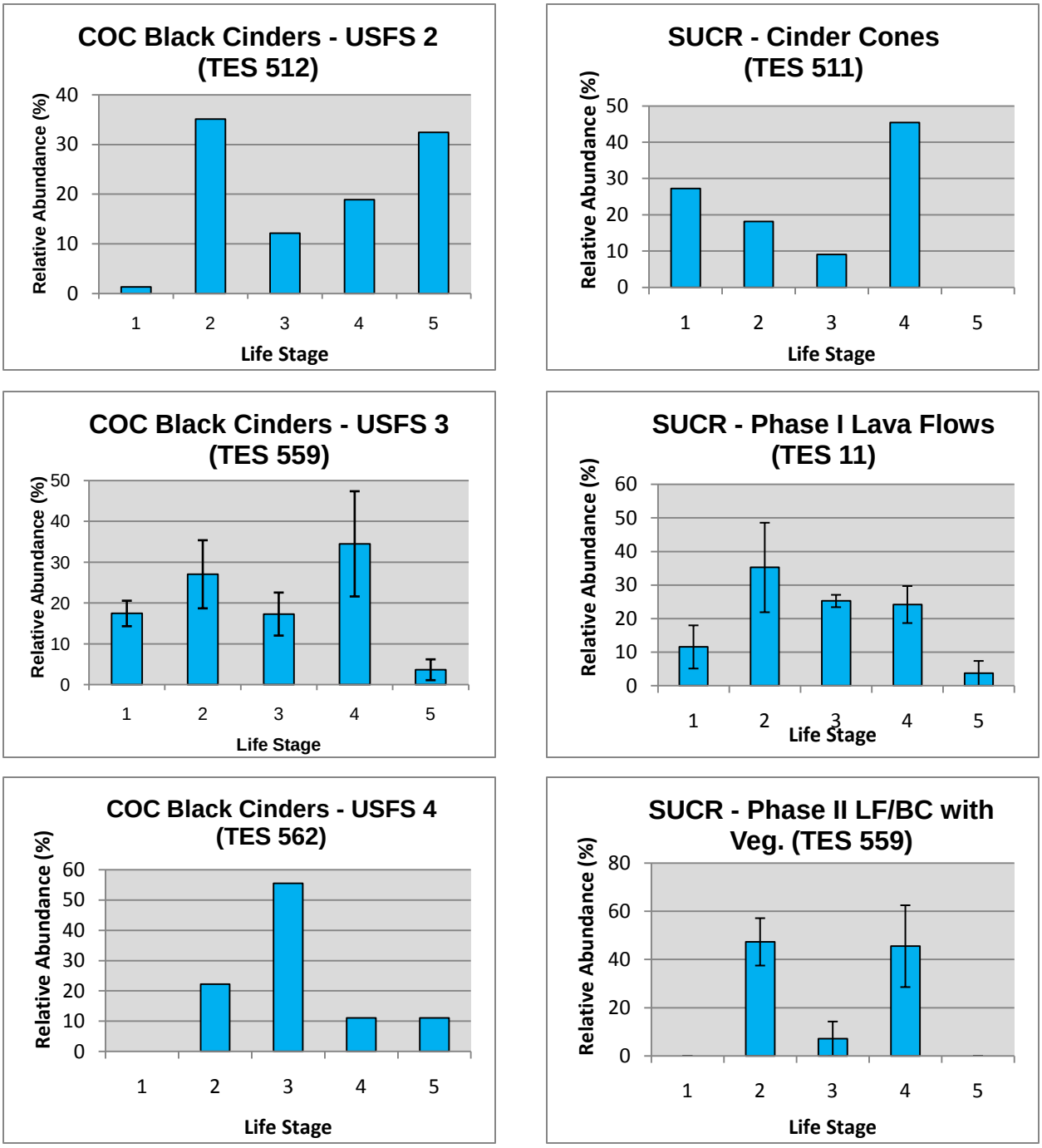

Figure 15 (cont.).

Population Density

The range of population densities for total *P. clutei* across the forest plots was 6-213 plants per 10 m^2 plot (average of 56.1 plants per plot or 5.61 plants/m^2). Plots in the monument ranged from 7-13 plants per 10 m^2 plot (average of 10 plants per plot or 1

plant/m^2). The range of live plants was 6-187 plants per 10 m^2 plot (average of 45.59 live plants/plot) in the forest and 7-11 live plants per 10 m^2 plot in the monument (average of 8.33 live plants/plot).

Community Data

A non-metric multidimensional scaling (NMS) analysis indicates that *P. clutei* grows across a wide range of diverse and variable habitats (Figure 16). The five Coconino NF plots from pinyon-juniper habitat clustered to the right of the graph, indicating a distinct plant community (COC 1, 10, 11, 17, 22) (locations mapped in Appendix C). The vectors indicate a relationship between dead trees and downed woody material and the plant community growing in the pinyon-juniper habitat. However, these relationships were not found to be significant in this study and warrant further exploration.

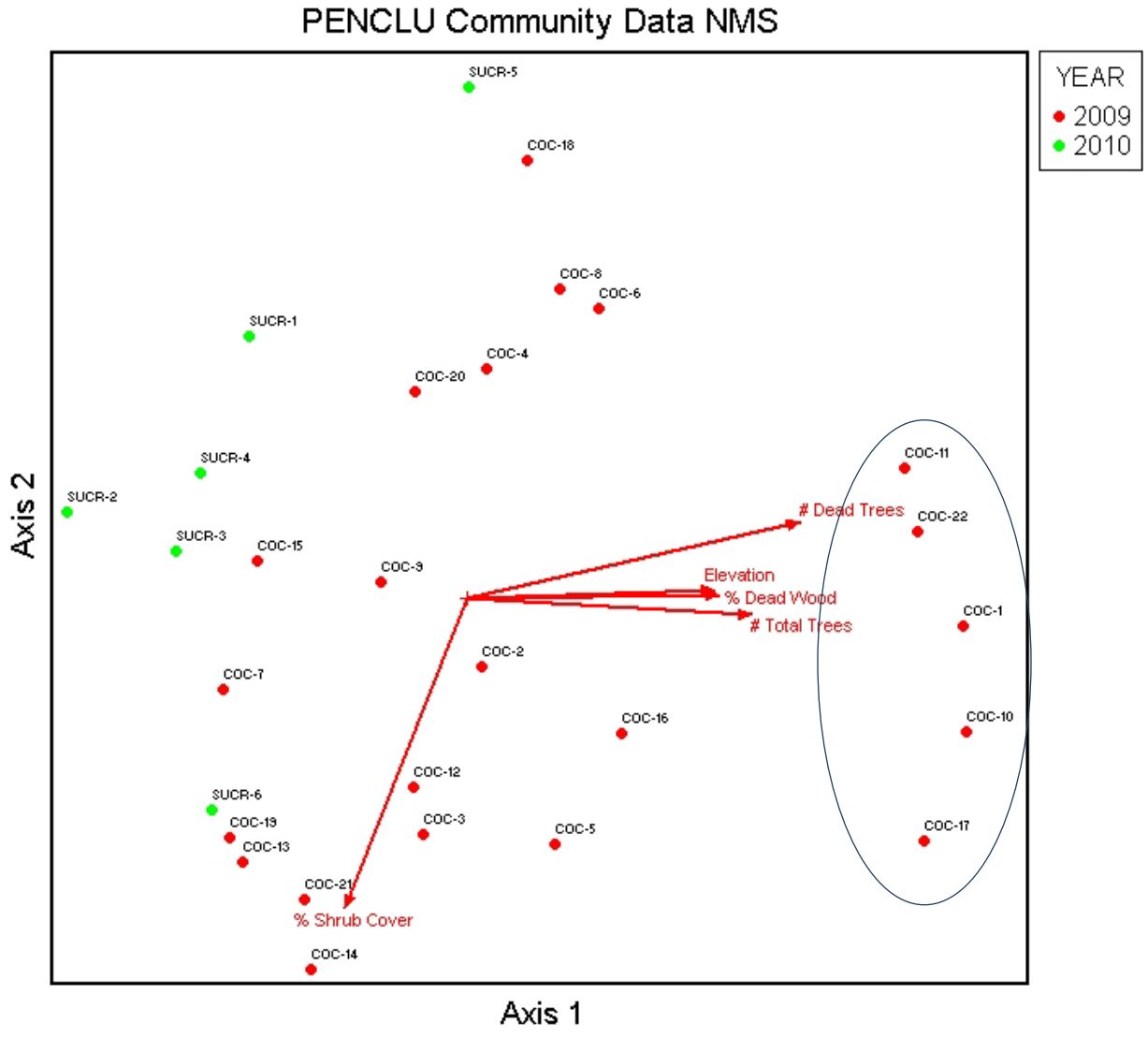

Figure 16. Non-metric multidimensional scaling (NMS) ordination of plant community data from *P. clutei* plots. Plots which are shown more closely to each other are more similar in

terms of their community composition. Environmental data were ordinated along with community data; vectors show correlations with an $r^2 > 0.2$, with the given environmental variable increasing in the direction indicated. The oval encloses plots located in pinyon-juniper woodlands.

Correlations with Environmental Factors

A correlational analysis revealed no significant relationships between the number of trees (either living or dead) and the number of *P. clutei* plants on the study plots (Table 2). Nor was there a significant relationship between tree canopy cover, shrub canopy cover, grass cover or downed woody material and the number of *P. clutei*.

Table 2. Simple correlation coefficients (r-values) for analysis of total number and number of live of P. clutei plants versus explanatory variables measured on field plots. Coefficients were estimated using the restricted maximum likelihood method.

Variable	Total PENCLU (no.)	Live PENCLU (no.)
Live trees (no.)	-0.0968	-0.0776
Snags (no.)	0.2838	0.3156
Tree cover (%)	-0.1230	-0.0837
Woody debris cover (%)	0.2228	0.2949
Shrub cover (%)	0.0964	0.0405
Grass cover (%)	*	*
OHV disturbance (%)	0.1688	0.1862

* Limited variation in explanatory variable prevented meaningful statistical analysis

Genetic Variability

Eight primer pairs that had been identified in a study by Kramer and Fant (2007) as suitable for assessing genetic diversity in the *Penstemon* genus were tested for amplification of loci using leaf tissue samples collected from 30 individuals in fifteen Coconino National Forest plots. Successful amplification of homologous PCR products for 8 loci had occurred in a sample of leaf tissue from two *P. clutei* individuals obtained from the Flagstaff Arboretum for the Kramer and Fant study (2007). In our current study, three of the eight primer pairs were successfully amplified (Pen 02, Pen 04, and Pen 18) and are likely suitable for determining genetic diversity in a future study of *P. clutei* (Figures 17-20). However, additional processing revealed that primer pair Pen 18 appears to be monomorphic with no variation in fragment length among the population. The bands for Pen 21 and 24 were faint, but consistent, and may be useful in providing additional power for making future inferences on population genetic structure.

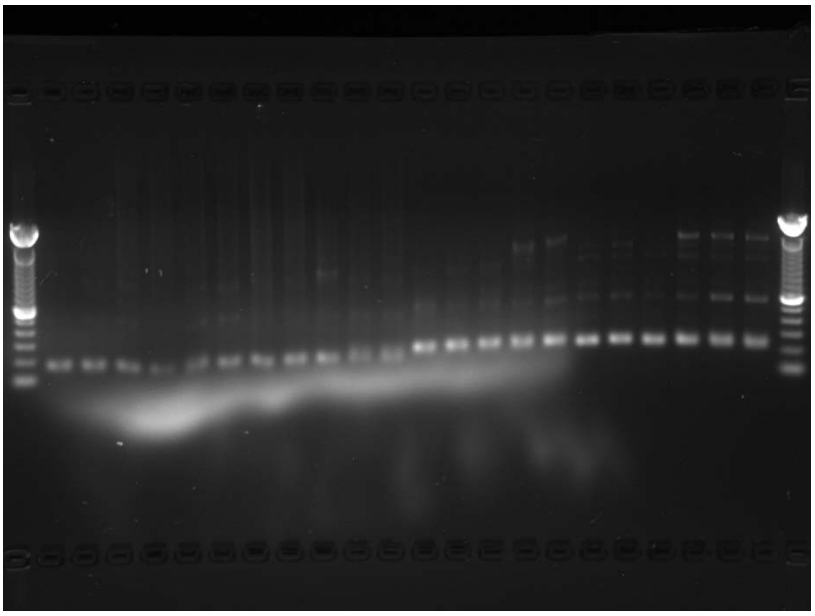

Figure 17. Amplified PCR products from a microsatellite primer trial (primer pair Pen 02 on left/Pen 04 on right). Each horizontal band represents an allele.

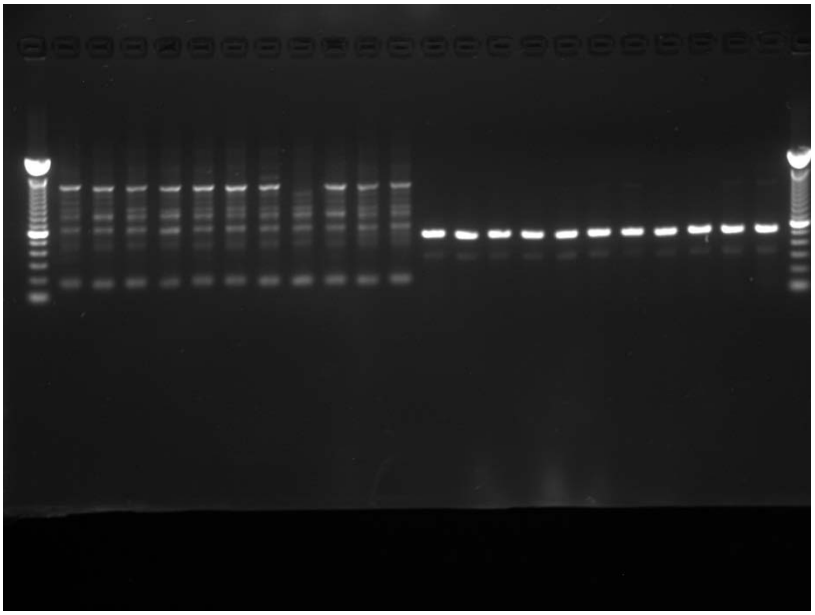

Figure 18. Amplified PCR products from a microsatellite primer trial (Pen 05/18). Primer 18 was most successfully amplified and was identified as suitable for future *P. clutei* genetic variability studies.

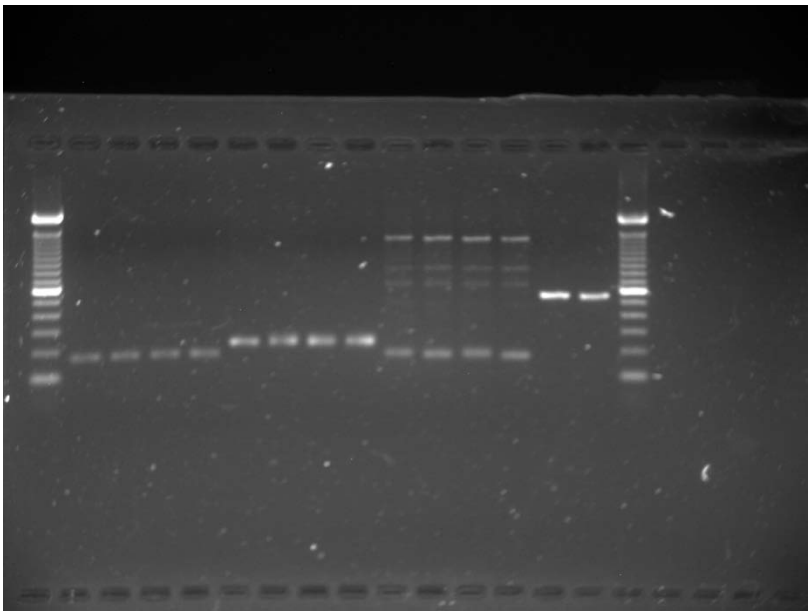

Figure 19. Amplified PCR products from a second microsatellite primer trial (Pen 02, 04, 05, 18).

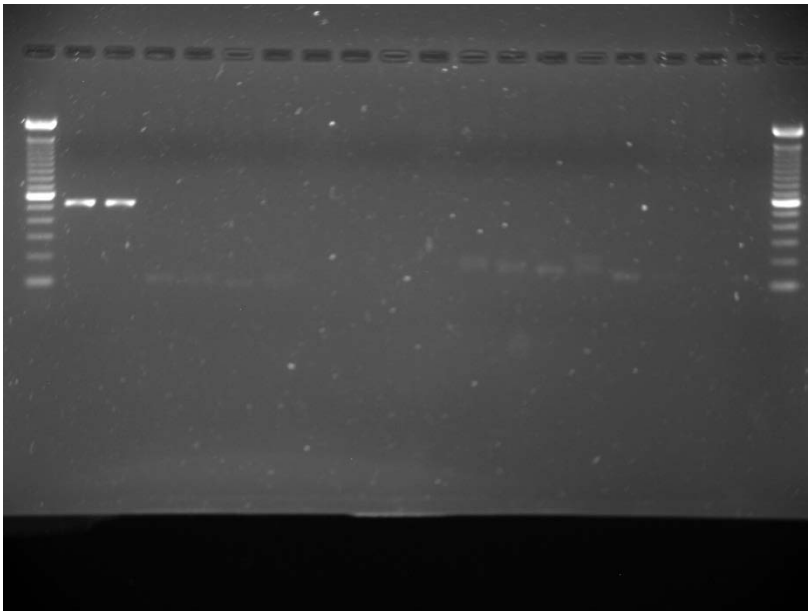

Figure 20. Amplified PCR products from second microsatellite primer trial (Pen 18, 21, 23, 24, 25)

Preliminary results from the microsatellite marked primer with an *a priori* determination of two distinct populations, suggest that there is one single population of *P. clutei* and that the two suspected populations are not significantly different or distinct from each other. Locus Pen 18 is monomorphic within the population, on both the east and west sides of US 89. The fixation index (FST), a measure of genetic difference, was .0437 for the Indian Flat individuals on the west side of US 89 and .0044 for the individuals on the east side of US 89. A value of 0 indicates panmixis (populations are interbreeding freely) and a

value of 1 indicates complete separation and an absence of genetic drift between the populations. These preliminary results suggest that gene flow is occurring between the two subpopulations on either side of the highway. However, it must be emphasized that these results are based on a small number of individuals (n=30) and are *very* preliminary.

Discussion

This study was designed to collect baseline data and to establish a long-term monitoring study of *P. clutei.* This species lives in a harsh environment, and long-term monitoring will assist in our understanding of long-term population dynamics in the absence of disturbance, as well as when significant disturbances occur, and may assist in our understanding of the impacts of climate change on a rare endemic species.

Population Structure and Abundance

The study was not designed to pinpoint causal relationships between environmental factors and population structure, although we investigated these links in order to gain insight regarding life history strategies and modes of persistence. Such information is critical in determining effective conservation strategies for species of concern.

The population structure results show an overall trend of a fairly high relative abundance of mature plants compared to seedlings and juveniles. Large numbers of seedlings were observed following root trenching in a previous study (Fulé et al. 2001) and were also observed in some of the plots sampled for this study. The lower relative abundance of seedlings is probably a reflection of high mortality during the seedling stage, particularly since the data in the forest plots was collected during a lengthy dry period in the late summer of 2009. Low relative abundance at the seedling stage may also be due to a lack of recent disturbance in many of these monitoring plots. Seedling mortality appears to be high, based on observations in the field, but once plants reach maturity they have adapted to the harsh, arid environment by means of thick leaves, a large woody taproot and occasionally thick lateral roots. Also, the six classes are not true age classes, because individual plants have not been tracked through time, and we do not know at what age this species is capable of reproductive maturity. The juvenile and young reproductive stages are thus probably artifacts of observing plants in the field at one point in time rather than distinct classes that each plant goes through, as it is quite possible for a plant to reach reproductive maturity at two years of age and quite probably by three years of age. In addition, it is unknown how long this species lives or for how many years a plant may remain reproductively mature. Future studies should follow individual seedlings through time and also incorporate experimental components that manipulate disturbances in a way that will affect competition by making additional water and nutrient resources available.

A lack of significant correlations between environmental factors and *P. clutei* may be due to the variability of habitats and microhabitats in which the penstemon successfully grows, or it may simply be due to the locations of the plots, which were situated around known populations. We did not install plots in locations in which *P. clutei* surveys were not conducted.

Conservation Concerns

P. clutei is a narrowly endemic species that faces a number of threats including climate change, unregulated OHV activity and potential hybridization with other penstemon species. Climate models predict a more arid climate in the southwestern U.S. in the coming decades (Seager et al. 2007). Because of the difficulty of experimentally manipulating a rare species and its habitat, long-term monitoring plots will allow us to track population changes through time in both the presence and absence of disturbance and will allow us to monitor for potential effects of climate change as well. A large portion of the range of *P. clutei* is occupied by a large OHV recreational area (Cinder Hills OHV Area) (Appendix A). Most of this area is not fenced and OHV use spills outside the boundaries shown in the map.

Although *P. clutei* appears to benefit from disturbance, whether disturbance is beneficial or detrimental depends on the type of disturbance and the amount of impact. No quantifiable data has yet been collected on the impacts of OHV activity on this species, but anecdotal evidence points to OHVs as a direct factor in *P. clutei* mortality (J.D. Springer, personal observations, 2008). OHV activity causes above- and belowground soil impacts, resulting in decreased soil moisture, increased soil bulk density, and increased water infiltration time, which have been shown to negatively impact plant species in the area, such as ponderosa pine (Kennedy 2005). Our hypothesis is that disturbance to competitors, such as trees, increases population growth, but disturbance to populations results in population declines. It is critical to our understanding of *P. clutei* ecology that disturbances affecting tree mortality are studied alongside those that disturb or compact the soil, such as might occur with OHV activity. Future studies should incorporate OHV impacts as an experimental variable.

P. bicolor, a rare endemic of the Mohave Desert in CA and NV has been observed to hybridize with *P. palmeri*, which was widely planted in the region for reclamation projects. The concern is that contamination of the gene pool and swamping via hybridization may lead to the loss of genetically "pure" populations of *P. bicolor* and may be the single greatest threat to this rare endemic, after habitat loss (Glenne 2003). Hybridization with garden and roadside plantings is also a potential concern for *P. clutei*. Additional studies beyond the preliminary work described in this report would be useful to determine the genetic integrity of the species in its natural environment and whether gene flow is occurring freely within the population.

P. clutei subpopulations are often widely dispersed across the landscape and the Indian Flat and Monument subpopulations are currently most protected from OHV activity. They are likely most important for long-term conservation efforts (Appendices C and D).

Future Monitoring and Research

In addition to continued occasional monitoring (perhaps every 3-5 years to minimize concerns over soil impacts due to trampling) of the long-term plots in the Coconino NF and the Monument, priority research needs include: 1) additional molecular studies that examine variability, potential for hybridization, and possible parental species to determine if *P. clutei* is a good species or a subspecies, 2) demographic studies that follow individual plants through time, and 3) the impact of two types of disturbance: those that affect tree competition and those that impact the soil, particularly OHV activity.

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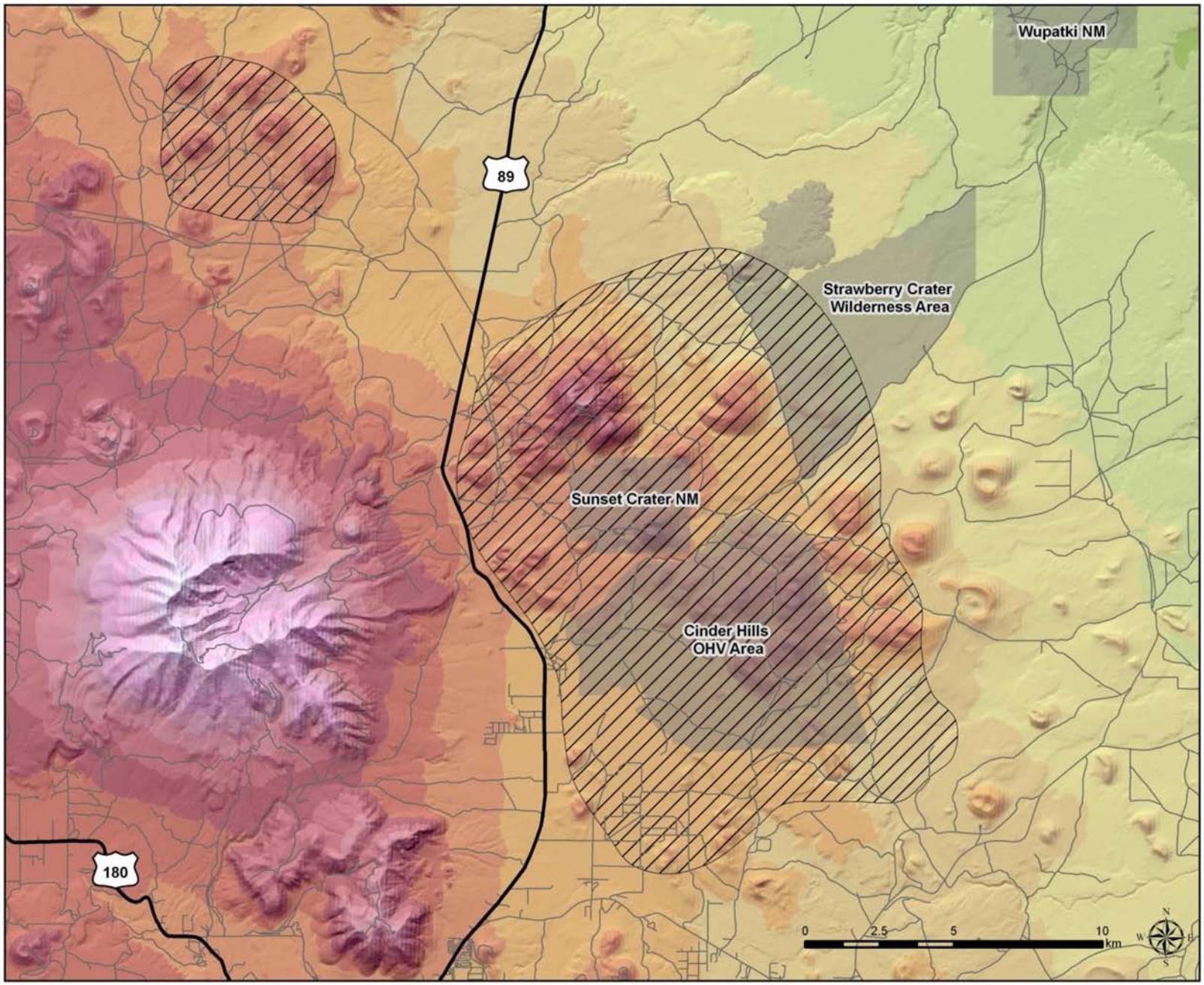
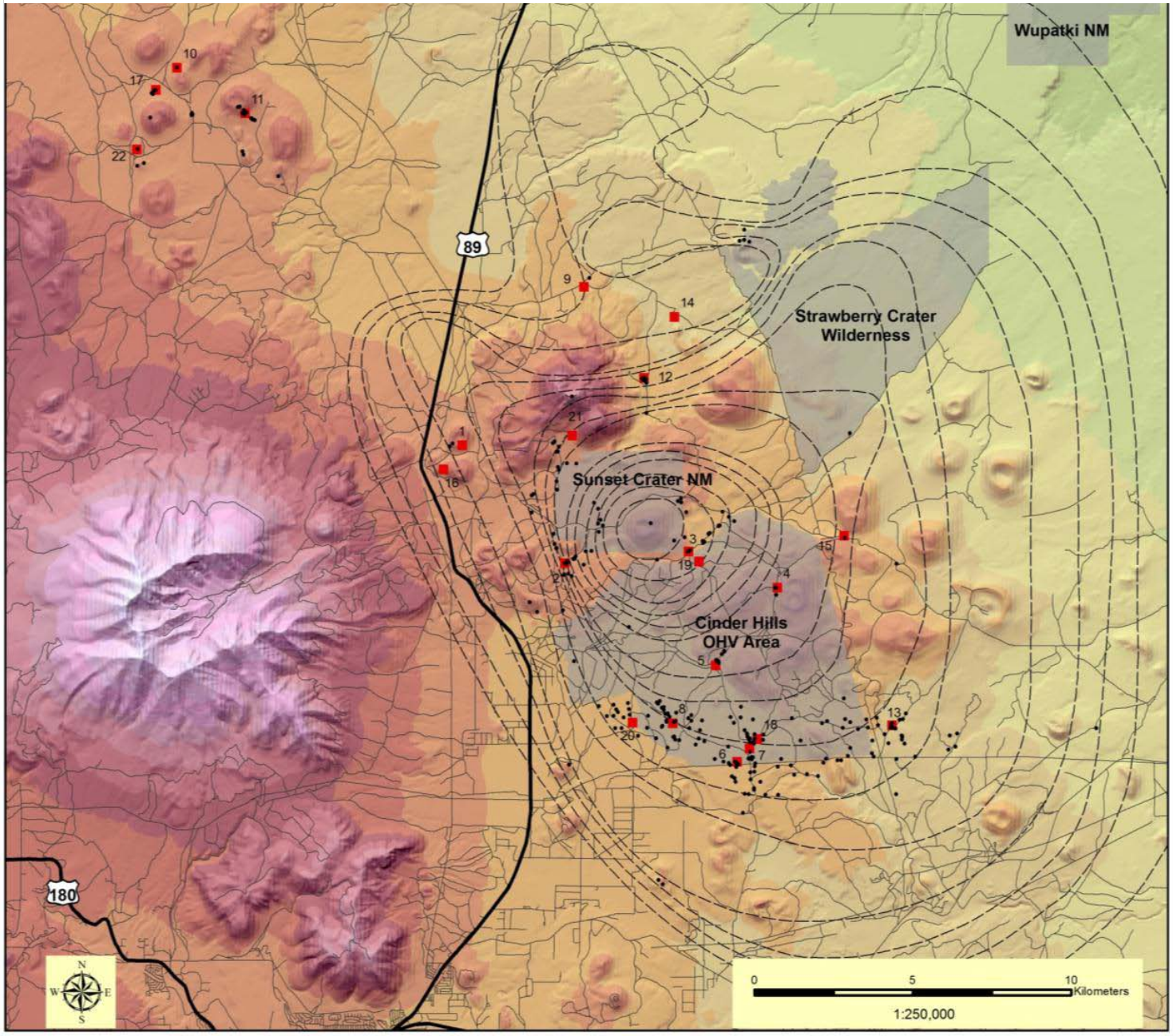
Appendix A. Approximate known range of *P. clutei* in northern Arizona.



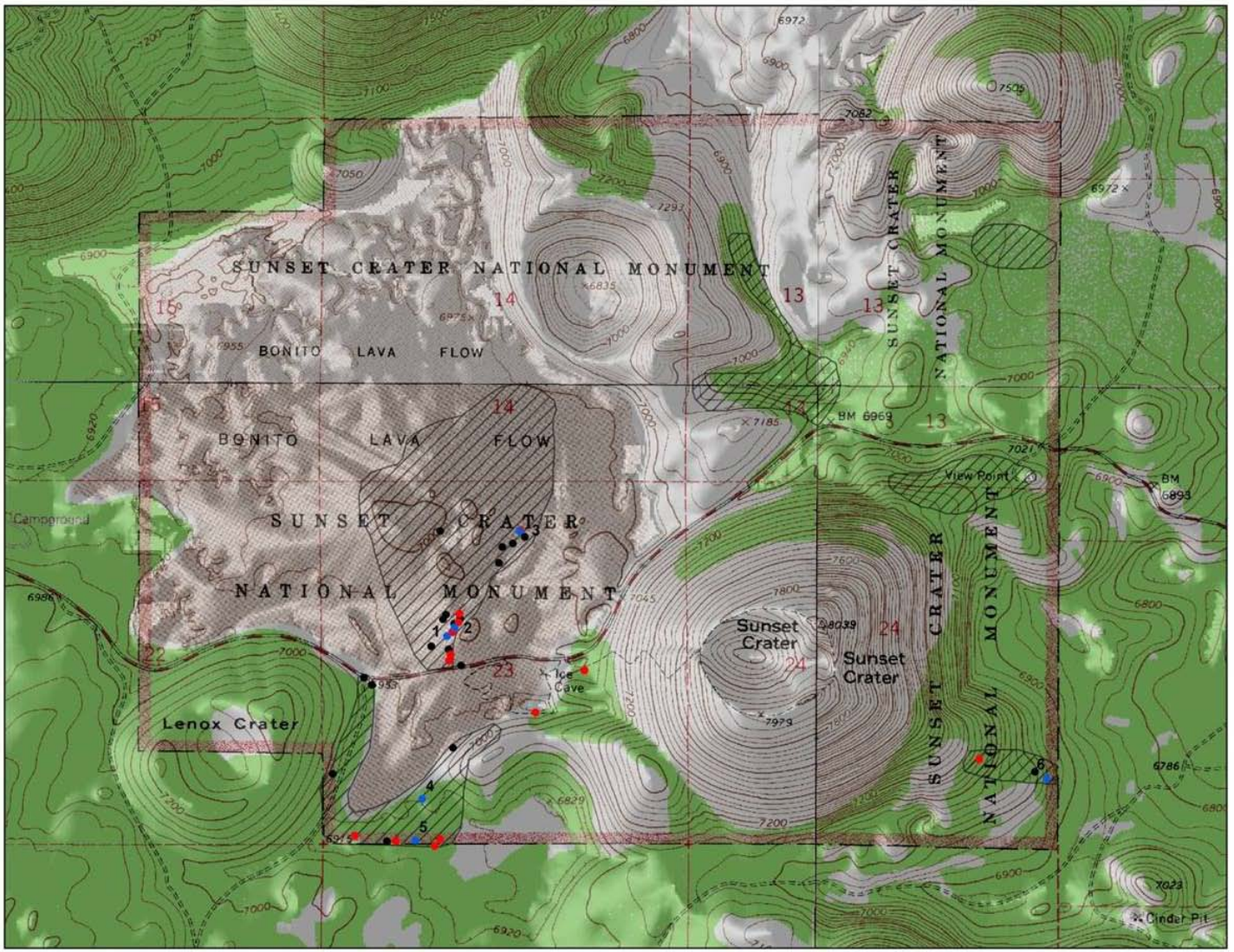
Appendix B. UTMS for long-term monitoring plots and TES mapping units containing long-term *P. clutei* monitoring plots in the Coconino National Forest and Sunset Crater Volcano National Monument (NAD 27, Zone 12).

SITE	PLOT	UTMX	UTMY	TES map unit	VEG	Soils	Landform	Aspect	Elev (M)	Slope (%)
COC RC-East	1	448607	3915911	441	Pied/Jude2/Jumo/Fapa	Basaltic cinders	Cinder cones	166	2252	18
	16	448021	3915156	441	Pied/Jude2/Jumo/Fapa	Basaltic cinders	Cinder cones	238	2290	29
COC RC - West	10	439600	3927838	450	Jumo/Pipos	Cinders	Cinder cones	317	2172	21
	11	441754	3926402	450	Jumo/Pipos	Cinders	Cinder cones	133	2252	29
	17	438932	3927144	450	Jumo/Pipos	Cinders	Cinder cones	59	2175	18
	22	438329	3925261	450	Jumo/Pipos	Cinders	Cinder cones	199	2239	12
COC RC - OHV	19	456077	3912245	561	Pipos/Fapa	Basaltic cinders	Cinder cones	89	2125	13
COC BC - USFS 1	14	455308	3919972	510	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Elevated plains	24	1894	6
COC BC - USFS 2	9	452446	3920918	512	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Elevated plains	353	1987	28
COC BC - USFS 3	2	451846	3912188	559	Pipos/Fapa	Basaltic cinders	Elevated plains	71	2144	5
	12	454345	3918042	559	Pipos/Fapa	Basaltic cinders	Elevated plains	82	2009	9
	13	462181	3907069	559	Pipos/Fapa	Basaltic cinders	Elevated plains	35	1946	6
	15	460664	3913060	559	Pipos/Fapa	Basaltic cinders	Elevated plains	348	2097	11
	20	453983	3907156	559	Pipos/Fapa	Basaltic cinders	Elevated plains	95	2023	2
COC BC - USFS 4	21	452076	3916226	562	Pipos/Psmeg	Basaltic cinders	Cinder cones	235	2221	42
COC BC - OHV 1	3	455754	3912557	559	Pipos/Fapa	Basaltic cinders	Elevated plains	106	2097	7
	5	456608	3908965	559	Pipos/Fapa	Basaltic cinders	Elevated plains	137	2093	4
	6	457292	3905913	559	Pipos/Fapa	Basaltic cinders	Elevated plains	135	2043	1
	7	457677	3906337	559	Pipos/Fapa	Basaltic cinders	Elevated plains	225	2049	2
	8	455259	3907135	559	Pipos/Fapa	Basaltic cinders	Elevated plains	188	2038	3
	18	457926	3906636	559	Pipos/Fapa	Basaltic cinders	Elevated plains	318	2060	4
COC BC - OHV 2	4	458561	3911419	561	Pipos/Fapa	Basaltic cinders	Cinder cones	276	2263	34
SUCR - PHASE I LAVA FLOW	1	453017	3913527	11	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Lava flows	336	2134	3
	2	452980	3913482	11	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Lava flows	278	2134	4
	3	453278	3913911	11	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Lava flows	109	2131	2
SUCR - PHASE II FLOW/CINDERS W/VEG	4	452846	3912727	559	Pipos/Fapa	Basaltic cinders	Elevated plains	199	2112	4
	5	452816	3912548	559	Pipos/Fapa	Basaltic cinders	Elevated plains	61	2106	2
SUCR - CINDER CONE	6	455592	3912817	511	Pipos/Pied/Jumo/Fapa	Basaltic cinders	Cinder cones	90	2119	20

Appendix C. Long-term monitoring plots installed in Coconino National Forest (n=22). Red dots denote plot centers. Black dots are previously recorded occurrences of *P. clutei*. Isograph lines represent the extent and depth of tephra immediately after the Sunset Crater eruption (lines courtesy of Michael Ort, NAU Dept. of Geology).



Appendix D. Long-term monitoring plots installed in Sunset Crater National Park (n=6). Blue dots denote plot centers. Red and black dots are previously recorded occurrences.



10-1-2009

Appendix E. Microsatellite primer trial methods. Second set of methods is from a repeat trial with slightly modified methods.

***Penstemon* MSAT Protocol** modified from Kramer. Molecular Ecology (2007) 7, 998-1001

Title: *Penstemon clutei* MSAT Protocol
Date: 05/24/10
User: acb

Purpose: Running an initial trial of 8 MSAT primer sets taken from a study of *P. rostriflorus* , across 10 samples with one duplicate and one NTC.

1. Setup Study Design

No. rxns	plus 10%	total rxns	vol./rxn	Total Volume
12	2	14	**10**	**140**

belowmust be 0 or more (do not add "NA")

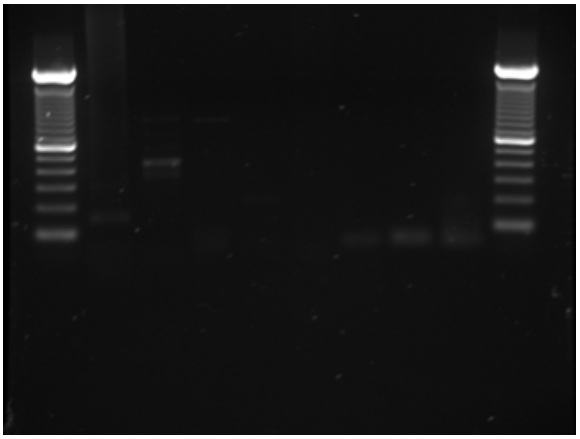
Template vol/rxn **1.0** µl

635 DNA total

Region Specific PCR:

	Reagents	[Starting]	units	[Final]	units	1	rxn	14	rxn's
1	dH_2O	q.s. for to total volume				5.40	µl	75.60	µl
2	PCR Buffer	10	X	1.00	X	1.00	µl	14.00	µl
3	MgCl2	50	mM	3.75	mM	0.75	µl	10.50	µl
4	DNTP's	5	mM each	0.40	mM	0.80	µl	11.20	µl
5	Primer F	5	uM	0.25	uM	0.50	µl	7.00	µl
6	Primer R	5	uM	0.25	uM	0.50	µl	7.00	µl
7	Taq	5	U/ul	0.25	U	0.05	µL	0.70	µl
8	DNA	50-100	ng/ul	Add directly		1.0	µL	14	µL
					Total:	10.00	µL	140.00	µL

Thermal Cycling conditions: **Cycle 1**: 94° for 5 mins **Cycle 2**: (10x) 94° for 30s, 60° for 30s, with 1 degree decrease every cycle, 2 min extension at 72° **Cycle 3:** (30x) 94° for 30s, 50° for 30s, 72° for 2 mins **Cycle 4**: 72° for 10 minutes **Cycle 5**: 4° hold infinitely



Gel 3

gel setup: (3ul DNA + 2ul 1x loading dye, 4ul 100bp ladder)

Plate Map													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen02
B	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen04
C	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen05
D	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen18
E	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen21
F	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen23
G	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen24
H	Plot3_1	Plot4_2	Plot6_1	Plot9_1	Plot10_1	Plot11_2	Plot13_1	Plot14_1	Plot16_2	Plot17_1	Plot17_1	NTC	Pen25

***Penstemon* MSAT Protocol** modified from Kramer. Molecular Ecology (2007) 7, 998-1001

Title: *Penstemon clutei* MSAT Protocol

Date: 05/24/10

User: acb

Purpose: The initial trial had multiple bands on most primer pairs. Rerunning 4 samples with a modified PCR protocol.

1. Setup Study Design

No. rxns	plus 10%	total rxns	vol./rxn	Total Volume
4	1	5	**10**	**50**

belowmust be 0 or more (do not add "NA")

Template vol/rxn **1.0** µl

635 DNA total

Region Specific PCR:

	Reagents	[Starting]	units	[Final]	units	1	rxn	5	rxn's
1	dH_2O	q.s. for to total volume				6.10	µl	30.50	µl
2	PCR Buffer	10	X	1.00	X	1.00	µl	5.00	µl
3	MgCl2	50	mM	2.00	mM	0.40	µl	2.00	µl
4	DNTP's	5	mM each	0.20	mM	0.40	µl	2.00	µl
5	Primer F	5	uM	0.25	uM	0.50	µl	2.50	µl
6	Primer R	5	uM	0.25	uM	0.50	µl	2.50	µl
7	Taq	5	U/ul	0.50	U	0.10	µL	0.50	µl
8	DNA	50-100	ng/ul	Add directly		1.0	µL	5	µL
					Total:	10.00	µL	50.00	µL

Thermal Cycling conditions: **Cycle 1**: 94° for 5 mins **Cycle 2**: (10x) 94° for 30s, 62° for 45s, with 1 degree decrease every cycle, 1 min extension at 72° **Cycle 3:** (30x) 94° for 30s, 55° for 55s, 72° for 1 mins **Cycle 4**: 72° for 7 minutes **Cycle 5**: 4° hold infinitely

Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Plot3_1	Plot3_1	Plot3_1	Plot3_1	Plot3_1	Plot3_1	Plot3_1	Plot3_1				
B	Plot3_2	Plot3_2	Plot3_2	Plot3_2	Plot3_2	Plot3_2	Plot3_2	Plot3_2				
C	Plot4_1	Plot4_1	Plot4_1	Plot4_1	Plot4_1	Plot4_1	Plot4_1	Plot4_1				
D	Plot4_2	Plot4_2	Plot4_2	Plot4_2	Plot4_2	Plot4_2	Plot4_2	Plot4_2				
E												
F												
G												
H												
	Pen02	Pen04	Pen05	Pen18	Pen21	Pen23	Pen24	Pen25				

gel setup: (3ul DNA + 2ul 1x loading dye, 4ul 100bp ladder):