



Conservation Assessment for Triple-ribbed Milkvetch (*Astragalus tricarlinatus*, Fabaceae) in Joshua Tree National Park

Natural Resource Report NPS/JOTR/NRR—2015/999



ON THE COVER

Triple-ribbed milkvetch, showing the cream-colored flowers and gray, well-spaced leaflets. Photo taken in Joshua Tree National Park, Upper East Deception Canyon, Little San Bernardino Mountains, Riverside County, California.
Photograph by: Tasha La Doux.

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Summary

Background

Triple-ribbed milkvetch (*Astragalus tricarinatus* A. Gray) is a perennial herb in the pea family (Fabaceae) endemic to southern California. It occurs in Riverside and San Bernardino counties in the transition zone between the Mojave and Sonoran deserts (CNDDB 2014, CCH 2014). *Astragalus tricarinatus* was listed by the federal government as endangered in 1998 due to its narrow distribution, limited number of individuals, and vulnerability to ground disturbance activities related to maintenance of an oil pipeline in Big Morongo Canyon (USFWS 1998, 2009). At the time of listing, *A. tricarinatus* had only been observed in four locations (Big Morongo Canyon, Whitewater Canyon, Mission Creek, and Aqua Alta Canyon) during the previous 20 years; however, more than one individual had only been observed in Big Morongo Canyon (USFWS 1998, 2009). Habitat and distribution of this species was thought to be limited to a few scattered individuals in canyons and sandy wash bottoms mostly of the southeastern San Bernardino Mountains and western Little San Bernardino Mountains. Fewer than 100 individuals had been documented by 1998. Since 2004, surveys have yielded more information on the distribution, habitat preference, and abundance of *A. tricarinatus* (Amsberry and Meinke 2007, Fraga and Pilapil 2012, White 2004a, 2004b). We currently estimate 3100 individuals represented by 45 occurrences, and ten of these occurrences host more than 50 individuals.

The purpose of this report is to provide a comprehensive review of *A. tricarinatus* biology, ecology, distribution, taxonomic history, conservation status, and to provide management recommendations for populations that occur within Joshua Tree National Park (Park or JOTR) based on all known current information and recent studies conducted by Rancho Santa Ana Botanic Garden (RSABG) and JOTR. Here we present original research investigating seed germination, population genetics, and a habitat model for *A. tricarinatus*, with emphasis on plants that occur within JOTR. Finally, a monitoring protocol is provided for a pilot study initiated in the Park for assessing demographics and reproductive capacity.

Original Research

Germination trials conducted between 2004 and 2013 indicate that seeds require some form of scarification to germinate. Clipping the seed coat at the hilum yielded 97–100% germination, whereas scarifying the seed coat using sandpaper yielded 68–96% germination. No treatment, a cold water soak prior to sowing, cold moist stratification, and boiling water followed by 24 hours of water soak had the lowest success rates with 0–4% germination rates.

The population genetic study utilized ISSR (inter simple sequence repeat) markers and found individual populations were generally cohesive; however, the populations did not form reciprocally monophyletic population groups. This suggests either historic or contemporary gene flow among all three populations sampled. All three populations have similar levels of genetic diversity, however each population was found to have unique genetic diversity.

The GIS habitat model utilized five parameters (elevation, slope, aspect, soil mapunit, and vegetation association) to assign probability values for potential habitat to the area within the Park. These

environmental datasets helped characterize occupied habitat for *A. tricarinatus*. The output from the model was used to prioritize areas for ground-truthing surveys, which yielded two new occurrences for the species.

Recommendations

Conservation efforts for *A. tricarinatus* must focus on building upon baseline data, especially where gaps exist, by supporting research, field surveys, and long-term monitoring. Proposed research includes expanding the current population genetic study to determine levels of genetic diversity and gene flow across the range, among central and peripheral populations, and to evaluate source-sink dynamics among populations, including the occurrences characterized as “waifs.” Other research that would be particularly useful for future conservation efforts include a phylogenetic study to determine the closest relative of *A. tricarinatus* and its phylogenetic uniqueness, as well as an ecophysiological study to investigate drought tolerance across the range of the species. In addition, pollinator syndrome and breeding mechanism studies would aid tremendously in developing future management strategies. Current management practices in JOTR will include implementing a standardized monitoring protocol, as presented in this report, as well as continued efforts to survey and map known occurrences. Field surveys utilizing the existing habitat model will be used to search for new populations as well as improve the model. Finally, a detailed management plan that identifies a Conservation Area with Core Habitat will be developed in accordance with the Coachella Valley Multiple Species Habitat Conservation Plan and the USFWS 5-year Review. Management actions will be developed to ensure protection of essential ecological processes and protection of biological corridors and linkages critical for the long-term survival of *A. tricarinatus*.

Acknowledgments

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Acronyms and Initialisms

JOTR	Joshua Tree National Park
RSABG	Rancho Santa Ana Botanic Garden
USFWS	United States Fish & Wildlife Service
CCH	California Consortium of Herbaria
CNDDDB	California Natural Diversity Database
CVMSHCP	Coachella Valley Multiple Species Habitat Conservation Plan
EO	Element Occurrence (location record from CNDDDB)
Herbaria acronyms:	C = Natural History Museum of Denmark; US = Smithsonian Institute; MO = Missouri Botanical Garden; UC = University of California Berkeley; RSA = Rancho Santa Ana Botanic Garden; UCR = University of California Riverside
RAWS	Remote Automatic Weather Station
TWC	The Wildlands Conservancy

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Introduction

Scope and Purpose

Two federally listed plant species occur within the Joshua Tree National Park (JOTR) boundaries (Figure 1): *Astragalus tricarinatus* (triple-ribbed milkvetch, Fabaceae) and *Erigeron parishii* (Parish's fleabane, Asteraceae). *Astragalus tricarinatus* was federally listed as endangered in 1998 primarily due to maintenance impacts from a crude oil pipeline running through occupied habitat in Big Morongo Canyon. Other factors contributing to the decision for federal protection included the restricted global distribution, limited number of individuals, and vulnerability to stochastic events (USFWS 1998). The purpose of this conservation assessment is to review and summarize all known background information on *A. tricarinatus* including taxonomic history, rarity and conservation status, habitat information, biology and ecology, known and potential distribution, and threats. We also report findings from germination trials and population genetic studies conducted at RSABG, in addition to results for a GIS-based habitat model developed at JOTR. Finally, we provide recommendations for management specific to JOTR, including a monitoring protocol to collect demographic and reproductive data, and suggestions for future research on *A. tricarinatus*.

Background

Astragalus tricarinatus (Fabaceae) is endemic to southern California and is restricted to Riverside and San Bernardino counties; it primarily occurs in the transition zone between the Mojave and Sonoran Deserts (Figure 2). Currently, there are 45 documented occurrences (CCH 2014, CNDDDB 2014, see Appendix D). Ten of these are considered historic (not observed in over 20 years), whereas at the time of listing (November 1998), there were 15 occurrences, eight considered historic, and only one had more than one individual (USFWS 2009). Since 2004, a number of new populations have been discovered during field surveys; this, in addition to research conducted at known populations, has yielded valuable information on the distribution, habitat preference, and abundance of *A. tricarinatus* throughout its range (Amsberry and Meinke 2007, Fraga and Pilapil 2012, White 2004a, 2004b). *Astragalus tricarinatus* is a perennial herb with white to cream flowers and a distinctive three keeled fruit that give the plant its common name, triple-ribbed milkvetch (Wojciechowski and Spellenberg 2012; Figure 3). It is a spring blooming species, generally between April and June, but can bloom as early as February. This species occurs between 390–1525 m (1285–5005 ft) in elevation, primarily on steep slopes, but sometimes in rocky and gravelly washes (Fraga and Pilapil 2012). Threats to this species include the presence of exotic plant species, trail and road maintenance, illegal off-highway vehicle activity, residential development, wildland fire and suppression activities, climate change, pipeline maintenance, habitat fragmentation, reduced pollinator services, and small population sizes (Fraga and Pilapil 2012, USFWS 2009).

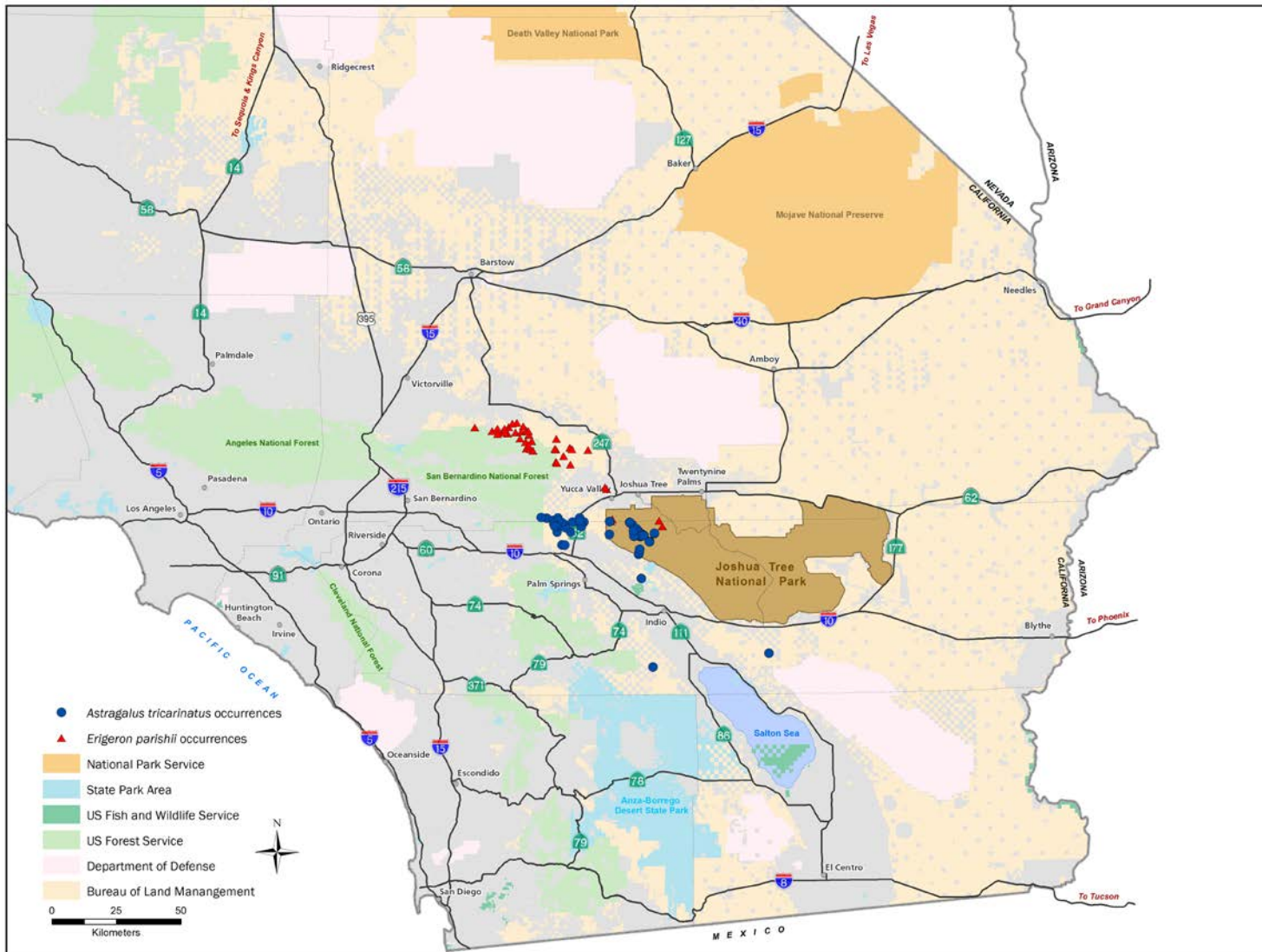


Figure 1. Joshua Tree National Park (shown in brown) is one of three large National Park Service units located in southern California. Two federally listed plants, *Erigeron parishii* (red triangles) and *Astragalus tricarlinatus* (blue circles), are found within the boundaries of Joshua Tree National Park.

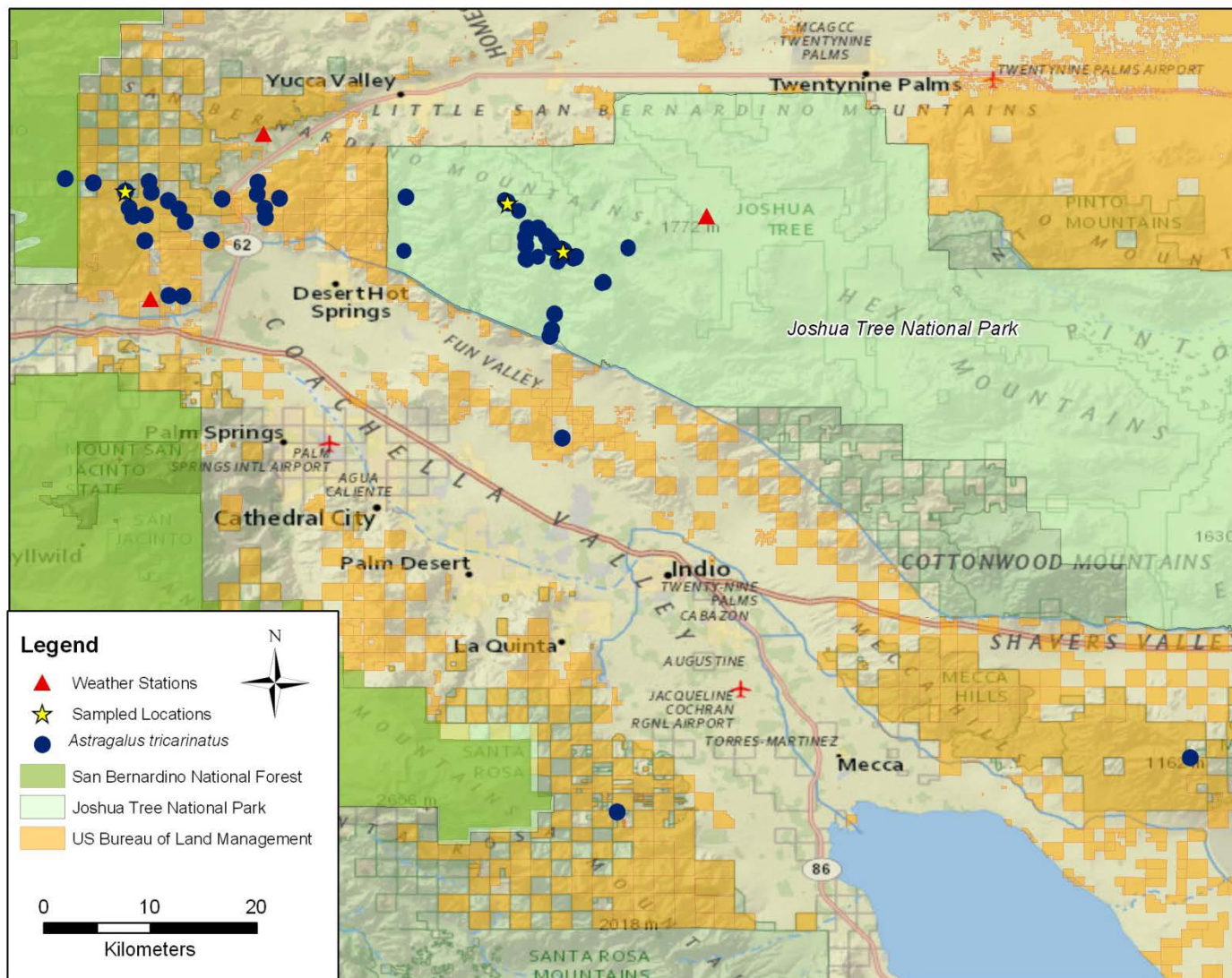


Figure 2. The known global distribution of *Astragalus tricarlinatus* is limited to 45 occurrences (blue circles) in San Bernardino and Riverside counties of southern California. Three localities, Upper Covington Flat, Eureka Peak, and Whitewater Canyon, were sampled (yellow stars) for the genetic study presented in Appendix B. The weather stations (red triangles) used for a climate analysis across the range of the species are presented in Table 4.

Species Description

Astragalus tricarinatus is a relatively short-lived, herbaceous perennial (Figure 3) in the pea family (Fabaceae) that is loosely tufted from a woody taproot (Barneby 1964, Wojciechowski and Spellenberg 2012). Plants are 5–70 cm tall and finely strigose throughout, with straight, appressed hairs. Numerous stems arise from the base and are more or less erect to ascending in clumps (Barneby 1964). During dry seasons and/or after fruiting, the stems and leaves die back to ground level. The leaves are 7–20 cm long, with 17–27 leaflets that are 3–12 mm long, well-spaced, more or less narrowly obovate, elliptic and ovate, silvery canescent adaxially, and more or less green abaxially (Barneby 1964, Wojciechowski and Spellenberg 2012). The inflorescence is a loose raceme, 6–18 cm long with 5–15 flowers that are widely spreading to ascending; the peduncles are erect to ascending, 9–20 cm long (Figure 4). The calyx is 6.1–7.6 mm with mixed black and white hairs; the calyx tube is 4.1–5 mm; the flowers are cream colored, the banner is 12.6–15.7 mm long, recurved at a more or less 45° angle, the keel is 9.7–11 mm long. The fruit is a legume with two locules; at maturity the body is ascending and 24–42 mm long, 3.5–5.5 mm wide, more or less linear, three sided, glabrous, thin and papery; the stalk-like base is 1–2.5 mm long, stout, and jointed at top; the upper suture is a narrow ridge (Barneby 1964, Jones 1923, Wojciechowski and Spellenberg 2012). There are 2–20 seeds per fruit. Chromosome counts are not reported for *A. tricarinatus*, however the new world *Astragalus* have chromosome numbers in an aneuploid series with base numbers of $x = 11, 12, 13, 14, 15$ (Spellenberg 1976).

Taxonomic History

Astragalus tricarinatus was first described by Asa Gray in 1876 from a herbarium specimen collected by Charles C. Parry in Whitewater Canyon, San Bernardino County, California (Gray 1876). In his revision of the North American species of *Astragalus*, Marcus Jones (1923) placed *A. tricarinatus* in section *Hamosi*, however this placement has not been followed by subsequent authors because section *Hamosi* was based on *A. hamosus* L., which is an annual European species and thought to be unrelated (Rydberg 1927). Dr. Per Axel Rydberg (1927) transferred *A. tricarinatus* to the genus *Hamosa* Medik., then created a new section, *Tricarinatae*, within *Hamosa* with *H. tricarinata* (A. Gray) Rydb. serving as the type (Barneby 1964). Subsequent authors have followed Gray's (1876) original treatment by placing *A. tricarinatus* in the genus *Astragalus* L. (Barneby 1964, 1968, Isley 1986, Wojciechowski and Spellenberg 2012). Rupert Barneby (1964) transferred *A. tricarinatus* from its earlier placement in section *Hamosi* of *Astragalus* to section *Leptocarpi* subsection *Tricarinati*, recognizing the section originally described by Rydberg in the genus *Hamosa* at the rank of subsection in *Astragalus*. Section *Tricarinatae* of *Hamosa* included about a dozen species, but as a subsection is now reduced to only include two closely related species: *A. tricarinatus* and *A. bernardinus* M.E. Jones.

Based on morphological similarities, *A. bernardinus* is considered the closest relative to *A. tricarinatus* (Barneby 1964). Their close affinity was first described by Jones (1895) and Jepson found the species to be so similar that he included *A. bernardinus* in his circumscription of *A. tricarinatus* (1936). These two species have nearly overlapping geographic distributions, but differ in

their habitat preference, growth habit, leaf morphology, flower color, and size of flower and fruit (Barneby 1964; Table 1, Figures 3, 4, and 5). *Astragalus pachyphus* has also been confused with *A. tricarinatus*, however the geographic range for these two species do not overlap and they are morphologically distinct. Specimens of *A. pachyphus* from the Central Valley in Kern County where previously misidentified as *A. tricarinatus* (Jones 1923, Jepson 1936). *Astragalus pachyphus* can be distinguished from *A. tricarinatus* by its narrower, linear, or linear-oblong leaflets and its laterally flattened fruit of almost woolly texture (Barneby 1964).



Figure 3. *Astragalus tricarinatus* fruit (left), flowers (center), and habit (right). Photos by Mitzi Harding, Tasha La Doux, and Duncan Bell (left to right).



Figure 4. A large tufted individual of *Astragalus tricarinatus* (left), erect inflorescences (left and center), flowers (center), and leaves (right). Photos by Mitzi Harding.



Figure 5. *Astragalus bernardinus* leaves (left), flowers (left), fruits (center), and weak, wiry stems often twining in shrubs (right). Photos by Mitzi Harding.

Table 1. Distinguishing morphological characters for *Astragalus bernardinus* and *A. tricarinatus*.

Character	<i>Astragalus bernardinus</i>	<i>Astragalus tricarinatus</i>
Habitat	stony areas among desert shrubs, juniper woodlands	exposed rocky slopes, canyon walls along desert washes
Habit	herbaceous perennial often twining in shrubs, inflorescence generally among branches	loosely tufted herbaceous perennial with woody caudex and erect inflorescences
Stem	weak, wiry, slender stems, 10–50 cm	woody caudex with erect, stiff stems, 5–70 cm
Leaflet number	7–19	17–27
leaflet shape	narrow to broadly lanceolate	narrowly obovate
flower color	Pale to dark lilac	white to cream
banner	7.1–10.2 mm	12.6–15.7 mm
keel	6.8–9.4 mm	9.7–11 mm
calyx tube	2.7–4.1 mm	4.1–5 mm
Fruit	three-sided, glabrous, 20–30 mm long, 4–5 mm wide, lower surface often flat or shallowly channeled	three-sided, glabrous, 24–42 mm long, 3.5–5.5 mm wide, narrow ridge on upper suture

Phylogenetic studies

Astragalus is one of the largest and most diverse genera of flowering plants in the world with over 2,500 annual and perennial herbaceous plant species occurring throughout the northern hemisphere and South America. Western North America is one of the centers of diversity for the genus with about 400 species reported to occur here (Sanderson 1991). The genus has received considerable attention from plant taxonomists and several studies have examined systematics and phylogenetic relationships within the North American species (Gray 1964, Jones 1923, Rydberg 1929, Barneby 1964, Isley 1986, Sanderson 1991, Liston 1992, Sanderson and Doyle 1993, Wojciechowski and Spellenberg 2012, Scherson et al. 2005). The aneuploid *Astragalus* species from the New World (chromosome numbers ranging from $x=11-15$) have been informally called Neo-*Astragalus* and form a monophyletic clade nested within old world *Astragalus* species which have euploid chromosome numbers ($x=8, 16, 32$; Sanderson and Doyle 1993). *Astragalus tricarinatus* likely occurs in the Neo-*Astragalus* clade based on the placement of other species that are thought to be relatively closely related, although *A. tricarinatus* has not been sampled in any published phylogenetic studies so relationships within the Neo-*Astragalus* clade remain largely unresolved (Scherson et al. 2005).

Biology and Ecology

Life History

Astragalus tricarinatus is a relatively short-lived perennial that is thought to survive 3–5 years (Amsberry and Meinke 2007, Sanders 1999, USFWS 2009). There are no published demographic studies that have determined average lifespan of plants, although preliminary observations of plants in JOTR support a lifespan of less than 5 years (La Doux and Harding, unpublished data, also see Appendix E). A pilot study to assess occurrence viability was conducted by Amsberry and Meinke in 2005 and 2006 at two locations in the Mission Creek watershed (Amsberry and Meinke 2007). They randomly selected 26 plants for a series of size parameter and reproductive capacity measurements. In 2005, they reported an overall mean stem length of 20.9 cm while the mean number of flowering stems per plant ranged from 18–46 throughout the season. They observed annual dieback at their study site with plants producing new shoots from a woody caudex (Figure 6). In addition, they reported that all reproductive plants appeared to be perennial, 38% had obvious woody bases (Amsberry and Meinke 2007).

Reports of seed production are relatively high for *A. tricarinatus* (estimated 2,759 seeds per plant) compared to reports from seed counts for other species of *Astragalus* (72–450 seeds; Amsberry and Meinke 2007, Gisler and Meinke 2001). An average of 12 viable seeds per fruit were estimated from eight plants at Catclaw Flat (Amsberry and Meinke 2005). Similarly, an assessment of seed accessions at RSABG found 2–20 viable seeds per fruit with an average of 11 seeds per fruit (Table 2). Based on these relatively high values of seed production per plant, it is likely that *A. tricarinatus* maintains a large soil seed bank *in-situ*. Mechanisms for seed dispersal have not been documented; however given that *A. tricarinatus* has larger populations at the heads of canyons and smaller, presumably waif, populations downstream, it is likely that some seeds are dispersed by water and/or gravity (USFWS 2009). Other vectors (e.g. ants, rodents, birds) for seed dispersal are of interest for future studies, as the upland sites appear to have abundant seed banks (White 2004a, 2004b, Amsberry and Meinke 2007).

Table 2. Average number of seeds per fruit for *Astragalus tricarinatus* estimated from two studies.

Study	Date	Location	# of sampled individuals	Fruit #	Total # seeds	Avg seed per fruit
RSABG accession 23184	05/04/2004	Wathier Landing	15	352	3808	10.82
Amsberry and Meinke 2007	05/05/2005	Catclaw Flat	8	59	714	12.10



Figure 6. New shoots emerging from the base of *Astragalus tricarinatus* (top photo). Dead branches are often present among new growth (bottom photo). Photos by Duncan Bell and Tasha La Doux, respectively.

Reproductive Biology

Astragalus tricarinatus has specialized flowers that are characteristic of the legume subfamily Papilionoidae (Fabaceae). The flowers are bilaterally symmetrical, the corolla being composed of five petals, including a generally broad banner that is held dorsally, two free wings (lateral to the banner), and a keel that consists of two fused petals that are opposite and positioned ventrally relative to the banner (Green and Bohart 1975). The flowers are borne on a raceme that is indeterminate and the inflorescence may bloom for several days to a week as individual flowers open along the axis (Barneby 1964, Green and Bohart 1975, Karron 1988, Figure 3). Sexual reproduction in the genus *Astragalus* is usually mediated by medium to large bees although pollinators for *A. tricarinatus* have not been observed. *Astragalus* flowers, like many papilionaceous legumes, have a “tripping” mechanism for pollination (Karron 1988). Pollination of a flower is triggered when a bee lands on the keel and inserts its head under the banner; the keel is depressed (tripped) forcing the sexual column (anthers and stigma) into contact with the bee. This action of tripping the flower allows pollen to be deposited on the bee, and at the same time the stigma can receive pollen brought by the bee from another plant.

Astragalus tricarinatus is a narrow endemic restricted to southern California; its global distribution falls within a 518 sq km (200 sq mi) area (CNDDB 2014, CCH 2014). Such a narrow distribution may have consequences for the reproductive biology of this perennial herb. Several studies have investigated reproductive strategies within the genus *Astragalus*, a genus with a large number of narrow endemics. These studies provided evidence that most narrowly distributed *Astragalus* species are self-compatible and by contrast, their more widely distributed congeners are often self-incompatible (Ralphs and Bagley 1988, Karron 1989, Kaye 1999). To the contrary for *A. lentiginosus* var. *coachellae*, a narrow endemic restricted to California’s Sonoran Desert, Meinke et al (2007) found that while bagged flowers were capable of setting fruits with viable seeds, the selfing rates were extremely low (<2%). These data support outcrossing as a primary means of seed production for *A. lentiginosus* var. *coachellae* despite its narrow geographic range. Data from a population genetic analysis for *A. tricarinatus* (reported in Appendix B) is similar to the *A. lentiginosus* var. *coachellae* results in that the genetic diversity values favor a self-incompatible reproductive system for *A. tricarinatus*. Two of the values we calculated for genetic diversity, θ^H and $G_{ST}B$, fall within a range more similar to outcrossing species than to selfing species (Appendix B). The breeding system of *A. tricarinatus* has not been studied; nonetheless *A. tricarinatus* is thought to be an outcrossing, perennial species of short to moderate life span. A study of the reproductive biology of *A. tricarinatus* involving bagging experiments, pollinator observations, and controlled pollinations could provide more information on pollination mechanisms, breeding system, and the ability to produce viable seed from self-pollination for this rare species.

Consistent with most species of *Astragalus*, *A. tricarinatus* does not show evidence of vegetative reproduction and relies on germination of seeds to recruit new individuals into populations (Barneby 1964, Karron 1989, Kaye 1999). Plants described as seedlings have been observed in multiple years in large populations, including years with above (2004, 2005) and below (2006) average precipitation, suggesting that germination of seeds may occur frequently (White 2004a, 2004b, Amsberry and Meinke 2007). However, this information is not sufficient to assess long-term viability

of populations and more information is needed on the relative importance of life stages and their contribution to the species life cycle (i.e. seed bank, seedling success rates, and reproductive capacity of various life stages).

Genetics

Long-term survival of any species is dependent upon its ability to endure adverse circumstances and adapt to environmental change. This ability is directly tied to the genetic diversity of the species. Knowledge of the breadth of genetic diversity and the distribution of that diversity is critical to management of populations. In ideal circumstances the entire breadth of genetic diversity would be preserved or protected, but this is rarely an option. Populations on the fringe of a distribution often experience more extreme environmental conditions than the species as a whole, and are more susceptible to random genetic drift and subsequent extirpation. Similarly, small populations are at greater risk of extinction due to stochastic events. Information on genetic diversity within and among populations can be used to infer historic gene flow and other evolutionary processes in addition to aiding in the management of individual populations. To better understand the genetic diversity of *A. tricarinatus* located within JOTR, a study of relative genetic diversity was undertaken using an analysis of ISSR genotype data. Two populations within JOTR, one near Eureka Peak (Population 1) and the other below Covington Flats (Population 2), were compared to a single population in Whitewater Canyon (Population 3) at Catclaw Flat on land managed by The Wildlands Conservancy (Table 3, Figure 2). Details of the study are provided in Appendix B.

Table 3. Sampled populations for population genetic study of *Astragalus tricarinatus*. EO= CNDDDB Element Occurrence, TWC= The Wildlands Conservancy.

Pop ⁿ ID	Location	EO#	Latitude	Longitude	Elevation m (ft)	Land Manager	Population size
1	Eureka Peak	32	34.029	-116.356	1486 (4875)	JOTR	~215
2	Covington Flats	28	33.989	-116.309	1435 (4710)	JOTR	~70
3	Catclaw Flat	20	34.039	-116.678	1035 (3395)	TWC	~100

No prior genetic work has been done on *A. tricarinatus*, though a few population genetic studies have been conducted on other species of *Astragalus* (Liston 1992, Karron et al. 1988, Travis et al. 1996, and Alexander et al. 2004). In general, the genus is notorious for low levels of genetic diversity (Liston 1992, Wojciechowski et al. 1999, Kazempour Osaloo et al. 2003, Wojciechowski et al. 2004). For example, in allozyme-based studies, variation is generally low ($G_{ST} < 0.10$, but see *Astragalus clarianus* and *A. pauperculus*; Liston 1990). However, recent population genetic studies using other fragment-based markers (ISSRs and AFLPs; Karron et al. 1988 and Alexander et al. 2004) have detected more diversity in some *Astragalus* species. Based on the findings of our study, *Astragalus tricarinatus* is intermediate in diversity relative to those fragment-based studies (Table B10 in Appendix B).

Each population was characterized by a number of unique ISSR bands, and the number of unique bands was similar across the three populations (14-20 bands). Fourteen unique bands were characterized in the Eureka Peak population, 18 unique bands in the Covington Flats population, and

20 unique bands in the Whitewater Canyon population. Population 2, from Covington Flats in JOTR, had the highest values for all population descriptors including the number of alleles (N_a), number of effective alleles (N_e), Shannon's Information Index (I), Nei's gene diversity (h), Nei's unbiased gene diversity (u_h), and percentage polymorphic loci (%P) (see Tables B3-B5 in Appendix B). Individual populations were generally cohesive, with the majority of samples from each population forming a clade or grade (see Figures B4-B6 in Appendix B). The populations did not form reciprocally monophyletic population groups however, suggesting either historic or contemporary gene flow among all three populations sampled.

According to our data, the Eureka Peak and Covington Flats populations are more closely related to each other ($NeiP = 0.034$; $uNeiP=0.028$) than either are to the Whitewater Canyon population, but they are also much closer geographically. Interestingly, the Covington Flats population is slightly more closely related to the Whitewater Canyon population ($NeiP=0.079-0.081$; $uNeiP=0.073-0.074$) than is the Eureka Peak population ($NeiP=0.082-0.086$; $uNeiP=0.076-0.080$) despite being further away. This is graphically demonstrated in both the PCoA plots (Figures B1-B3 in Appendix B) and the UPGMA trees (Figures B4-B6 in Appendix B). All three populations have similar levels of genetic diversity. These analyses provide evidence for past or contemporary gene flow among all three populations, and that gene flow appears to be high based upon the low G_{ST} values.

Habitat

Astragalus tricarlinatus was thought to occur primarily on sandy or gravelly soils in washes and canyon bottoms, often at the base of scree slopes (Sanders 1999, USFWS 2009). However, since the discovery of a large population at the head of Whitewater Canyon in 2004, the preferred habitat for this species is now thought to be steep, rocky slopes and ridge tops (White 2004a, 2004b). This shift in understanding has led to several additional occurrences documented in similar upland habitats (White 2004a, 2004b, Fraga and Pilapil 2012). Because *A. tricarlinatus* occurrences in washes and canyon bottoms are transitory, and usually consist of few plants, it is thought that plants in these habitats are waifs washed down from upland source populations. Documented occurrences of *A. tricarlinatus* range in elevation between 390–1525 m (1285–5005 ft) (CCH 2014, CNDDB 2014).

Astragalus tricarlinatus is found primarily on southern and westerly slopes with a gradient of 17° or more (Fraga and Pilapil 2012). In canyon bottoms the slopes are gentler with a gradient of 9° or less. Based on data collected from 50 plants in Upper East Deception Canyon (see Appendix E) of the Little San Bernardino Mountains, plants were found on SE- to SW-facing slopes with an average slope of 43° (median = 42°, minimum = 20°, maximum = 90°). Surveys and monitoring for this species is extremely difficult due to the steep slopes and unstable soils (Figure 7).

The *A. tricarlinatus* substrate in Whitewater Canyon has been described as “unproductive looking gravelly soil” and “unusual metamorphic rock” (White 2004a, 2004b). According to Alice Miller (JOTR Vegetation Branch Chief between 2008-2010), the largest occurrences in JOTR “occur on shallow to deep, poorly developed, sandy or sandy-skeletal soils over weakly to moderately cemented bedrock derived from granite and gneiss. These soils are very friable and erosive, and somewhat excessively drained with medium to high runoff; they have low organic content, and low water holding capacity. Soil surfaces are dominated by gravel and fine gravel and have gravelly sand

or gravelly loamy sand textures.” Although the soil at known population locations have not been analyzed, and therefore any superficially “unique” characteristic cannot be defined at this time, it has a distinctive white to gray-green color that can be identified from a distance during field surveys (Figure 7). Soil analysis is needed to determine if soil properties are similar across the range of the species and if exceptional soil properties exist at locations with the largest occurrences. An effort to define which soil series is present at known populations across the range of the species would be very informative, as the current soil map used for the park only utilizes the soil “mapunit”, which describes an area of similar topography, climate, soil, and vegetation, but is quite coarse in scale. According to the NRCS soil map of JOTR (Houdeshell et al 2014), there are three soil mapunits associated with typical *A. tricarinatus* habitat within JOTR:

- 1) Xeric Torriorthents-Bigbernie association: This mapunit is very common throughout the Little San Bernardino Mountains (16,519 acres) and therefore captures the majority of the known occurrences in the park, (i.e. Upper East Deception Canyon). This mapunit is composed of 45% Xeric Torriorthents and similar soils, 25% Bigbernie and similar soils, and 30% dissimilar minor components. The landform is described as steep mountain slopes (30-75%) with east to southwest aspects. The parent material is colluvium derived from granitoid and/or gneiss over residuum weathered from granitoid and/or gneiss. The Xeric Torriorthents are somewhat excessively drained soils, as are the other soils listed below, and are classified as Xeric Torriorthents. Bigbernie soils are classified as sandy-skeletal, mixed, thermic Typic Torriorthents.
- 2) Smithcanyon gravelly sand: This mapunit is very restricted, with only 2,806 acres in the Little San Bernardino Mountains. The Eureka Peak occurrences are found on this mapunit, which is composed of 80% Smithcanyon, dry and similar soils, as well as 20% dissimilar minor components. The landform is described as steep mountain slopes (30-75%) with east to southwest aspects. The parent material is colluvium derived from granitoid and/or gneiss over residuum weathered from granitoid and/or gneiss. Smithcanyon, dry soils are classified as being mixed, thermic, shallow Xeric Torripsamments.
- 3) Smithcanyon-Stubbespring-Rock outcrop complex: This mapunit is not very common in the park with only 4,075 acres designated in the Little San Bernardino Mountains. The two occurrences near Quail Mountain are found on this mapunit, which is composed of 40% Smithcanyon and similar soils, 25% Stubbespring and similar soils, 20% rock outcrop, and 15% dissimilar minor components. The landform is defined as hilly backslopes or side slopes with southeast to northeast aspects and 15–50% slopes. Parent material is colluvium derived from granitoid over residuum weathered from granitoid. Stubbespring soils are classified as loamy, mixed, superactive, thermic, shallow Xeric Haplargids.

Astragalus tricarinatus is mapped as occurring on the following geologic substrates: mafic, felsic, gneiss, granodiorite, and alluvium (USGS 2009, NPS GRI 2014; Figures 8 and 9). According to the unpublished digital geologic map of JOTR (based upon USGS source maps), the majority of known localities are found on the geologic unit titled “*Klmiff* - Layered mafic, intermediate, and felsic rocks, foliated (Late Cretaceous)”, which is generally dark-colored with equal abundance of mafic and

felsic rocks. This geologic unit is widespread throughout the Little San Bernardino Mountains (NPS GRI 2014, Figure 9).

The largest occurrences known in JOTR occur on the *Kmif* geologic unit near Eureka Peak as well as the *Kggif* geologic unit near Quail Mountain. The latter is defined as “granitic, granodioritic, and intermediate rocks, foliated (*Kggif*)”. it is a light-colored substrate derived from granitoid rock material. *Kmif* is defined as “mafic and intermediate plutonic rocks.” It is fine- to coarse-grained and medium to dark gray in color. These rocks range in composition from granodiorite to tonalite and quartz diorite and contain biotite and biotite-hornblend, which could explain the slightly greenish-gray hue of the substrate at the largest *A. tricarinatus* populations (Figure 7). Both *Kggif* and *Kmif* are widespread throughout the Little San Bernardino Mountains. (NPS GRI 2014, Figure 9).

There are a handful of localities in sandy wash corridors representing waif or transient occurrences. Although this habitat does not seem to support large populations, the waif or transient occurrences may play a role in gene flow between occurrences in isolated canyons. These occurrences are found on the *Qyaly* geologic unit, which are present on piedmonts and pediment aprons, and defined as “young alluvium, light-sourced piedmont apron, younger unit.” The material consists of unconsolidated young sand and pebbly sand derived from quartz-rich light-colored granite (NPS GRI 2014; Figure 9). The sandy wash soils are Carrizo, which are very deep sandy-skeletal hyperthermic Typic Torriorthents (Houdeshell et al 2014).

To better understand the habitat preferences of *A. tricarinatus*, and to be able to remotely identify probable habitat of this species, in 2012 a habitat model for JOTR (see Appendix C) was created in ArcGIS Desktop using a Python script (v2.6, © Copyright 1990-2014, Python Software Foundation). The model uses up to five environmental parameters (elevation, slope, aspect, soil mapunit, and/or vegetation association) to assign probability values of suitable habitat to the area within the park. A data layer representing occupied *A. tricarinatus* habitat within JOTR was created using a 15 m radius buffer around known point localities. The model uses this layer to narrow the values of the input parameters to only those that characterize at least 95% of the occupied habitat, and creates a weighted overlay of the results. Surveys in 2013 designed to ground truth the model yielded the discovery of 2 new populations in areas identified as highly probable habitat. The model was designed to be employed iteratively as more occurrences are discovered, and/or more detailed layers describing environmental parameters become available (such as soil series).

An early attempt at creating the model involved manually identifying ranges of the environmental parameters which best characterize occupied habitat in JOTR. It was determined that there are four major vegetation associations (Single-leaf Pinyon Pine / Muller’s Oak Woodland Association, California Juniper / Blackbush Association, Muller Oak — California buckwheat — Narrowleaf goldenbush Association, and Catclaw Acacia — Desert Almond Association) and two soil mapunits (Xerric Torriorthents-Bigbernie association, and Smithcanyon gravelly sand) associated with >94% of known *A. tricarinatus* habitat in JOTR. The soil “mapunit” describes an area of similar topography, climate, soils and vegetation. It was also determined that more than 63% of the mapped occupied habitat is between 1370–1470 m (4495–4823 ft), 96% of occurrences have slopes ranging between 5–84% (3–40°), and 68% showed an aspect ranging between 155°–262° (SSE–W).



Figure 7. Habitat for *Astragalus tricarinatus* in the Little San Bernardino Mountains of Joshua Tree National Park. Top photo shows Erin Babich (red arrow) scrambling up a steep slope in Upper East Deception Canyon while surveying for *A. tricarinatus*. Bottom photo shows a closer view of the unique soil where these plants occur; a permanent plot was established in 2013 at this location near Eureka Peak. Photos taken by Mitzi Harding (top) and Tasha La Doux (bottom).

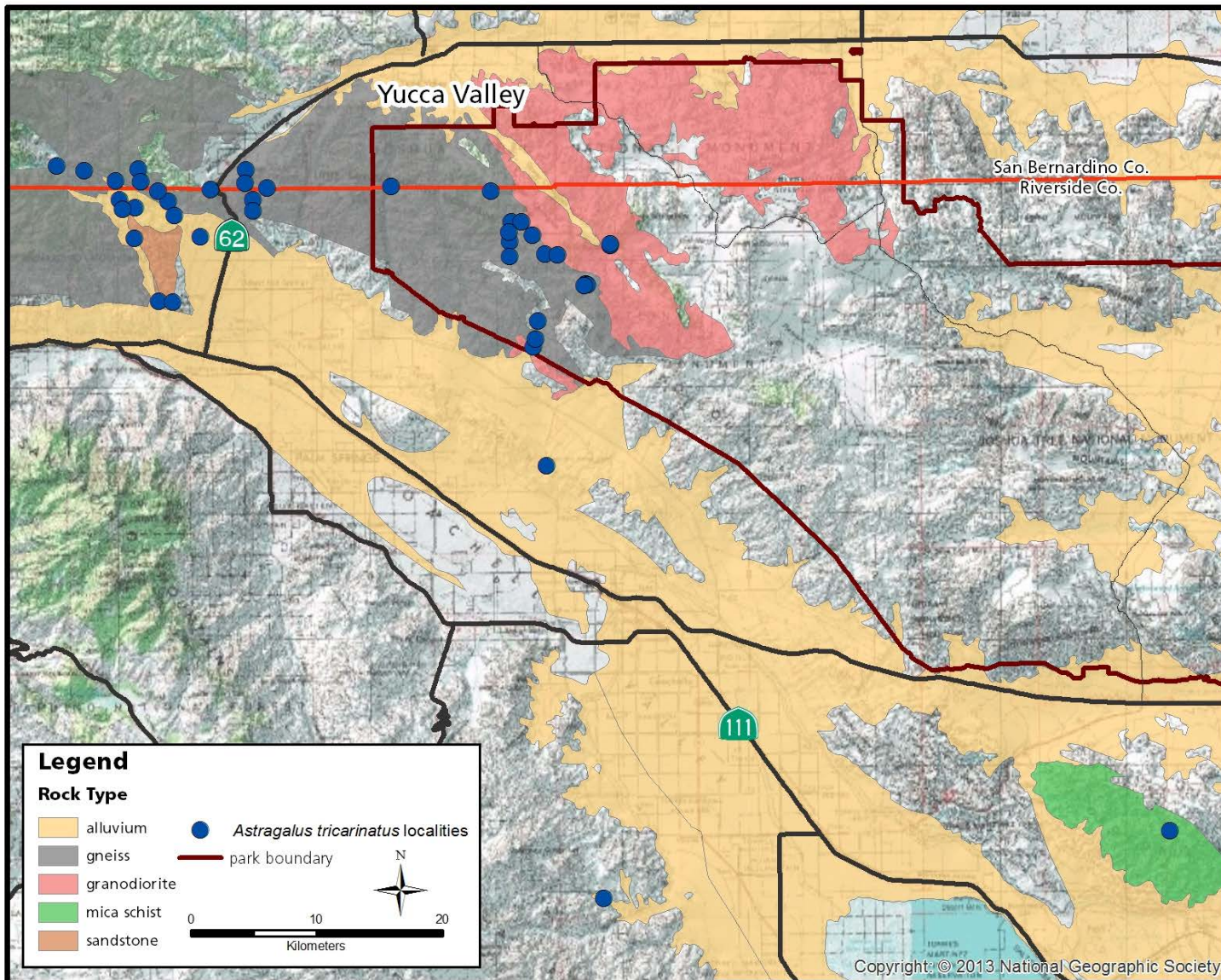


Figure 8. Geologic map of southern California (USGS 2009), showing parent materials that *Astragalus tricarinatus* occurs on throughout its range, including within Joshua Tree National Park.

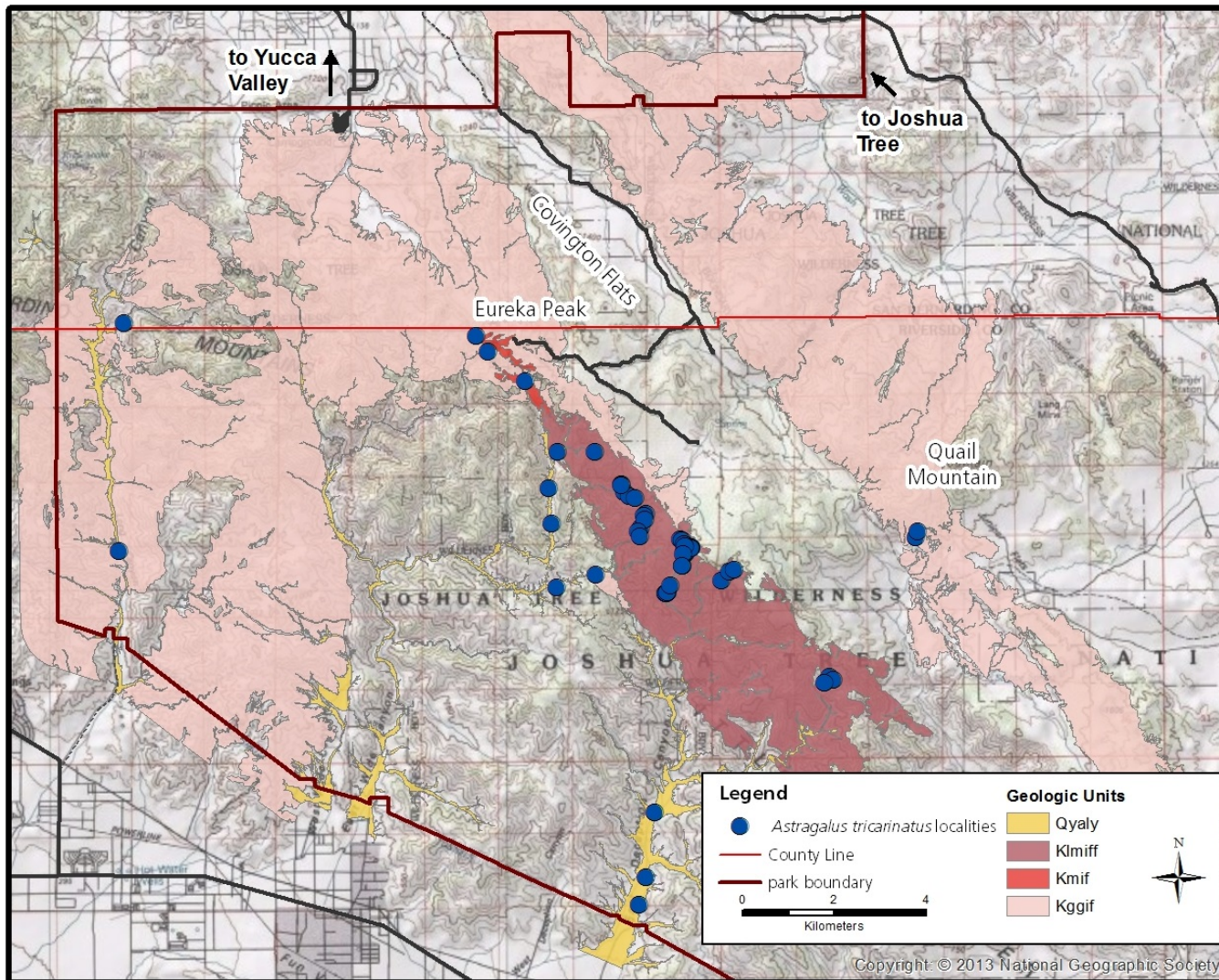


Figure 9. Geologic units associated with *Astragalus tricarinatus* locations in Joshua Tree National Park. Geologic units, listed youngest to oldest: Qyaly - Young alluvium, light-sourced piedmont apron, younger unit (Holocene); Klmiff - Layered mafic, intermediate, and felsic rocks, foliated (Late Cretaceous); Kmif - Mafic and intermediate rocks, foliated (Late Cretaceous); Kggif - Granitic, granodioritic, and intermediate rocks, foliated (Cretaceous) (NPS GRI 2014).

Climate

Long-term persistence of *A. tricarinatus* within its native habitat and current distribution is of concern due to its highly restricted global distribution, small population sizes, and geographic isolation of known occurrences. The global distribution of *A. tricarinatus* is narrow, with the core populations (excluding Agua Alta Canyon and the Chuckwalla/ Orocopia Mountains) spanning approximately 40 km (25 mi) east to west and 20 km (12 mi) north to south. For this reason, it is not unexpected that the weather conditions would be similar in such a localized region, which might suggest that even minor shifts in temperature or rainfall patterns will impact the species. We report mean annual precipitation, average air temperature, minimum air temperature and maximum air temperature from data gathered at three Remote Automatic Weather Stations (RAWS) across the range of *A. tricarinatus* (Table 4, Figure 2). Based on the data from these RAWS, average precipitation ranges from 14.71–17.22 cm (5.79–6.78 in) and average annual temperature ranges from 16.04°–19.49°C (60.88–67.08°F). The White Water RAWS Station occurs at the lowest elevation and is only 1 km west of a known occurrence in the lower portion of Whitewater Canyon; this station reports the warmest maximum temperature based on averages across the last six years (Table 4). The Morongo Valley and White Water Stations report the widest temperature fluctuations; 11–12°C (19–22°F) difference between maximum and minimum temperatures, compared to 3°C (5°F) at the Lost Horse Station. The Lost Horse RAWS Station is 6.7 km northeast of the nearest known occurrence of *A. tricarinatus* in JOTR; however it is at a similar elevation to occurrences near Covington Flat. The Lost Horse weather station reports the coolest average air temperature, but is also the driest with 14.71 cm (5.79 in) average annual precipitation. Current and future climate conditions may impact the long-term survival of the species. Unfortunately, no studies have been done to determine thresholds for temperature extremes, nor do we know the impact of changes in precipitation patterns and/or amounts.

Table 4. Weather data from three RAWS stations (WRCC 2014) spanning the geographic and elevation range of *Astragalus tricarinatus* (Figure 2). National Weather Service identification number is in parentheses following the weather station name.

RAWS Weather station (NWS ID):	Lost Horse (45614)	Morongo Valley (45863)	White Water (45628)
Mean annual precipitation cm (in)	14.71 (5.79)	17.22 (6.78)	15.40 (6.06)
Mean avg. air temp °C (°F)	16.04 (60.88)	15.78 (60.4)	19.49 (67.09)
Minimum avg. air temp °C (°F)	14.29 (57.72)	11.54 (52.78)	14.44 (58.26)
Maximum avg. air temp °C (°F)	17.07 (62.72)	23.73 (74.72)	25.11 (77.19)
Elevation m (ft)	1280 (4200)	780 (2561)	776 (2546)
# of years data collected	22	6	6

Vegetation and Associated species

The upland habitats most frequently associated with *Astragalus tricarinatus* include: *Larrea tridentata* Shrubland Alliance, *Pinus monophylla* Woodland Alliance, *Quercus cornelius-mulleri* Shrubland Alliance, and *Juniperus californica* Woodland Alliance; whereas the lowland wash habitats are generally classified as *Ambrosia salsola* Shrubland Alliance, *Chilopsis linearis* Woodland Alliance, and *Acacia greggii* Shrubland Alliance (CNDDB 2014, Sawyer et al. 2009, USFWS 2009, Keeler-Wolf et al. 2005, Evens et al. 2012, La Doux et al. 2013).

According to the vegetation classification for JOTR (Keeler-Wolf et al. 2005, Evens et al. 2012, La Doux et al. 2013), *A. tricarinatus* most commonly occurs in the following vegetation associations: *Pinus monophylla* / *Quercus cornelius-mulleri* Woodland Association, *Juniperus californica* / *Coleogyne ramosissima* Association, *Quercus cornelius-mulleri* — *Eriogonum fasciculatum* — *Ericameria linearifolia* Association, *Acacia greggii* — *Prunus fasciculata* Association.

The following species are commonly associated with *A. tricarinatus* throughout its range:

<i>Acmispon glaber</i> (Vogel) Brouillet	deer weed
<i>Ambrosia salsola</i> (Torr. & A. Gray) Strother & B.G. Baldw.	cheese bush
<i>Bebbia juncea</i> (Benth.) Greene var. <i>aspera</i> Greene	sweet bush
<i>Chilopsis linearis</i> (Cav.) Sweet subsp. <i>arcuata</i> (Fosberg) Henrickson	desert willow
<i>Crossosoma bigelovii</i> S. Watson	ragged rock flower
<i>Dudleya lanceolata</i> (Nutt.) Britton & Rose	lance-leaved live forever
<i>Echinocereus engelmannii</i> (Engelm.) Lem.	hedgehog cactus
<i>Encelia farinosa</i> Torr.	brittle bush
<i>Eriodictyon trichocalyx</i> A. Heller	yerba santa
<i>Ericameria linearifolia</i> (DC.) Urbatsch & Wussow	golden bush
<i>Eriogonum fasciculatum</i> Benth.	California buckwheat
<i>E. saxatile</i> S. Watson	hoary wild buckwheat
<i>Juniperus californica</i> Carrière	California juniper
<i>Hesperoyucca whipplei</i> (Torr.) Trel.	chaparral yucca
<i>Hilaria rigida</i> (Thurb.) Scribn.	big galleta grass
<i>Keckiella antirrhinoides</i> (Benth.) Straw	yellow bush snapdragon
<i>Larrea tridentata</i> (DC.) Coville	creosote bush
<i>Mirabilis laevis</i> (Benth.) Curran	desert wishbone bush
<i>Opuntia basilaris</i> Engelm. & J.M. Bigelow var. <i>basilaris</i>	beavertail cactus
<i>Peritoma arborea</i> (Nutt.) H.H. Iltis	bladder pod
<i>Psoralea arborescens</i> (A. Gray) Barneby	Mojave indigo bush
<i>Quercus cornelius-mulleri</i> Nixon & K.P. Steele	Muller's oak
<i>Rhus ovata</i> S. Watson	sugar bush
<i>Salvia apiana</i> Jeps.	white sage
<i>Senegalia greggii</i> (A. Gray) Britton & Rose	cat claw
<i>Sphaeralcea ambigua</i> A. Gray	globe mallow
<i>Stephanomeria pauciflora</i> (Torr.) A. Nelson	wire lettuce
<i>Stipa coronata</i> Thurb.	crested needle grass
<i>Yucca schidigera</i> Ortgies	Mojave yucca
<i>Ziziphus parryi</i> Torr. var. <i>parryi</i>	Parry's jujube

Within JOTR, *A. tricarinatus* is often associated with the following species (in addition to those listed above):

<i>Acmispon heermannii</i> (Durand & Hilg.) Brouillet	Heermann's lotus
<i>Acmispon rigidus</i> (Benth.) Brouillet	shrubby deervetch
<i>Arctostaphylos glauca</i> Lindl.	bigberry manzanita
<i>Asclepias subulata</i> Decne.	rush milkweed
<i>Cercocarpus betuloides</i> Nutt.	mountain mahogany
<i>Chrysothamnus viscidiflorus</i> (Hook.) Nutt.	yellow rabbitbrush
<i>Encelia actoni</i> Elmer	Acton's brittlebush
<i>Ericameria paniculata</i> (A. Gray) Rydb.	black-banded rabbitbrush
<i>Ericameria teretifolia</i> (Durand & Hilg.) Jeps.	round leaf rabbit brush
<i>Eriogonum nudum</i> Benth. var. <i>pauciflorum</i> S. Watson	little-flower wild buckwheat
<i>Eriogonum wrightii</i> Benth. var. <i>nodosum</i> (Small) Reveal	knot-stem bastard sage
<i>Lepidospartum squamatum</i> (A. Gray) A. Gray	scale-broom
<i>Linanthus pungens</i> (Torr.) J.M. Porter & L.A. Johnson	granite gilia
<i>Lupinus andersonii</i> S. Watson	Anderson's lupine
<i>Lupinus excubitus</i> M.E. Jones	grape soda lupine
<i>Nolina parryi</i> S. Watson	Parry's beargrass
<i>Pinus monophylla</i> Torr. & Frem.	singleleaf pinyon pine
<i>Prunus fasciculata</i> (Torr.) A. Gray	desert almond
<i>Psoralea schottii</i> (Torr.) Barneby	indigo bush
<i>Quercus john-tuckeri</i> Nixon & C.H. Mull.	Tucker oak
<i>Salvia mohavensis</i> Greene	Mojave sage
<i>Stipa speciosa</i> Trin. & Rupr.	desert needle grass
<i>Yucca brevifolia</i> Engelm.	Joshua tree

Distribution and Abundance

The distribution of *A. tricarinatus* spans 126 km (78 mi) east to west, from the foothills of the San Bernardino Mountains, east to the Chuckwalla Mountains (Figures 1 and 2) and encompasses about a 518 sq km (200 sq mi) area (CNDDDB 2014, CCH 2014). We estimate that *A. tricarinatus* has a global population of nearly 3100 individuals distributed among 45 known occurrences in ten watersheds throughout its geographic range (CNDDDB 2014, Fraga and Pilapil 2012). For comparison, at the time of listing *A. tricarinatus* was mostly known from historical occurrences (had not been seen in >20 years) within seven watersheds, representing seventeen occurrences and fewer than 100 individuals (USFWS 1998).

Status of Populations

Most occurrences (the CNDDDB definition for an “occurrence” includes all individuals within ¼ mile of each other) of *A. tricarinatus* are on public lands or private preserves including: Bureau of Land Management, Joshua Tree National Park, the San Bernardino National Forest, and The Wildlands Conservancy White Water and Mission Creek Preserves (CNDDDB 2014). The two largest populations occur on land managed by The Wildlands Conservancy at Wathier Landing (estimated 1300 individuals in 2010; EO# 14) and 1 mi WNW of Wathier Landing (estimated 900 individuals in 2010; EO# 36). The area southwest of Eureka Peak in JOTR has the third largest occurrence with 215 plants observed in 2011. Most known occurrences (70% or 30 occurrences) have ten or fewer individuals documented at the site or have an unknown number of individuals because the occurrence is “historic”, i.e. they have not been observed in over 20 years (CNDDDB 2014). The remaining 11 occurrences have population sizes that range from 21 individuals to 96. A frequency distribution graph shows the disparity in population size at documented occurrences (Figure 10). Small populations likely represent transient or waif populations that are washed down from larger populations higher in the watershed.

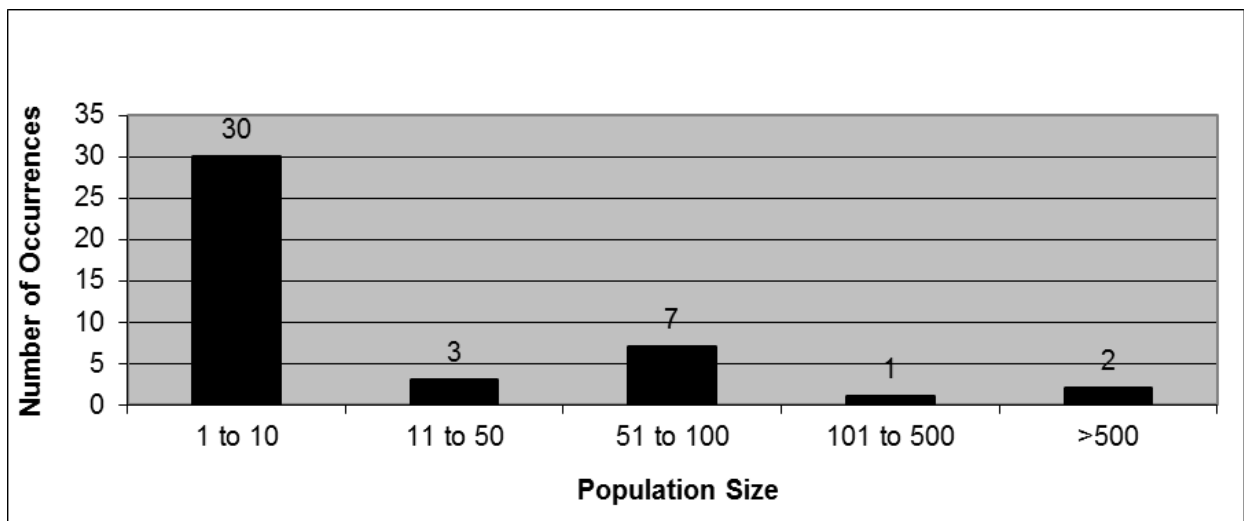


Figure 10. Frequency distribution graph for population size of *Astragalus tricarinatus*. Most occurrences (70%) have 10 or fewer individuals and only two occurrences have more than 500 individuals.

Population trends for *A. tricarinatus* are difficult to discern for lack of systematic monitoring for the species across its range. Ten of the occurrences recorded in the CNDDDB are historic and four of these have such vague locality information that they have been difficult to relocate (CNDDDB 2014).

According to the data in CNDDDB (2014), only one known occurrence was recorded as having Excellent (A) habitat quality (Wathier Landing, EO# 36); it has no threats listed. Eight occurrences are rated as good (B), including the largest population at Catclaw Flat (EO# 14), as well as three occurrences in JOTR near Covington Spring (EO#s: 27, 28, 30). Seven occurrences are rated as fair (C), and nine occurrences are rated as poor (D). Twelve occurrences, including several historic occurrences, are ranked as unknown. Several of the low ranks are presumably due to small population size at those locations since few threats are listed in CNDDDB (2014).

In JOTR, there are 18 known *A. tricarinatus* occurrences, 12 of which have six or fewer individuals and are likely better categorized as waif occurrences (i.e. not likely to be effective populations). There is one occurrence with 24 individuals and five occurrences with ≥ 65 individuals recorded (only one of these exceeds 100 individuals). The long-term viability of *A. tricarinatus* in JOTR, therefore, is considered uncertain due to the small number of individuals and their isolation from one another. Within the Little San Bernardino Mountains in JOTR, there are approximately 12 acres of occupied habitat with an estimated 560 plants. The occurrence near Eureka Peak (EO#32) contains the highest number of plants, estimated at 215 individuals.

Prior to 2006, presence of *Astragalus tricarinatus* in JOTR was only known from three vouchers:

1. Patrick Leary and J. Hogan, 3 May 1975, Long Cyn, 1.5 mi N of Joshua Tree NM boundary; T2SR5ES22, 1800 ft.; Riverside County (UNLV9780).
2. Harlan Lewis, 28 April 1940, Joshua Tree National Monument, Keys View; Riverside County (LA205753). Recently annotated to *Astragalus bernardinus* (Tom Huggins pers. comm.).
3. J. B. Feudge (1462), 9 May 1926, Keyes Ranch, Little San Bernardino Mts, 1220 m; Riverside County (POM147871).

In 2006, three new occurrences were discovered in JOTR; two along the southern boundary in East Deception Canyon and one in Long Canyon. All three occurrences were found in sandy washes and are considered waif occurrences (EO#s: 21, 22, 23). The discovery of these waifs prompted additional surveys upstream and eventually led to the discovery of a larger population (~80 individuals total) in Upper East Deception Canyon in 2008 (EO#s: 28, 29). These occurrences grow on a unique light-colored substrate (as described earlier under the Habitat section), which provided a much stronger search image for field surveys. The Park was then able to launch a concerted effort for targeted surveys in similar habitats. Since 2008, eleven occurrences have been documented, including the third largest population known for the species (EO#32).

Threats

Current threats to *A. tricarinatus* are not well documented, in part because accessing its habitat makes it a challenging species to study. The greatest threat to this species is likely its small isolated populations with a limited global distribution - rendering it vulnerable to stochastic events (Holsinger 2013). Threats associated with small population sizes (<500 individuals) are applicable to most (95%) of the occurrences (Figure 10). While it is likely that additional occurrences will be documented with further field efforts, the chances seem low for discovering additional large populations (>500 individuals) simply based on the low number of large populations found to date, despite many years of concerted field efforts. In addition, the remoteness of suitable habitat makes new discoveries of large populations less likely.

At listing, threats to *A. tricarinatus* included maintenance activities for the crude oil pipeline in Big Morongo Canyon, vehicle use, and vulnerability due to small population sizes (USFWS 1998, 2009). The listing rule recorded past instances of plants destroyed during road grading activities and plants impacted in pipeline realignment procedures (USFWS 1998). These activities remain a threat in Big Morongo Canyon. Additional threats that have been listed for *A. tricarinatus* include mining activity, residential development, illegal off highway vehicle activity, the presence of exotic plant species, trail and road maintenance, wildland fire and suppression activities, and climate change (Fraga and Pilapil 2012, USFWS 2009).

There is little significant mining activity within the range of *A. tricarinatus*, but one occurrence (EO#18) is near an active quarry. There is a substantial amount of potentially minable gravel in Whitewater Canyon, therefore future mining is a concern (USFWS 2009).

Much of the known occupied habitat is extremely rugged and difficult to access; therefore most occurrences are not subject to direct impacts from development pressure (e.g. housing), however, the occurrences in Lower Mission Creek are currently threatened by development. There is concern for the indirect effects of residential or commercial development occurring nearby, especially along the southern boundary of JOTR, starting at state highway 62 all the way to Indio. With an increase in human population in this region, we can expect increased illegal OHV activity, spread of non-native or invasive species, disrupted pollinator services by native pollinators, and the potential for increased fire frequency. In addition, threats of urbanization and development into habitats downstream from large populations, such as in Lower Mission Creek, could prove quite pervasive. The role of waif or transient populations found along alluvial corridors remains unknown. It is possible that these transient occurrences, for example in East Deception Canyon, are in fact contributing to the flow of genes among populations that may otherwise be isolated from one another.

Non-native species that are associated with and may pose a threat to *A. tricarinatus* include *Bromus madritensis* L. subsp. *rubens* (L.) Husn. (red brome) and *Brassica tournefortii* Gouan (Saharan mustard). However, direct impacts caused by the presence of exotic or invasive species have not been documented, nor are disease and predation known to be threats affecting *A. tricarinatus* (USFWS 2009). In addition, the impact of local or global climatic changes on seed bank ecology,

mortality/recruitment rates, longevity of individuals, reproductive success, or other life-history and ecological traits remain unknown for this species.

Conservation Status

Astragalus tricarinatus was listed by the federal government as endangered in 1998 and is considered under the Coachella Valley Multiple Species Habitat Conservation Plan (CVMSHCP), a regional conservation plan developed under section 10(a)(1)(B) of the Endangered Species Act (CVMSHCP 2007, 2014). Critical habitat for *Astragalus tricarinatus* has not been designated (USFWS 2009), and no recovery plan or outline has been published for *A. tricarinatus* (USFWS 2014, species profile website).

The current rarity ranking of this species is provided below (CNPS 2014).

- Federal Listing Status: Endangered
- State Listing Status: None
- State Rank: None. S1
- California Native Plant Society: 1B.2 (Fairly endangered in California)
- Global Rank: G1

Ex-situ Conservation: Seed Bank Holdings

Conservation of plant species can be achieved by protection of habitats and populations in nature (*in-situ*), or by the preservation of genetic diversity outside of an organism's natural habitat (*ex-situ*), often in gene banks, botanic gardens, zoos, or translocation sites (Cohen et al. 1991). *Ex-situ* conservation provides back-up for biological diversity that might otherwise be lost in nature due to human induced environmental change or natural extinction. Seed banks are considered one of the most economical forms of *ex-situ* conservation. It not only provides an excellent opportunity for maintaining genetic diversity in vulnerable populations, but they also provide a low impact means for research opportunities. In other words, without depleting or impacting the natural populations, seed bank material could be grown in a greenhouse setting to study some very important ecological questions, such as mating system, climate thresholds, seed viability, germination requirements, and fire ecology. Although there are no plans to augment native populations of *A. tricarinatus* at this point, this may be considered in the future if significant declines in population numbers are documented.

There are four seed accessions of *A. tricarinatus* stored in the seed bank at RSABG (Table A1 in Appendix A); two of these are from populations within JOTR. Germination studies of seeds for *A. tricarinatus* have been conducted to assess baseline seed viability and germination requirements. Seed germination trials conducted at RSABG report variable rates that range from 0 to 100% germination depending on treatment (RSABG internal data). Nicking or clipping the seeds using a knife or scalpel produced the highest germination rate consistently, with 97-100% germination rate. The lowest germination rates (i.e. no seeds germinating) were observed after “no treatment” or when

seeds were soaked in water prior to sowing. Many *Astragalus* are well known for having hard impermeable seed coats that cause physical dormancy and prevent germination. The hard seed coats prevent water absorption, permeation of gases, or they may constrain the embryo, all of which can cause dormancy (Baskin and Quarterman 1969). Seeds with this dormancy mechanism require abrasion or scarification by chemical or mechanical means in order for germination to occur. Based on the trials presented here, it appears that clipping the seed coat provides the most reliable method for triggering germination in *A. tricarinatus*. The oldest *A. tricarinatus* seed accession currently in storage was collected in 2004. During germination trials in 2014, these seeds germinated at 97% (29 of 30) after clipping, however 10 of the germinated seeds had poor cotyledon development and died, which lowered the post-germination survivorship to 63%. Whether the poor survivorship is due to the age of the seeds cannot be determined without further testing, therefore it is unknown if seeds will retain high viability in cold storage (-20 C) for long periods of time. Given that these seeds are likely to remain dormant in natural soil seed banks, we suspect that the seeds will remain viable in dry cold storage for many years. Additional details for the germination studies conducted at RSABG are summarized in Appendix A.

Research and Management Recommendations

Research

Many knowledge gaps exist with regard to the life history, geographic distribution, habitat preference and ecology of *A. tricarinatus*. Research questions outlined here aim to advance our knowledge of this species to inform management strategies and long term conservation efforts.

Population-level and phylogenetic studies

We recommend pursuing population genetic and phylogenetic research that would further our current knowledge of the genetic distinctiveness and diversity present within and among populations throughout the range. These studies could aid in prioritizing conservation efforts. Examples include:

1. Expanding the population genetic study presented in this report to include additional populations across the range. The initial ISSR study (Appendix B) indicates that there are unique alleles present in the JOTR populations, i.e. alleles not present in the San Bernardino Mountains. However, due to the limited sampling used in the initial study it is possible these alleles are not limited to JOTR. Expanding this study will provide information regarding the uniqueness of the JOTR populations.
2. There are no published phylogenetic studies that have sampled *A. tricarinatus* or its close relatives. It is hypothesized that *A. bernardinus* is the closest relative based on morphology, however additional information is needed regarding the phylogenetic position of *A. tricarinatus* relative to other species of *Astragalus* and its evolutionary history.
3. Levels of gene flow among populations of *A. tricarinatus* are not well understood and the parameters that were analyzed in the current study are not well suited to study contemporary gene flow. An expanded paternity analysis utilizing the genotypes of the mother plants and

their offspring compared with a pool of potential fathers within a population would provide information on contemporary gene flow and directionality.

4. The role of transitory populations or waif individuals for gene flow among populations would be of great interest to long-term protection of the species. If these transitory gene pools play an important role in connecting populations, conservation of the supporting habitats might become a greater priority.

Life-history, ecology, and reproductive biology studies

We recommend ecological studies addressing any gaps in current knowledge of basic biology for *A. tricarinatus*. In particular, studies that would significantly add to the long-term management and conservation of the species include, but are not limited to:

1. Long-term population viability, including waifs and transient populations found in the wash corridors.
2. Determine rates of survivorship for individuals and determine meaningful demographic size classes (e.g. seedlings, juveniles, and adults).
3. Identify effective pollinators and learn more about pollinator biology, including timing of pollination, life cycles, pollen dispersal distances, host plants, etc.
4. Evaluate potential seed dispersal vectors (animals, wind, etc.)
5. Determine whether self-pollination is possible, or whether any reproductive barriers are present (e.g. self-incompatibility, inbreeding depression).
6. Conduct a more detailed soil analysis. For example, define which soil series are present at populations and determine unique chemical or physical properties across the range of the species. Also, determine if exceptional soil properties exist at the locations that hold the largest populations.
7. Document physiological response to various rainfall patterns (amount/timing of rains, monsoonal rains), as well as changing environmental conditions such as shifting seasons or higher/lower temperature extremes.
8. Determine germination rates after fire (smoke treatment on seeds), as well as the ability to resprout from the base post-fire.
9. Determine germination and survivorship rates for seeds of varying ages, under various storage methods, and further testing on scarification methods to mimic natural conditions (both physical and chemical treatments).
10. Test for changing environmental conditions in a controlled environment. For example, the effect of extended periods of drought or increased competition from non-native species on survivorship.

11. Improve our understanding of preferred habitat parameters by conducting additional surveys and analyzing with habitat models.
12. Determine whether rhizobium and mycorrhizae relationships are necessary for or enhance growth for this species through cultivation experiments.

Park Management

Field Surveys and Habitat Modeling

Continued efforts to locate new populations utilizing the habitat model will remain a priority, as well as improving upon the habitat model. Field surveys will focus on high probability areas according to the maps produced by the current model (see Appendix C). Because the model is meant to be iterative, any new data points must be uploaded immediately, and the model updated before another field survey takes place. According to the current habitat model, geospatial data is most effective if taken for each individual more than 15 m apart, so time permitting, additional geospatial data will be collected. Future habitat models, if possible, will incorporate absence points, as well as providing a means to weight the parameters differently. In addition, we hope to include habitat information from the entire geographic range in future habitat models.

Finally, future efforts will prioritize then revisit all known occurrences in order to document population status and changes over time. Revisits will likely need to occur every 3–5 years, however the revisitation rate will need to be adapted according to any observed trends.

Annual Monitoring

As recommended in the CVMSHCP (2007, 2014) and by the USFWS (2009) 5-year Review, annual monitoring will occur in JOTR. We propose following the protocol presented in Appendix F at the two permanent plots for a minimum of five years, or until better data can be used to adapt improved protocols. The frequency of monitoring could be reassessed after the first five-year period. With consistent annual data collection, results from statistical analyses on demographics and reproductive biology for this species will be much more effective at predicting any correlative relationships. In particular, long-term viability of the two *A. tricarinatus* occurrences being monitored, as well as longevity of individuals, will be addressed. Adding waif or transient populations found in wash corridors to the annual monitoring would provide valuable information about their role in the long-term viability of the species. Finally, there are a number of recommendations presented in Appendix E pertinent to annual monitoring that will be followed or addressed.

Protection

Complete protection should be attempted for all individuals and occupied habitat of *A. tricarinatus*, in accordance with the Endangered Species Act (USFWS 2014). Collecting of specimens can be allowed for the purpose of research or recovery efforts, but must be permitted by NPS and reported to USFWS.

Areas within JOTR with known populations or individuals will be designated as a strict “no disturbance” zone, which would exclude any kind of ground disturbance, vegetation pulling, trimming, or removal. In particular, fire suppression activities (e.g. hand lines) should not be allowed

in and around the known populations. Any newly proposed trails or access routes will require field surveys and GIS analysis prior to plan approval in order to ensure the route avoids all known individuals and potential habitat.

Joshua Tree National Park was identified in the Coachella Valley Multiple Species Habitat Conservation Plan (CVMSHCP) as a potential Conservation Area (CVMSHCP 2007, 2014). Now with the current information in place, a detailed management plan adopting the goals and objectives of the CVMSHCP will be developed. Specifically, the plan will need to specify a Conservation Area with Core Habitat in accordance with the CVMSHCP and the USFWS 5-year Review, as well as develop management actions that will ensure protection of essential ecological processes and protect biological corridors and linkages (CVMSHCP 2014, USFWS 2009).

Finally, as a measure to protect the genetic diversity found within JOTR, an ex-situ seed bank will be established. Seeds will be collected from all (or most) known populations and placed in long-term storage at RSABG. All seed collection, viability testing, and seed processing will follow ethical and current protocols, such as those described in the Seeds of Success (2014) technical report.

Appendix A: Germination Study

By Naomi Fraga, Michael Wall, and Evan Meyer, Rancho Santa Ana Botanic Garden

Seeds in the legume family (Fabaceae), including *Astragalus*, are well known for having hard impermeable seed coats that prevent germination and cause physical dormancy. Hard seed coats can cause dormancy because they prevent absorption of water, permeation of gases, or they may constrain the embryo (Baskin and Quarterman 1969). Hard seed coats generally become permeable and seeds often germinate after the seed coat is abraded or scarified by chemical or mechanical scarification. In nature, seeds of *Astragalus tricarinatus* are likely scarified through a heating/cooling cycle, which may include some type of chemical interaction from the soil, and/or the physical action of sand or gravel abrading the seed coat during rain events. Regardless of how the seed coat is broken, doing so allows germination to occur when seeds are moistened.

Currently, there are four seed accessions of *Astragalus tricarinatus* held at RSABG (Table A1), all localities are from the Little San Bernardino Mountains. Two accessions are from within Joshua Tree National Park (JOTR), collected in 2011 from the Eureka Peak and Upper Covington Flat areas. The other two accessions are from Wathier Landing and Whitewater Canyon area, collected in 2004 and 2011, respectively.

Amsberry and Meinke (2007) conducted a pilot study on germination requirements for *A. tricarinatus*. Although the methods used for scarification were not included in the report, they found that 80% of viable seeds germinated within 72 hours after “scarification” and wetting. Between 2004 and 2013 a variety of treatments were tested at RSABG, success rates varied from 0–100%, depending on the pre-treatment of seeds (Table A2). All germination tests at RSABG were conducted on 0.5% agar solution on clear plastic examination plates maintained at 11 hrs. light at 20°C and 13 hrs. dark at 12°C. Prior to germination, seeds were sterilized using a 20% bleach and 1% tween solution. Unsuccessful treatments included: “no-treatment” and “water soak”, both yielding 0% germination; boiling water followed by a 24-hour soak yielded 2% germination; and cold moist stratification yielded 4% germination. More successful treatments included: scarification of seed coat using abrasion (sandpaper rubbed for 3 min), which yielded 68-96% germination; and clipping the seed coat at the hilum using a scalpel, which yielded 97–100%.

Additional germination trials could focus on longevity of seeds in the seed bank and analyzing the potential contributors to a chemical scarification process.

Table A1. Current seed accession information for *Astragalus tricarinatus* at the RSABG seed bank.

ACC #	Date	Collector	State	County	Land Manager	Location
23439	14-Jun-11	Mitzi Harding	California	Riverside	JOTR	Little San Bernardino Mountains; approximately 1 mile west of Eureka Peak
23438	14-Jun-11	Mitzi Harding	California	Riverside	JOTR	Little San Bernardino Mountains; approximately 1.5 miles south of Upper Covington Flats parking area/backcountry trailhead
21384	29-Apr-04	Michael Wall	California	San Bernardino	The Wildlands Conservancy	Little San Bernardino Mountains; ESE of Mount San Gorgonio, headwater region of N fork of Whitewater River; E end of Catclaw Flat at Wathier Landing, ca. 6.0 mi. WNW along trail from stone house
23425	3-May-11	Duncan S. Bell	California	Riverside	The Wildlands Conservancy	Little San Bernardino Mountains; just off the Pacific Crest Trail between the West Fork of Mission Creek and Whitewater Canyon. USGS Quad: Catclaw Flat

Table A2. Germination trial information for seeds stored at the RSABG seed bank.

ACC #	Date tested	Treatment	# Tested	Total Germ.	%	Observations
23425	16-Apr-12	CL	15	15	100	all seedlings with very healthy root and cotyledon development
23439	19-Jul-11	CL	20	20	100	healthy root and cotyledon development
23425	05-Jul-11	CL	24	24	100	all seedlings with healthy vigorous root and cotyledon development
21384	21-Jun-04	CL	30	29	97	19 of 30 with healthy root/ cotyledon; 10 of 30 with stunted, deformed root and/or shoot or had cotyledons detach from root
23425	18-Mar-13	SC	50	48	96	48 with healthy root and cotyledon. 1 with stunted root, normal cotyledon. 1 ungerminated seed hard, probable that seed coat was insufficiently scarified.
23425	02-Aug-11	SC	50	34	68	healthy root and cotyledon development; severe mold on plate rapidly occurred killing seedlings
23425	02-Aug-11	CS1	50	2	4	healthy root and cotyledon development; ungerminated seeds hard
23425	02-Aug-11	HW1	50	1	2	healthy root and cotyledon development; ungerminated seeds hard
23425	02-Aug-11	WS	50	0	0	all seeds hard, no imbibition
23425	02-Aug-11	NT	50	0	0	all seeds hard, no imbibition
CL	Clip, rupture seed coat using a pin knife or scalpel. Seeds punctured at hilum					
NT	No Treatment					
CS1	Cold Moist stratification; up to 14 days					
WS	Water soak prior to sowing					
HW1	Boiling water; soak cooling 24 hours					
SC	Scarification of seed coat using abrasion (sandpaper rubbed for ca. 3 min)					

Appendix B: Genetic study

By Linda Prince and Naomi Fraga, Rancho Santa Ana Botanic Garden

Astragalus tricarlinatus occurs primarily on mafic, felsic, gneiss, granodiorite, and alluvium substrates in the Little San Bernardino Mountains. Historically, waif populations were thought to be the only known locations for this rare endemic of southern California. However, since 2004 a number of new populations have been discovered during field surveys, including large populations in Joshua Tree National Park (JOTR), yielding valuable information on the distribution, habitat preference, and abundance of *A. tricarlinatus* (Amsberry and Meinke 2007, Fraga and Pilapil 2012, White 2004a, 2004b). No prior genetic work has been done on *Astragalus tricarlinatus*, and the genus is notorious for low levels of genetic diversity in general (Liston 1992, Wojciechowski et al. 1999, Kazempour Osaloo et al. 2003, Wojciechowski et al. 2004). A few population genetic studies have been conducted on other *Astragalus* species (Liston 1992, Karron et al. 1988, Travis et al. 1996, and Alexander et al. 2004), but no prior information is available for this taxon. Numerous studies have compared the variability of data generated by allozymes and repeat based markers (AFLPs, RAPDs, and ISSRs in particular), and shown that higher genetic diversity can be detected by repeat based markers, especially within populations (see the recent reviews by Nybom and Bartish 2000, and Nybom 2004). Based on prior studies showing low levels of diversity within the genus, we employed ISSR markers to gather baseline genetic diversity estimated for two populations located within JOTR, and compared them to a single population from outside the park. Populations sampled include Population 1 from Eureka Peak (-116.3557 / 34.0290, elev. 1486 m), Population 2 near Covington Flats (-116.3089 / 33.9889, elev. 1435 m), and Population 3 in Whitewater Canyon (-116.67905 / 34.03819, elev. 1033 m). For a comprehensive list of all known populations, see Table D1 in Appendix D; also see Figure 2 in the main report to see localities for sampled populations.

Fragment based methods such as ISSRs have some limitations relative to allozymes. Because they are primarily dominant data, it is not possible to determine heterozygosity, thus the statistical tests that may be applied are more limited. It is possible to assess relative levels of diversity. Dominant markers such as ISSRs, RAPDs, and AFLPs cannot be analyzed using the same measures as co-dominant markers such as microsatellites and allozymes. Some scientific publications frequently report heterozygosity, gene diversity, and other indices that cannot be calculated from dominant data in which heterozygosity and homozygosity are unknown. The only valid data that can be reported include the percentage of polymorphic loci (%P), Φ_{ST} (an analog of F_{ST} based on genetic distance), genetic distance (GD), and Shannon's information index (I) (Culley 2014.).

Inappropriate measures include number of alleles per locus (A), number of alleles per polymorphic locus (A_p), proportion of observed heterozygotes (H_o), proportion of expected heterozygotes under Hardy-Weinberg equilibrium (H_e), proportion of the total diversity that is partitioned among populations (F_{ST}), analog of F_{ST} that incorporates effects of small and unequal population/sample sizes (Θ), and an analog of F_{ST} but calculated differently under the assumption of Hardy-Weinberg equilibrium (G_{ST}). These measures cannot be used because they rely on knowing the number of homozygous and heterozygous individuals. With dominant data it is impossible to calculate the

frequency of dominant alleles (Culley 2014). Nonetheless, these measures are often provided within peer-reviewed publications, therefore we provide them here for relative comparison purposes.

Experimental Design

Three populations of *Astragalus tricarlinatus* were sampled; two from JOTR (Eureka Peak and Covington Flats) and a single population from Whitewater Canyon (see Figure 2 in the main report). For each population, 2 to 4 leaves from a minimum of 30 individuals were collected and immediately placed in silica gel desiccant. Plants were sampled haphazardly, including extreme edges of the population. Sampled individuals were also geo-referenced using hand-held GPS units accurate to approximately 3 m. Voucher specimens for all populations are housed in the herbarium collection of RSABG and JOTR. All laboratory work was conducted in the Laboratory of Molecular Evolution and Systematics (molecular lab) at RSABG. Any remaining leaf material was saved and stored in the molecular lab at RSABG.

DNA was extracted following a minor modification of the Doyle and Doyle (1987) method (inclusion of a 50°C incubation with Proteinase K (Life Technologies, Grand Island, New York, U.S.A.) for 20 min prior to the 65°C incubation step). DNA was resuspended in 100µL 1X TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5). No secondary cleaning methods were employed. Quantification was carried out using 2µL of the stock DNA extraction with a NanoVue spectrophotometer (GE Healthcare Biosciences, Pittsburgh, Pennsylvania, U.S.A.). All DNA was diluted in water, to a working concentration of 10 ng/µL. Remaining DNAs are stored in the -80°C freezer collection of the molecular lab at RSABG.

Data collection

DNA for three to four individuals per population were selected, based upon quantity of DNA available, for ISSR marker screening. Approximately 30 different fluorescently tagged ISSR primers were amplified using Phusion high fidelity polymerase (with the 5X GC Buffer; New England Biolabs, Ipswich, Massachusetts, U.S.A.). Because ISSR primers are anchored microsatellite primers, polymerase stutter or slipping is expected to be a problem. When the polymerase attempts to copy the template DNA, it can slip, thereby adding an additional repeat, or skip a repeat, leading to the generation of incorrect fragment sizes. The use of Phusion polymerase minimizes the likelihood of these fragments. Annealing temperature varied based upon primer composition, as indicated in Table B1. Fluorescently tagged fragments (1-3 µL) were diluted in 10 µL Hi-Di formamide and co-loaded with 0.5-0.75µL Liz 1200 internal size standard, electrophoresed and visualized on an Applied Biosystems Inc. (Carlsbad, CA, U.S.A.) 3130xl Genetic Analyzer (50 cm array, POP7 polymer) following the manufacturer's directions. Applied Biosystem's GeneMapper v. 4.0 software was used to score the data using the built-in AFLP module, with automatic binning.

Data were reviewed for each sample and the number and variability of bands per sample per primer noted. A number of primers either failed to produce any fragments or were virtually invariant across the samples tested. The results of the initial screening are summarized in Table B1. From the primers screened, the three primers with the most variability and larger number of fragments were selected for population wide analysis (ISSR 813FAM, 815VIC, 880VIC). Only peaks with heights greater than 100 in at least one individual were scored, and only peaks with height >50 were scored as

present. All samples were amplified and run in triplicate. Only peaks that reproducibly produced scorable peaks (as described above) were analyzed. Background noise ranged from 5-7 with a few up to 10. A (primarily) binary data matrix (0, 1, ?) was generated and analyzed under several different criteria. The matrix included 6 monomorphic loci: 26, 30, 50, 144, 145, and 146. In the first set, all loci and all samples were analyzed, including 3 samples with large amounts of missing data (due to single primer failure OR no scorable fragments). Missing data were scored as ?. In the second set, the 3 samples that contained large amounts of missing data had the missing data changed from ? to 0. The third set excluded those 3 samples from the analyses. These different analyses were conducted to assess the impact of missing data on the overall population diversity and structure estimates.

The phylogenetic software package PAUP*4.0 (test version a129, Swofford 2002) was used to create a pairwise distance matrix under the “RFLP/AFLP” option [Nei-Li (fragments); L=17]. Distance dendrograms were also generated using both Neighbor Joining (NJ) and the unweighted pair group method with arithmetic mean (UPGMA; under both average distance and total distance). Both NJ and UPGMA are simple agglomerative or bottom-up data clustering methods used in bioinformatics for the creation of phylogenetic trees. Branch support was estimated using 1000 bootstrap replicates (NJ or UPGMA as appropriate).

The number of different alleles (N_a), number of effective alleles (N_e), Shannon's information index (I , Lewontin 1972), Nei's (1973) gene diversity (h) and unbiased gene diversity (u_h), and percentage polymorphic loci (%P) were calculated in GenAlEx v. 6.5 (Peakall and Smouse 2006). Estimates of genetic diversity (h), genetic structure (θ^I , an F_{ST} analog; θ^{II} , an estimate most similar to Nei's G_{ST}), and $G_{ST}B$ (a Bayesian estimate of G_{ST}) were estimated in HICKORY v. 1.1 (Holsinger and Lewis 2007). HICKORY values were estimated using the “f-free” model since estimates of f from dominant data can be unreliable (Holsinger et al. 2002). Each HICKORY analysis was run in triplicate to ensure the Bayesian estimation had reached stationarity. Genetic diversity measures were estimated in two different programs as the numbers differ slightly, with HICKORY consistently providing lower estimates of diversity. A genetic distance matrix was generated in GenAlEx (for use in Principal Coordinates Analyses (PCoA or PCO; also conducted in GenAlEx) to identify groups of samples with the highest allelic similarity. Plots (based on the first two axes) for each analysis are provided in Figures B1-B3.

Results

Effect of missing data

Population 2, from Covington Flats in JOTR, had highest values for all population descriptors including the number of alleles (N_a), number of effective alleles (N_e), Shannon's Information Index (I), Nei's gene diversity (h), Nei's unbiased gene diversity (u_h), and percentage polymorphic loci (%P), regardless of how missing data were treated (see Tables B3-B5). On the other hand, population 1, from Eureka Peak in JOTR, had the lowest values for all population descriptors.

Population genetic distance (see Table B6) and genetic structure (see Tables B7-9), as measured by θ^I , θ^{II} , and $G_{ST}B$, values ranged from 0.163-0.164 for $G_{ST}B$, 0.258-0.260 for θ^{II} , and 0.437-0.438 for

θ^I . The values were similar regardless of how missing data were treated and the variation did not change with replicate analyses.

Similar topologies were found for NJ (data not shown) and UPGMA analyses (see Figures B4-B6 for UPGMA dendrograms) regardless of the treatment of missing data. Most individuals from Eureka Peak and Whitewater Canyon group together within their respective populations, with the Covington Flats population forming an intermediate grade between the other two. There is very little branch support for the topology. Analysis 2 (missing data = 0) resulted in a topology with the most intermingling of individuals from different populations, but those differences were not supported by bootstrap values $\geq 50\%$. The effect of missing data was most obvious in the PCO graph of Analysis 1 (Figure B1), where individuals with large amounts of missing data (scored as ?) formed an outlying cluster.

Population Diversity

Each population was characterized by a number of unique ISSR bands not found in the other populations sampled, with 14 unique bands in the Eureka Peak population, 18 unique bands in the Covington Flats population, and 20 unique bands in the Whitewater Canyon population. Additionally, the Covington Flats-JOTR population had a high frequency (20-22 individuals out of the 31 sampled) of two bands (#131 and 132) that were either absent from the Eureka Peak population or present in very low frequency (1-2 out of the 31 individuals sampled) in the Whitewater Canyon population. The same was true for the Whitewater Canyon population, with 28 out of 31 individuals exhibiting band #54 and #59, which were absent in the other two populations.

Population Structure

Individual populations were generally cohesive, with the majority of samples from each population forming a clade or grade (see Figures B4-B6), regardless of how missing data were treated. The populations did not form reciprocally monophyletic population groups however, suggesting either historic or contemporary gene flow between all three populations sampled. Principal Coordinates Analysis (PCoA) showed a similar pattern (Figures B2-B3), with the bulk of the samples from any given population clustering together and small areas of overlap among the populations. As noted above, this pattern was slightly obscured under Analysis 1, where individual samples with large amounts of missing data were included in the analysis as “?” (Figure B1). In this case, the two outlying clusters correspond to data from two markers: ISSR-813 or ISSR-815. In all three PCoA analyses, coordinates 1-3 explained approximately 70% of the variation.

A variety of measures for population structure (θ^I , θ^{II} , and $G_{ST}B$) were examined to assess the distribution of individuals and gene flow within *Astragalus tricarlinatus*. The Bayesian estimate of G_{ST} ($G_{ST}B$) is considered the most conservative measure, followed by θ^{II} , and then θ^I . Values, based on three independent Bayesian runs, ranged from 0.163-0.164 for $G_{ST}B$, 0.258-0.260 for θ^{II} , and 0.437-0.438 for θ^I . Different methods of coding missing data had little effect on values obtained. θ^{II} is the most relevant statistic as it provides information on the extent of genetic differentiation among contemporaneous populations and is less sensitive to incomplete population sampling.

The Eureka Peak and Covington Flats populations are more closely related to each other ($NeiP = 0.034$; $uNeiP=0.028$) than either are to the Whitewater Canyon population, but they are also much closer geographically. Interestingly, the Covington Flats population is slightly more closely related to the Whitewater Canyon population ($NeiP=0.079-0.081$; $uNeiP=0.073-0.074$) than is the Eureka Peak population ($NeiP=0.082-0.086$; $uNeiP=0.076-0.080$) despite being slightly further away. This is graphically demonstrated in both the PCoA plots (Figures B1-B3) and the UPGMA trees (Figures B4-B6).

Discussion

Prior genetic work in *Astragalus* supported the claim that the genus is notorious for low levels of genetic diversity (Liston 1992, Wojciechowski and Hu 1999, Kazempour Osaloo et al. 2003, Wojciechowski et al. 2004). However, contrary to earlier genetic studies using comparative DNA sequences, allozyme studies have successfully investigated population level variation in a number of North American species (Table B10). The variation is still generally low ($G_{ST} < 0.10$, with two exceptions: *A. claranus* and *A. pauperculus*). Recent population genetic studies using other fragment-based markers (ISSRs and AFLPs; Karron 1988 and Alexander et al. 2004) have detected significantly more diversity. *Astragalus tricarinatus* is intermediate in diversity relative to those fragment-based studies (Table B10).

The Bayesian estimates of population structure are typical of those found in other studies (Nyblom 2004), but are not conclusive in terms of their predictive value, with $G_{ST}B=0.163-0.164$, $\theta^{II}=0.258-0.260$, and $\theta^I=0.437-0.438$. As a reminder, θ^I is an F_{ST} analog, θ^{II} is an estimate most similar to Nei's G_{ST} , and $G_{ST}B$, a Bayesian estimate of G_{ST} . The latter is the most conservative of the three. The $G_{ST}B$ values are consistent with long-lived perennials of narrow geographic range that are outcrossers, animal-dispersed (via consumption), and considered of late successional status. In other words, based on these data *A. tricarinatus* appears to have enough gene flow (past or present) that the populations have not greatly differentiated genetically. The θ^I values however, fall within the expected range for selfing species with gravity induced seed dispersal that are early successional annuals with a wide geographic range. As stated above, $G_{ST}B$ is very conservative, and θ^I very generous, especially if the study fails to sample all extant populations. θ^{II} values are intermediate between reported values for short- and long-lived perennials and species with narrow to regional geographic distributions. They are also closer in value to outcrossing species than to selfing species. *Astragalus tricarinatus* is thought to be an outcrossing perennial species of short to moderate life span. It occurs in unstable or disturbed habitats (due to natural erosional processes) and appears to be restricted to a specific substrate.

As stated above, each population was characterized by a number of unique ISSR bands, and the number of unique bands was similar across the three populations (14-20 bands). All three populations have similar levels of genetic diversity as well. There is evidence for past or contemporary gene flow among all three populations and that gene flow appears to be high based upon the low G_{ST} values.

Table B1. ISSR primers screened for population genetic analysis of *Astragalus tricarlinatus*, including annealing temperature employed and approximate number of major bands observed. Bold typeface indicates primers used in this study.

Primer Number	Base Composition	Dye	Anneal Temp. (°C)	Number of Bands
807	AGA GAG AGA GAG AGA GT	FAM	50.0	1-9
808	AGA GAG AGA GAG AGA GC	VIC	52.0	0
809	AGA GAG AGA GAG AGA GG	FAM	52.0	8+
811	GAG AGA GAG AGA GAG AC	VIC	52.0	7
812	GAG AGA GAG AGA GAG AA	VIC	50.0	0
813	CTC TCT CTC TCT CTC TT	FAM	50.0	13+
814	CTC TCT CTC TCT CTC TA	NED	50.0	5
815	CTC TCT CTC TCT CTC TG	FAM	52.0	10-16
817	CAC ACA CAC ACA CAC AA	VIC	50.0	0
818	CAC ACA CAC ACA CAC AG	FAM	52.0	4
820	GTG TGT GTG TGT GTG TC	VIC	52.0	0
821	GTG TGT GTG TGT GTG TT	PET	50.0	0
822	TCT CTC TCT CTC TCT CA	VIC	50.0	4
823	TCT CTC TCT CTC TCT CC	NED	50.0	4-10
825	ACA CAC ACA CAC ACA CT	VIC	50.0	1
826	ACA CAC ACA CAC ACA CC	PET	54.0	0
828	TGT GTG TGT GTG TGT GA	FAM	50.0	0
830	TGT GTG TGT GTG TGT GG	VIC	54.0	0
861	ACC ACC ACC ACC ACC ACC	FAM	60.0	0
863	AGT AGT AGT AGT AGT AGT	FAM	48.0	2
866	CTC CTC CTC CTC CTC CTC	FAM	60.0	>20
868	GAA GAA GAA GAA GAA GAA	VIC	48.0	3
869	GTT GTT GTT GTT GTT GTT	PET	48.0	0
873	GAC AGA CAG ACA GAC A	NED	48.0	0
874	CCC TCC CTC CCT CCC T	FAM	54.0	10-15
880	GGA GAG GAG AGG AGA	VIC	50.0	>15
881	GGG TGG GGT GGG GTG	VIC	54.0	0

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JOTR – Eureka Peak; Population 2 (ML678-ML708) = JOTR – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ?.

	Locus																																		
Individ.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
ML646	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML647	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML648	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML649	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML650	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML651	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML652	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML653	0	0	1	0	1	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML654	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML655	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML656	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML657	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML658	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML659	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML660	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML661	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML662	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML663	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML664	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML665	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML666	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML667	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML668	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML669	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML670	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML671	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML672	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML673	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML674	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML675	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML676	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML678	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	0	1	0	0	1	1	0
ML679	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML680	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0
ML681	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML682	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML683	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML684	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML685	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML686	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML687	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML688	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML689	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML690	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0
ML691	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	1	1	1	0	0	0	0	0
ML692	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	1

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																																		
Individ.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
ML693	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	0	1	1	1	0	0	0	0
ML694	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0
ML695	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML696	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0
ML697	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML698	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
ML699	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML700	0	0	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0
ML701	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML702	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
ML703	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML704	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	1	1	1	0	0	0	0
ML705	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML706	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML707	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML708	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML734	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	1	1	1	0	0	0
ML735	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
ML736	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	1	1	0	1	0	0	0
ML737	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	0
ML738	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1	0	1	0	0	0	1	1	1	0	0	0	0
ML739	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML740	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
ML741	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0
ML742	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0
ML743	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML744	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	0
ML745	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0
ML746	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	0	0
ML747	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	0
ML748	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
ML749	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	1	0	0	1	1	1	0	0	0	0	0
ML750	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0
ML751	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0
ML752	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML753	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML754	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0
ML755	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML756	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0
ML757	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	1	1	1	0	0
ML758	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	0	0	1	1	1	0	0	0	0
ML759	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	1	1	0	0	0
ML760	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	0	1	1	1	0	0	0	0	0
ML761	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML762	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML763	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0
ML764	1	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	0

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																																																																			
Individ.	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68																																			
ML646	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML647	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML648	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML649	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML650	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0	0	0	0	0																																			
ML651	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML652	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML653	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML654	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML655	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML656	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML657	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML658	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML659	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML660	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML661	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML662	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML663	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML664	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML665	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML666	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML667	0	0	0	0	1	1	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?																																			
ML668	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML669	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML670	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML671	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML672	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML673	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0																																			
ML674	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML675	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?																																			
ML676	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML678	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML679	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML680	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML681	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML682	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML683	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML684	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML685	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML686	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML687	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML688	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML689	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML690	1	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML691	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML692	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																																																																			
Individ.	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68																																			
ML693	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0																																			
ML694	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML695	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML696	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML697	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML698	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML699	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML700	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML701	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML702	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML703	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	1	0	1	1	0	1	0	0	0																																			
ML704	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0																																			
ML705	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML706	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML707	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML708	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML734	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML735	0	0	1	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?																																			
ML736	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML737	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML738	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML739	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML740	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	0	?	0	0	0																																			
ML741	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	0	1	1																																			
ML742	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML743	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML744	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML745	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML746	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML747	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML748	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML749	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML750	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML751	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML752	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML753	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML754	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML755	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	?	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML756	0	0	0	0	0	0	0	0	0	0	1	?	0	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML757	1	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0																																			
ML758	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0																																			
ML759	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0																																			
ML760	0	0	0	0	1	0	1	0	1	0	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0																																			
ML761	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0																																			
ML762	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	0																																			
ML763	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0																																			
ML764	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0																																			

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																																					
Individ.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101					
ML646	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	0	0	1	1					
ML647	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	1	1					
ML648	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	1					
ML649	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	1					
ML650	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0	1	1					
ML651	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	1	1					
ML652	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1	1	0	0	1	1					
ML653	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0	1	1					
ML654	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	0	1	1					
ML655	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1					
ML656	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	0	1	1	0	1	1	1					
ML657	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	1	1	1	0	0	1	1					
ML658	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1					
ML659	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0					
ML660	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	1	1	0	0	1	1					
ML661	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	1	1	0	0	0	1	1					
ML662	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0					
ML663	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	1					
ML664	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0	0	0					
ML665	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	1	1					
ML666	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	0	0	1	1					
ML667	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?					
ML668	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0	0	0	1	1					
ML669	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	1	1					
ML670	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	1	1	1					
ML671	0	0	0	1	1	0	0	0	0	0	0	0	0	0	?	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	1	1					
ML672	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	1	1					
ML673	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1					
ML674	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0					
ML675	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?					
ML676	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	1					
ML678	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	1					
ML679	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0					
ML680	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	?	1	0	0	0	1	1					
ML681	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	1	1	0	0	1	0					
ML682	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	1	1	0	0	0	0					
ML683	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0					
ML684	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0					
ML685	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0					
ML686	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	1					
ML687	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0					
ML688	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	1					
ML689	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0					
ML690	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0					
ML691	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0					
ML692	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	0	0	1	1	0	0	0	0					

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																																	
Individ.	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	
ML693	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	1
ML694	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
ML695	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML696	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0
ML697	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML698	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	1
ML699	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0
ML700	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	1	1	1	1
ML701	0	0	0	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML702	0	0	0	1	0	1	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML703	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	
ML704	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML705	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ML706	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ML707	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	0	0	0	1	1	0	0	1	0	0
ML708	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	1	1	1	1
ML734	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML735	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
ML736	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML737	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML738	0	0	0	1	1	1	1	0	?	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML739	0	0	?	1	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML740	0	0	1	1	1	1	1	1	?	0	0	0	0	0	0	0	0	0	0	?	0	?	0	0	0	0	0	0	0	?	0	1	1	
ML741	1	1	0	1	1	1	1	0	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML742	0	0	1	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML743	0	0	0	1	1	1	1	0	0	?	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML744	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML745	0	0	?	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML746	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML747	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML748	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1
ML749	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	1
ML750	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1
ML751	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML752	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML753	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML754	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1
ML755	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1
ML756	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	1
ML757	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	1	1
ML758	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML759	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML760	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML761	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
ML762	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
ML763	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1
ML764	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																											
Individ.	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	
ML646	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	0	1	
ML647	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	
ML648	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML649	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	1	
ML650	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML651	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML652	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	
ML653	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML654	1	0	0	0	1	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML655	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	
ML656	1	1	0	0	1	1	0	0	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	
ML657	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML658	1	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	1	
ML659	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML660	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1
ML661	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1
ML662	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	
ML663	0	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML664	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	
ML665	1	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	1	
ML666	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	1	1	0	1	
ML667	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	0	0	0	1	0	1	0	1	
ML668	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML669	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	1	0	1	
ML670	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	0	0	1	0	1	0	1	
ML671	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML672	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML673	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	
ML674	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	0	0	1	0	1	0	1	
ML675	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	0	0	0	1	1	1	0	1	
ML676	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	0	1	
ML678	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	
ML679	0	0	0	?	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML680	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	
ML681	0	0	0	1	0	0	0	0	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	1	1	0	1	
ML682	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0	0	0	1	
ML683	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	
ML684	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	
ML685	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML686	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	1	0	1	
ML687	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	
ML688	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	1	0	0	1	1	0	1
ML689	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	1	0	0	0	1	0	1	
ML690	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	1	
ML691	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	0	0	
ML692	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	1	1	

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

	Locus																											
Individ.	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	
ML693	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	0	1	0	1	
ML694	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	1	1	0	1	0	1	0	1	0	1	
ML695	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	1	1	1	
ML696	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	1	
ML697	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	1	
ML698	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	1	0	0	0	0	0	0	0	1	0	1	
ML699	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1	
ML700	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	1	
ML701	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	1	0	1	0	1	
ML702	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	
ML703	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	1	0	0	
ML704	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1	0	0	0	1	0	1	0	1	
ML705	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	0	1	1	0	1	1	1	0	1	1	0	
ML706	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	0	0	0	1	1	1	0	0	1	1	
ML707	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	1	0	1	
ML708	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	
ML734	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	1	0	
ML735	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	0	0	0	0	0	1	0	0	
ML736	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	
ML737	0	1	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	
ML738	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML739	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML740	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
ML741	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	
ML742	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	
ML743	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	1	0	
ML744	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	
ML745	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML746	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	1	0	0	0	0	
ML747	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	
ML748	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	1	0	1	0	0	
ML749	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	
ML750	1	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	
ML751	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML752	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	
ML753	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	
ML754	1	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML755	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML756	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	
ML757	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	
ML758	1	1	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	
ML759	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
ML760	1	1	0	0	1	0	0	0	0	1	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	
ML761	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	
ML762	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	1	
ML763	1	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	0	1	
ML764	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	1	1	1	0	0	0	0	0	1	0	0	

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

Individ.	Locus																	
	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146
ML646	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1	1	1
ML647	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
ML648	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1
ML649	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	1	1
ML650	1	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	1	1
ML651	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1
ML652	0	1	0	0	0	0	0	1	0	0	0	1	1	0	1	1	1	1
ML653	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1
ML654	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1
ML655	0	1	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML656	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1
ML657	1	0	0	0	0	0	0	1	0	0	0	1	1	0	1	1	1	1
ML658	0	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1	1	1
ML659	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1
ML660	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1
ML661	1	0	0	0	0	0	0	1	0	1	0	1	1	1	1	1	1	1
ML662	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1
ML663	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1
ML664	1	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1	1	1
ML665	1	0	0	0	0	0	0	1	1	0	0	1	1	0	1	1	1	1
ML666	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1
ML667	1	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML668	1	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1	1
ML669	1	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1
ML670	1	0	0	0	0	0	0	1	1	1	0	1	1	0	1	1	1	1
ML671	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML672	1	0	0	1	0	0	1	1	0	0	0	1	0	1	1	1	1	1
ML673	1	0	0	0	0	0	0	1	1	1	0	1	1	0	1	1	1	1
ML674	1	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML675	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML676	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1
ML678	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1
ML679	1	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	1	1
ML680	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	1
ML681	1	0	1	1	0	0	0	0	1	1	1	0	1	0	1	1	1	1
ML682	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1
ML683	0	0	1	0	0	0	0	1	0	0	1	1	0	1	1	1	1	1
ML684	1	0	1	1	1	1	0	1	0	1	0	1	0	0	1	1	1	1
ML685	1	0	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	1
ML686	1	0	1	1	1	0	0	1	1	0	0	1	1	0	1	1	1	1
ML687	0	0	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1
ML688	0	0	1	1	1	1	0	1	1	0	0	1	1	0	1	1	1	1
ML689	1	0	1	1	1	0	0	0	0	1	1	0	1	1	1	1	1	1
ML690	1	0	1	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1
ML691	1	0	1	1	0	0	0	1	1	0	0	1	0	0	1	1	1	1
ML692	1	0	1	1	1	0	0	0	0	1	1	0	1	1	1	1	1	1

Table B2. ISSR data matrix (analysis 1 version) for *Astragalus tricarlinatus*. Population 1 (ML646-ML676) = JTNP – Eureka Peak; Population 2 (ML678-ML708) = JTNP – Covington Flats; Population 3 (ML734-ML764) = Whitewater Canyon. Character states = 0, 1, ? (continued).

Individ.	Locus																	
	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146
ML693	0	0	1	1	1	1	0	0	1	0	1	0	0	0	1	1	1	1
ML694	1	0	1	1	1	1	0	0	1	0	0	0	1	0	1	1	1	1
ML695	1	0	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1	1
ML696	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1
ML697	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1
ML698	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1
ML699	1	0	1	1	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML700	1	0	1	1	1	0	0	1	0	1	1	1	0	1	1	1	1	1
ML701	1	0	1	1	1	1	0	1	0	1	0	1	0	0	1	1	1	1
ML702	1	0	1	1	0	0	0	1	1	0	0	1	1	0	1	1	1	1
ML703	1	0	0	1	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML704	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	1
ML705	1	1	1	1	1	1	0	0	0	0	1	0	0	0	1	1	1	1
ML706	0	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1
ML707	1	0	1	1	1	1	0	0	1	0	1	0	1	0	1	1	1	1
ML708	0	0	0	0	0	0	0	0	0	1	0	1	0	1	1	1	1	1
ML734	0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1
ML735	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML736	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1
ML737	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1
ML738	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML739	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML740	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1
ML741	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1
ML742	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1
ML743	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
ML744	0	1	1	1	1	0	0	0	0	1	0	0	1	1	1	1	1	1
ML745	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML746	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1
ML747	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1
ML748	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1
ML749	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1
ML750	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML751	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1
ML752	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML753	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1
ML754	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML755	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML756	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML757	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1
ML758	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
ML759	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	1	1
ML760	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
ML761	0	0	1	0	1	1	0	0	1	1	0	0	1	1	1	1	1	1
ML762	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	1	1
ML763	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
ML764	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	1

Tables B3-B5. Various population statistics and descriptors, under Bayesian allele frequency (BAFP; Lynch and Milligan 1994) criteria, for *Astragalus tricarlinatus* based on ISSR data. Results based on GenAlEx analyses. Highest values are in bold typeface, lowest values underlined. Number of individuals sampled (SS), number of alleles (Na), number of effective alleles (Ne), Shannon's Information Index (I), Nei's gene diversity (h), Nei's unbiased gene diversity (uh), percentage polymorphic loci (%P). h*1-3 are values of h from three, independent HICKORY analyses.

B3. Analysis #1 with missing data scored as ?.

Population	SS		Na	Ne	I	h	h*1	h*2	h*3	uh	%P
1. JOTR-EP	31	Mean	<u>1.164</u>	<u>1.197</u>	<u>0.199</u>	<u>0.124</u>	<u>0.138</u>	<u>0.138</u>	<u>0.138</u>	<u>0.129</u>	<u>52.74</u>
		SE	0.077	0.025	0.020	0.014	0.005	0.005	0.005	0.014	
2. JOTR-CF	31	Mean	1.432	1.313	0.289	0.187	0.189	0.189	0.189	0.193	68.49
		SE	0.072	0.030	0.022	0.016	0.007	0.007	0.007	0.016	
3. WC	31	Mean	1.288	1.227	0.231	0.144	0.150	0.150	0.151	0.149	60.27
		SE	0.076	0.025	0.020	0.014	0.007	0.007	0.007	0.014	
Total		Mean	1.295	1.246	0.239	0.152	0.159	0.159	0.159	0.157	60.50
		SE	0.044	0.016	0.012	0.008	0.005	0.005	0.005	0.009	4.55

B4. Analysis #2 with missing data scored as 0.

Population	SS		Na	Ne	I	h	h*1	h*2	h*3	uh	%P
1. JOTR-EP	31	Mean	<u>1.185</u>	<u>1.207</u>	<u>0.208</u>	<u>0.130</u>	<u>0.138</u>	<u>0.138</u>	<u>0.138</u>	<u>0.135</u>	<u>54.79</u>
		SE	0.078	0.025	0.020	0.014	0.005	0.005	0.005	0.014	
2. JOTR-CF	31	Mean	1.432	1.313	0.289	0.187	0.189	0.189	0.189	0.193	68.49
		SE	0.072	0.030	0.022	0.016	0.007	0.007	0.007	0.016	
3. WC	31	Mean	1.329	1.232	0.239	0.149	0.151	0.151	0.151	0.153	64.38
		SE	0.077	0.025	0.020	0.014	0.007	0.007	0.005	0.014	
Total		Mean	1.315	1.251	0.245	0.155	0.159	0.159	0.159	0.160	62.56
		SE	0.044	0.016	0.012	0.008	0.005	0.005	0.005	0.009	4.06

B5. Analysis #3 with taxa for which large amounts of data are missing excluded from the analyses.

Population	SS		Na	Ne	I	h	h*1	h*2	h*3	uh	%P
1. JOTR-EP	29	Mean	<u>1.144</u>	<u>1.196</u>	<u>0.196</u>	<u>0.123</u>	<u>0.138</u>	<u>0.138</u>	<u>0.138</u>	<u>0.128</u>	<u>51.37</u>
		SE	0.077	0.025	0.020	0.014	0.005	0.005	0.005	0.014	
2. JOTR-CF	31	Mean	1.432	1.313	0.289	0.187	0.189	0.189	0.189	0.193	68.49
		SE	0.072	0.030	0.022	0.016	0.007	0.007	0.007	0.016	
3. WC	30	Mean	1.288	1.229	0.232	0.145	0.151	0.151	0.151	0.150	60.27
		SE	0.076	0.025	0.020	0.014	0.007	0.007	0.007	0.014	
Total		Mean	1.288	1.246	0.239	0.152	0.159	0.159	0.159	0.157	60.05
		SE	0.044	0.016	0.012	0.008	0.005	0.005	0.005	0.009	4.94

Table B6. Pairwise population genetic distances for *Astragalus tricarinatus* based on analyses of ISSR data. Analysis 1: missing data = ?; analysis 2: missing data = 0; analysis 3: taxa with large amounts of missing data excluded. Highest values are in bold typeface, lowest values underlined. NeiP = Nei's genetic distance; uNeiP = Nei's unbiased genetic distance. Pop1 = JOTR – Eureka Peak; Pop2 = JOTR – Covington Flats; Pop3 = Whitewater Canyon.

Pairwise Comparison	Analysis 1		Analysis 2		Analysis 3	
	NeiP	uNeiP	NeiP	uNeiP	NeiP	uNeiP
Pop1-Pop2	<u>0.034</u>	<u>0.028</u>	<u>0.034</u>	<u>0.028</u>	<u>0.034</u>	<u>0.028</u>
Pop1-Pop3	0.086	0.080	0.082	0.076	0.085	0.080
Pop2-Pop3	0.081	0.074	0.079	0.073	0.081	0.074

Tables B7-B9. *Astragalus tricarinatus* estimates of population genetic structure based on HICKORY analysis results of ISSR data analyses. θ^I is an F_{ST} analog, θ^{II} is an estimate most similar to Nei's G_{ST} , and G_{STB} is a Bayesian estimate of G_{ST} .

B7. Analysis #1 with missing data scored as ?.

	Run 1			Run 2			Run 3		
	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}
Mean	0.437	0.260	0.164	0.437	0.259	0.164	0.438	0.259	0.164
SE	0.026	0.029	0.012	0.025	0.029	0.012	0.025	0.029	0.012

B8. Analysis #2 with missing data scored as 0.

	Run 1			Run 2			Run 3		
	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}
Mean	0.438	0.259	0.164	0.437	0.260	0.164	0.437	0.260	0.164
SE	0.025	0.029	0.012	0.025	0.029	0.012	0.025	0.029	0.012

B9. Analysis #3 with taxa (for which large amounts of data are missing) excluded from the analyses.

	Run 1			Run 2			Run 3		
	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}	θ^I	θ^{II}	G_{STB}
Mean	0.438	0.258	0.163	0.438	0.259	0.163	0.438	0.259	0.164
SE	0.025	0.029	0.012	0.026	0.029	0.012	0.025	0.028	0.012

Table B10. Comparison of *Astragalus* species with population level genetic studies. Habit = Annual (A) or Perennial (P).

Taxon	Habit	Distribution	Marker	F _{ST} or G _{ST}	Ref
<i>A. brewerii</i>	A	Narrow	Allozyme	0.000 (G _{ST})	1
<i>A. claranus</i>	A	Narrow	Allozyme	0.331 (G _{ST})	1
<i>A. nyensis</i>	A	Narrow	Allozyme	0.000 (G _{ST})	1
<i>A. pauperculus</i>	A	Narrow	Allozyme	0.725 (G _{ST})	1
<i>A. rattanii</i> var. <i>jepsonianus</i>	A	Narrow	Allozyme	0.053 (G _{ST})	1
<i>A. rattanii</i> var. <i>rattanii</i>	A	Narrow	Allozyme	0.058 (G _{ST})	1
<i>A. tener</i> var. <i>tener</i>	A	Narrow	Allozyme	0.059 (G _{ST})	1
<i>A. tener</i> var. <i>titi</i>	A	Narrow	Allozyme	0.000 (G _{ST})	1
<i>A. acutirostris</i>	A	Widespread	Allozyme	0.254 (G _{ST})	1
<i>A. gambelianus</i>	A	Widespread	Allozyme	0.211 (G _{ST})	1
<i>A. nuttallianus</i>	A	Widespread	Allozyme	0.000 (G _{ST})	1
<i>A. cremnophylax</i>	P	Narrow	AFLP	0.41 (F _{ST})	2
<i>A. linifolius</i>	P	Narrow	Allozyme	0.11 (F _{ST} *)	3
<i>A. oniciformis</i>	P	Narrow	ISSR	0.1130-0.1134 (G _{ST})	4
<i>A. osterhouti</i>	P	Narrow	Allozyme	0.14 (F _{ST} *)	3
<i>A. tricarinatus</i>	P	Narrow	ISSR	0.164 (G _{ST})	here
<i>A. nothoxys</i>	P	Widespread	Allozyme	0.000 (G _{ST})	1
<i>A. pattersoni</i>	P	Widespread	Allozyme	0.01 (F _{ST} *)	3
<i>A. pectinatus</i>	P	Widespread	Allozyme	0.07 (F _{ST} *)	3

1=Liston 1992, 2=Travis et al. 1996, 3=Karron et al. 1988, 4=Alexander et al. 2004. F_{ST}* = sum of CE+GOT values.

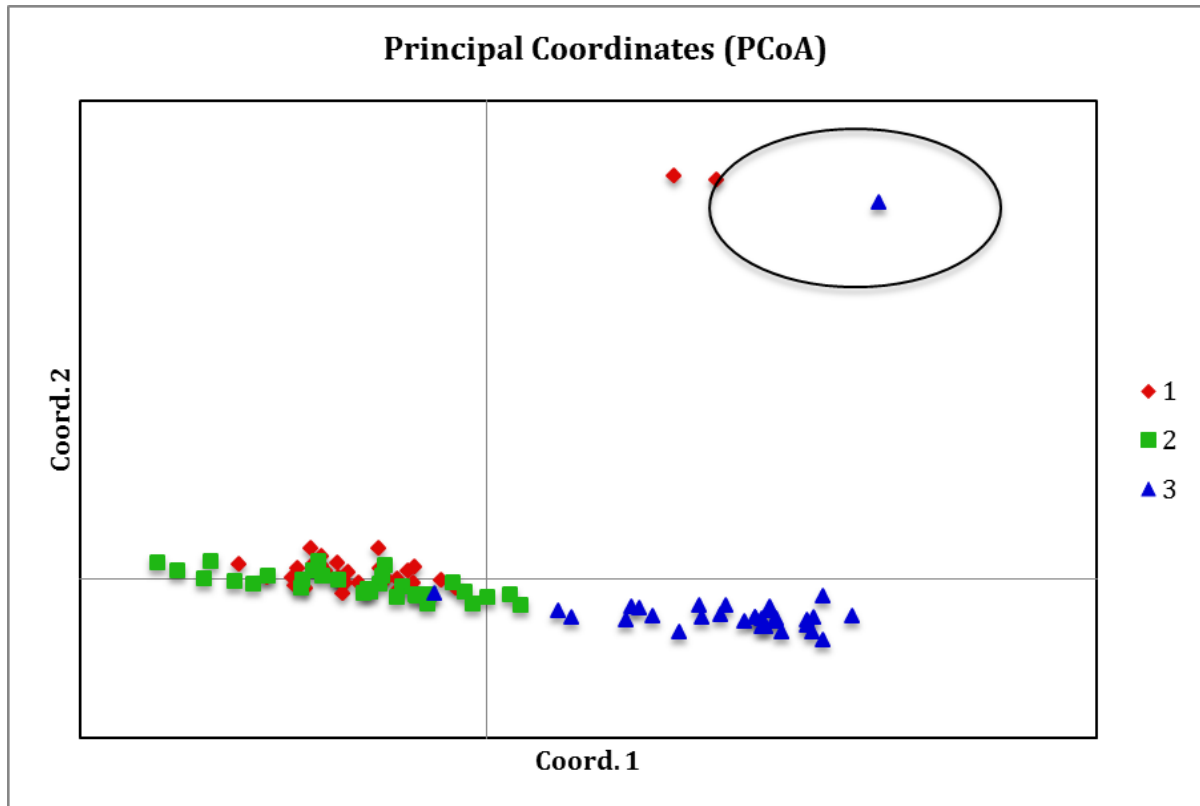


Figure B1. Principal coordinates analysis of ISSR data for *Astragalus tricarinatus* (analysis 1: missing data = ?). Coordinates 1 + 2 explain 60% of the variation, + coordinate 3 = 72%. Population 1 = JOTR – Eureka Peak (red); Population 2 = JOTR – Covington Flats (green); Population 3 = Whitewater Canyon (blue). Note the circled, outlying cluster. These are samples with large amounts of missing data. Circle = missing data for marker ISSR-815.

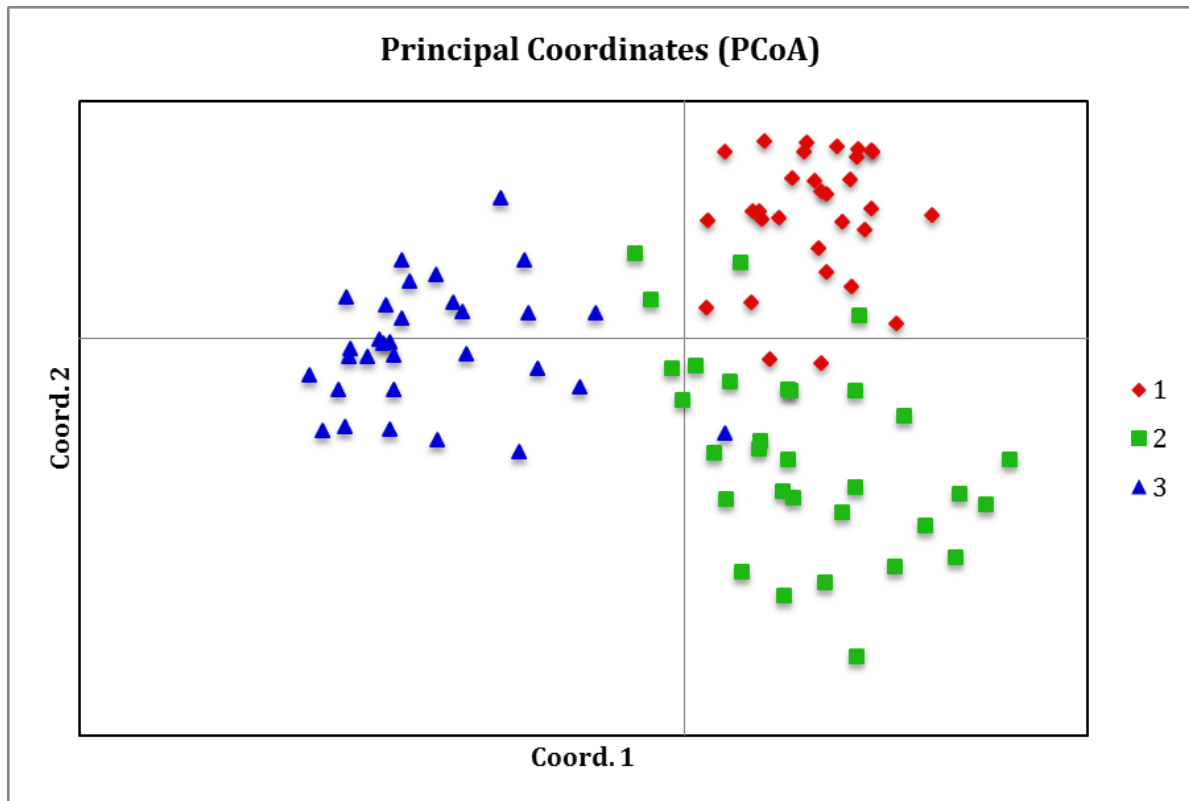


Figure B2. Principal coordinates analysis of ISSR data for *Astragalus tricarinatus* (analysis 2: missing data = 0). Coordinates 1 + 2 explain 54% of the variation, + coordinate 3 = 67%. Population 1 = JOTR – Eureka Peak (red); Population 2 = JOTR – Covington Flats (green); Population 3 = Whitewater Canyon (blue).

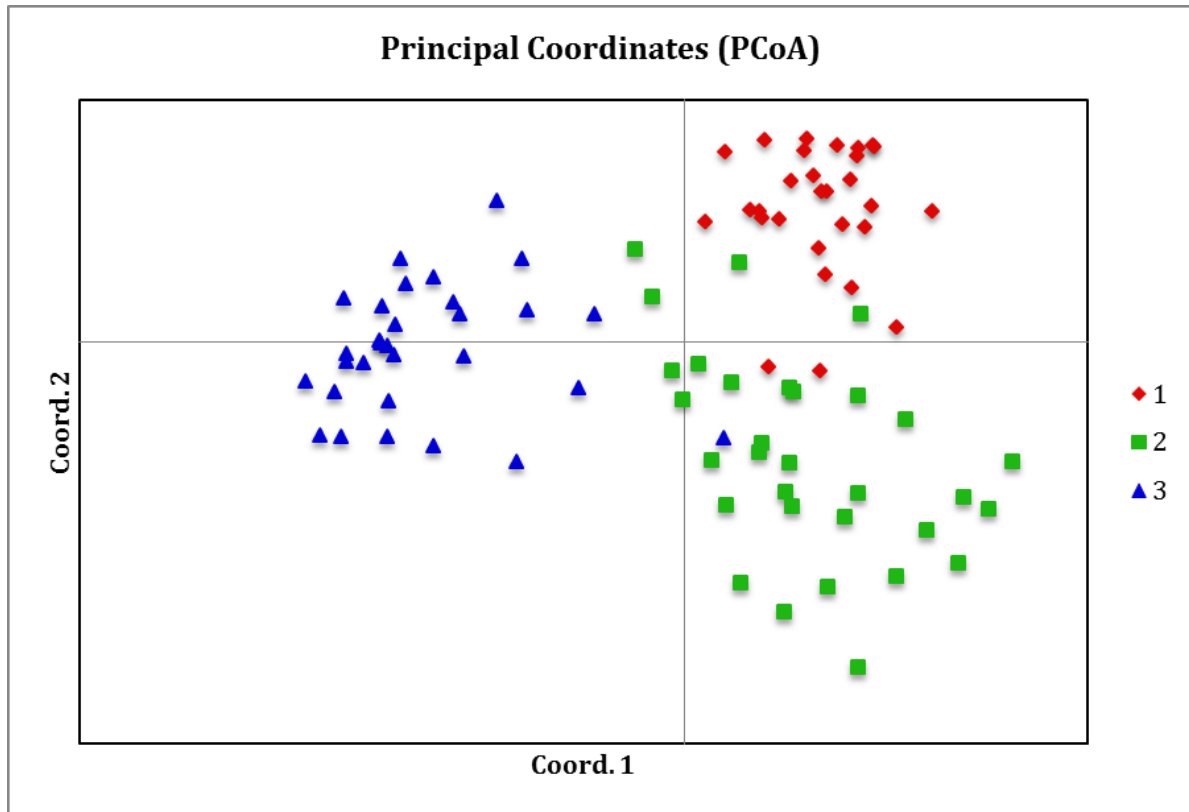


Figure B3. Principal coordinates analysis of ISSR data for *Astragalus tricarinatus* (analysis 3: excluding taxa with missing data). Coordinates 1 + 2 explain 56% of the variation, + coordinate 3 = 68%. Population 1 = JOTR – Eureka Peak (red); Population 2 = JOTR – Covington Flats (green); Population 3 = Whitewater Canyon (blue).

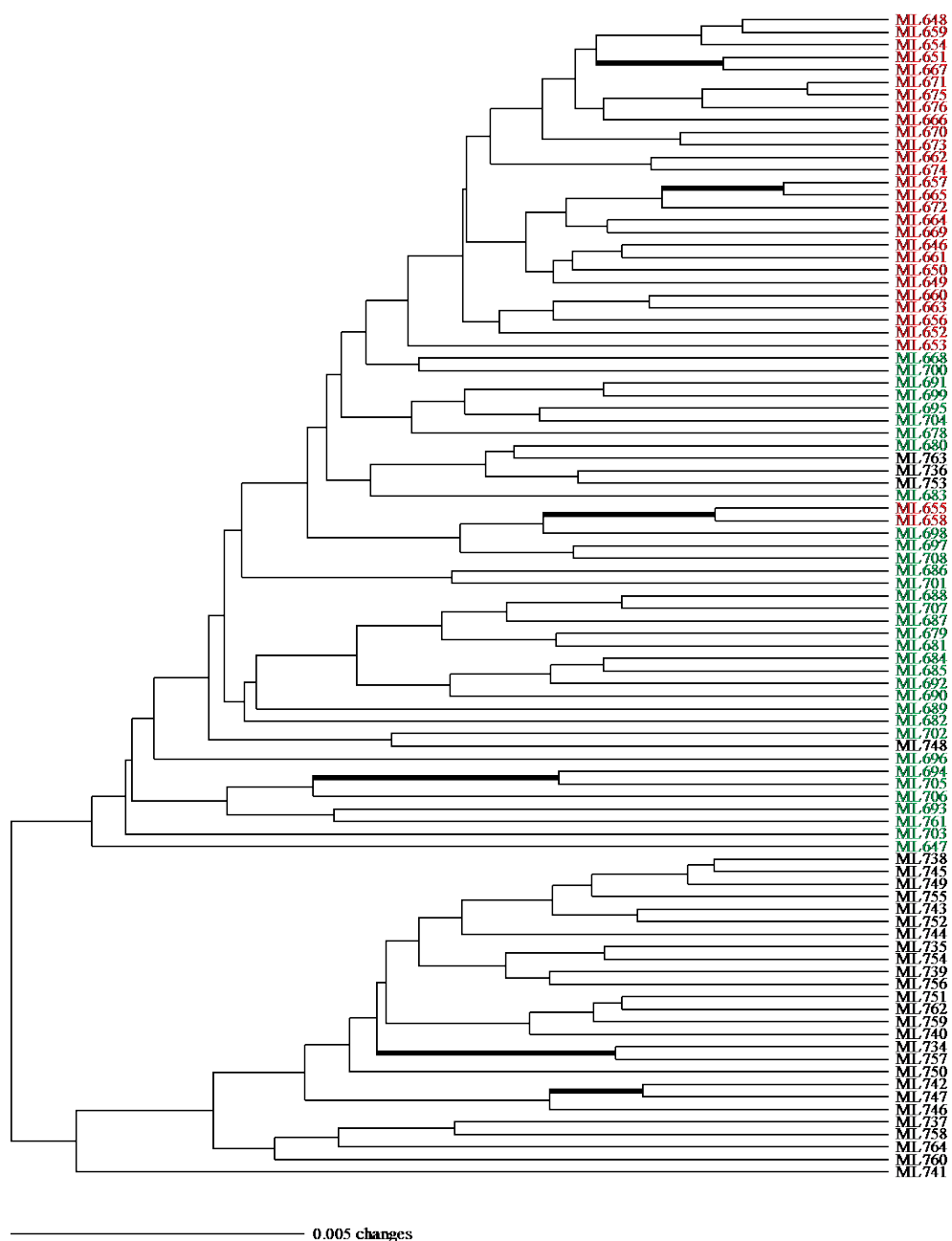


Figure B4. UPGMA analysis of ISSR data for *Astragalus tricarinatus* (analysis 1: missing data = ?). Population color code: JOTR – Eureka Peak (red); Pop 2 = JOTR – Covington Flats (green); Pop 3 = Whitewater Canyon (black). Bold lines indicate branches with bootstrap support $\geq 50\%$.

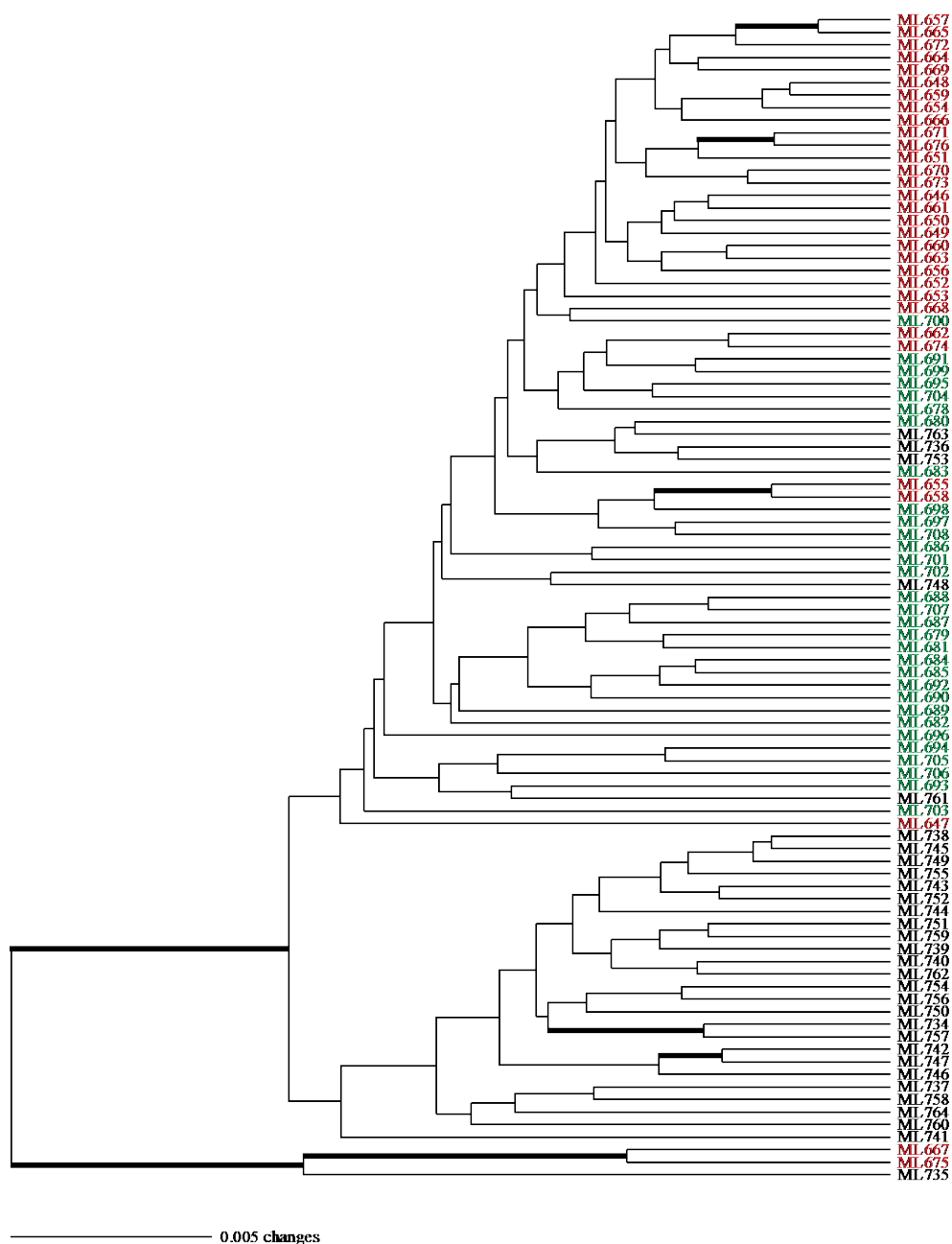


Figure B5. UPGMA analysis of ISSR data for *Astragalus tricarinatus* (analysis 2: missing data = 0). Population color code: JOTR – Eureka Peak (red); Pop 2 = JOTR – Covington Flats (green); Pop 3 = Whitewater Canyon (black). Bold lines indicate branches with bootstrap support $\geq 50\%$.

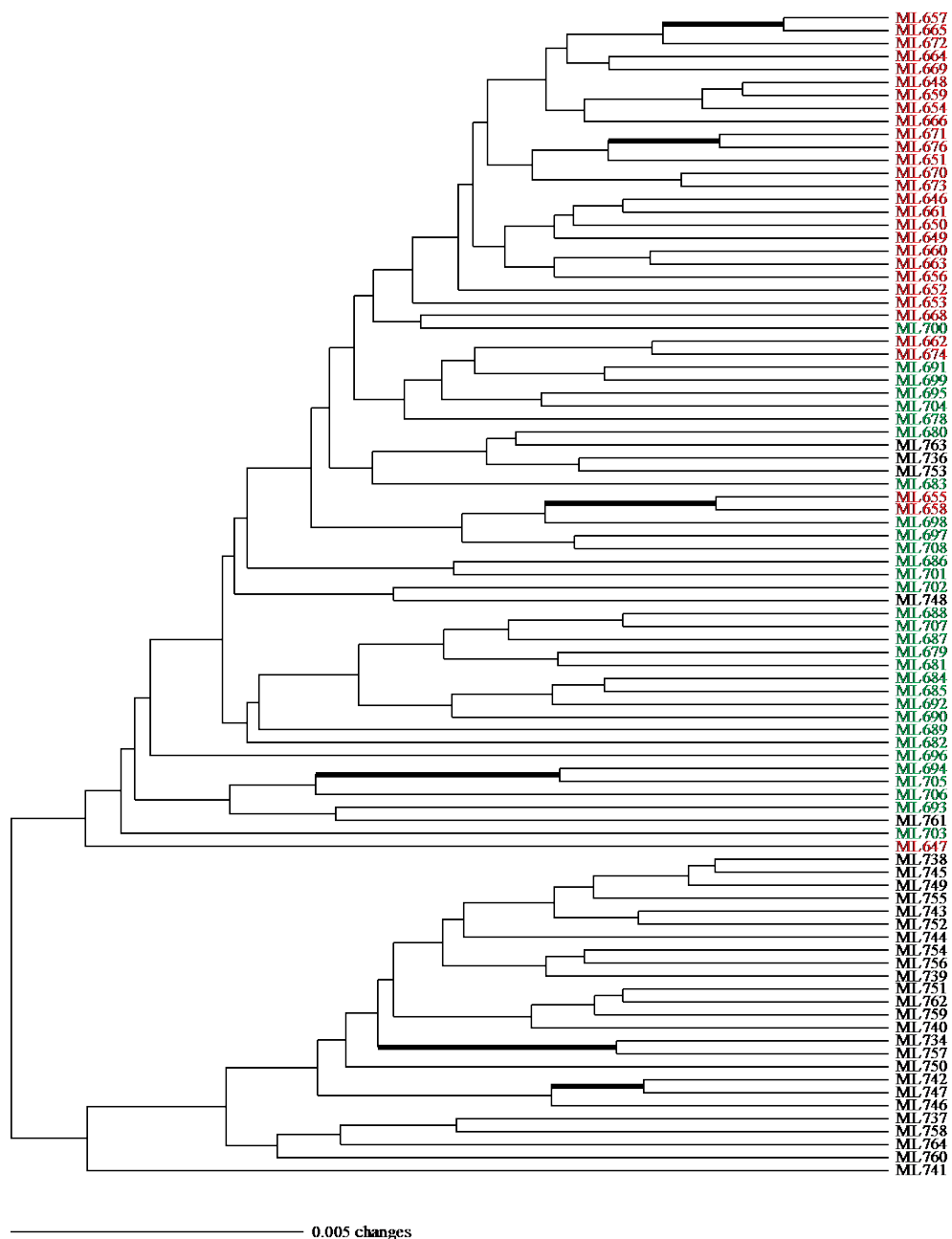


Figure B6. UPGMA analysis of ISSR data for *Astragalus tricarinatus* (analysis 3: excluding taxa with missing data). Population color code: JOTR – Eureka Peak (red); Pop 2 = JOTR – Covington Flats (green); Pop 3 = Whitewater Canyon (black). Bold lines indicate branches with bootstrap support $\geq 50\%$.

Appendix C: GIS habitat model for *Astragalus tricarinatus*

By Sean Murphy, Mitzi Harding, and Tasha La Doux, Joshua Tree National Park

Summary

A habitat model was developed to identify potential habitat for *Astragalus tricarinatus* in Joshua Tree National Park (JOTR). The model is designed to highlight probable habitat based on quantitative characteristics associated with known localities. Parameters used in developing the probabilities include: slope, aspect, elevation, soil mapunit, and vegetation association. Geographic locations are based on voucher specimens, field surveys, and CNDDDB occurrences. After a preliminary model was developed, high probability areas lacking presence points were targeted for a ground truthing exercise. The final model can be accessed as an ArcGIS Toolbox, labeled “Rare Plant Model” and is available on the JOTR Resource Division network shared drive.

Methods

The habitat model was developed in a python environment using Python v2.6 (© Copyright 1990-2014, Python Software Foundation) because the tools available in ArcToolbox and capabilities in ModelBuilder (ArcGIS v10.1) were not sufficient to complete the task. The python script provided the flexibility to look at raster files, query properties of those raster files, and then implement dynamic statistics based on those properties. A script tool interface was created in ArcGIS Desktop, an environment familiar to GIS users, for the user to specify input parameters. The *Astragalus tricarinatus* Habitat Model script can run one, some, or all of the following parameters based on user preferences: elevation, slope, aspect, soil mapunit, and/or vegetation association. A “presence point buffer” shapefile was produced in ArcGIS by creating circular polygons centered on each known locality with a 15-meter radius. The area within each polygon is then used to collect data for each of the parameters (e.g. averages, minimum/maximum). Also, the script was developed with the idea that it would be implemented iteratively with a ground truthing process, as it is based on presence of known locations. Absence points were not informative for the model and therefore are not incorporated into the script. Please note that the script requires an ArcInfo level of licensing and the Spatial Analyst extension.

A preliminary model was produced in ArcGIS by manually performing the functions now automated by the *A. tricarinatus* Habitat Model script. Range limits were manually calculated for each parameter using the buffered presence point layer. The ranges of these values were then used to create a limited raster for each parameter. Finally, we performed a weighted overlay of those limited rasters to create the final habitat model. The ranges of values used in creating the intermediate rasters were determined by narrowing the range for each parameter to include a minimum threshold of 60% of the area within the known habitat layer (buffered presence points). This threshold was determined to create range limits that best captured our understanding of the known habitat for the species, more specifically:

Four vegetation associations were included in the first model: Single-leaf Pinyon Pine / Muller’s Oak (*Pinus monophylla* / *Quercus cornelius-mulleri*) Woodland Association, California Juniper /

Blackbush (*Juniperus californica* / *Coleogyne ramosissima*) Association, Muller Oak — California buckwheat — Narrowleaf goldenbush (*Quercus cornelius-mulleri* — *Eriogonum fasciculatum* — *Ericameria linearifolia*) Association, Catclaw Acacia — Desert Almond (*Acacia greggii* — *Prunus fasciculata*) Association. These vegetation associations accounted for 95.2% of the area within the presence point buffer layer.

Two soil mapunits were used for the calculations: Xerric Torriorthents-Bigbernie association, Smithcanyon gravelly sand. These two soil mapunits account for 94% of the area within the presence point buffer layer.

Elevation range was limited to 1370–1470 m, which accounted for 63.4% of the area in the presence point buffer layer.

Slope range was limited to 3°–40°, which accounted for 95.8% of the area in the presence point buffer layer.

Aspect range was limited to 155°–262°, which accounted for 68% of the area in the presence point buffer layer.

The output from this model was then used to prioritize areas for ground truthing surveys, which yielded two new occurrences for the species (see Figure C1). Originally, we had thought that using absence points would be helpful in producing the model, however, in the end this was not the case. It is possible that absence points could be useful in a model that focuses on a narrower range of field values for the various parameters (i.e. limit the analysis to one watershed). The first model was informative for the development of a more user-friendly and automated script that is less time-intensive and reduces user error.

The final model is set up so that the user is not required to manually establish the range limits for the various parameters, as this method is tedious and inconsistent. The user only needs to select the various source data layers to be used in the analysis through a user-friendly interface, after which the model will perform calculations according to the specific scripts for each parameter (read below for details). Essentially, the model will create range limits for raster data such as slope, aspect, and elevation, capturing 95% of the area within the presence point buffer layer. For vector data such as soil and vegetation, only the type that represents the most area within the presence point buffer layer will be selected for. These limitations can be overcome either by adjusting the script (only to be done by advanced GIS/modeling specialists) or by creating source data layers that somehow combine relevant data. For example, by assigning a common code to all vegetation associations represented by the presence point buffer layer, the analysis could better represent the range of vegetation associations associated with the occurrence of the species. This type of manipulation to the source data layers can also offset any bias associated with an unequal number of data points in any given area. Because the model is based on weighted averages, it will always be prone to bias the results according to the habitat associated with the highest number of presence points. While this type of limitation may be appropriate for narrow habitat specialists, it can be misleading for plants that occur in a variety of habitats. For this reason, it may be reasonable to exclude certain presence points, if

they represent anomalous habitat types (for example, a waif found in the wash below the main population on the slope). Conversely, it is important to continue to add points to the presence point buffer layer, as each additional point will hopefully increase the accuracy of the output. In particular, it is important to make sure that GPS points are recorded in the field for each individual plant, or a group of plants isolated by a 15-meter radius.

The model is meant to be iterative. In other words, the output of each model will guide field surveys to target high probability habitat, then as new populations/individuals are added to the database, the model will increase in accuracy. Also, since a date field is collected with each point, users can select data in a date range and rerun the model based on the subset. Doing so will allow the user to see how the model accuracy changes over time as the amount of presence points are increased.

Scripts

Elevation: When the user selects elevation as an analysis parameter and specifies a digital elevation model to use in the analysis, the script applies the corresponding elevation statistic calculations. First, it extracts elevation values that are within the presence point buffer. Second, it calculates the elevation standard deviation and average of the extracted values. Third, it assigns a negative standard deviation elevation value and a positive standard deviation elevation value. This is done by taking the calculated standard deviation and subtracting or adding two standard deviations from the average elevation value, accordingly. Based on the standard deviation range, the script's last step takes the original digital elevation model and selects the elevation values within the standard deviation range to create a new raster. This raster is used in the last step of the script during the weighted overlay.

Slope: When the user selects slope as an analysis parameter and specifies a slope surface analysis product to use in the analysis, the script applies the corresponding slope statistic calculations. First, it extracts slope values that are within the presence point buffer. Second, it calculates the slope standard deviation and average of the extracted values. Third, it assigns a negative standard deviation slope value and a positive standard deviation slope value. This is done by taking the calculated standard deviation and subtracting or adding two standard deviations from the average slope value, accordingly. Based on the standard deviation range, the script's last step takes the original slope raster and selects the slope values within the standard deviation range to create a new raster. This raster is used in the last step of the script during the weighted overlay.

Aspect: When the user selects aspect as an analysis parameter and specifies an aspect surface analysis product to use in the analysis, the script applies the corresponding aspect statistic calculations. Aspect values are cyclic values, meaning 0° and 360° are the same value, and not a range that starts with a low value and stops at the high value. Because aspect values are cyclic, a few more steps needed to be incorporated into the script. First, it extracts aspect values that are within the presence point buffer. Second, it isolates the values ranging from zero to 180° by setting values less than one and greater than 180° to null. Third, it calculates the aspect standard deviation and average extracted values subset. Fourth, it assigns a negative standard deviation aspect value and a positive standard deviation aspect value. This is done by taking the calculated standard deviation and subtracting or adding two standard deviations from the average aspect value, accordingly. Next, the third and fourth step are repeated for aspect values ranging from 180 to 360 degrees – a subset is

created, average and standard deviation are calculated, and the low and high value in the standard deviation range are calculated. Based on the standard deviation range, the script's last step takes the original aspect raster and selects the aspect values within both standard deviation ranges and creates a new raster. This raster is used in the last step of the script during the weighted overlay.

Soil: When the user selects soil as an analysis parameter and specifies soil polygons to use in the analysis, the script applies the corresponding soil statistic calculations. First, it clips soil mapunit to the presence point buffer. Second, it calculates coverage area for each soil mapunit. Third, it sorts through the area totals and isolates the soil mapunit with the most coverage (the maximum). Lastly, the maximum soil mapunit is selected out from the original soil polygons and converted into a raster format. This raster is used in the last step of the script during the weighted overlay.

Vegetation

When the user selects vegetation as an analysis parameter and specifies vegetation polygons to use in the analysis, the script applies the corresponding vegetation statistic calculations. First, it clips vegetation associations to the presence point buffer. Second, it calculates coverage area for each vegetation association. Third, it sorts through the area totals and isolates the vegetation association with the most coverage (the maximum). Lastly, the maximum vegetation association is selected out from the original vegetation polygons and converted into a raster format. This raster is used in the last step of the script during the weighted overlay.

Weighted Overlay

The last part of the script takes the five parameters, or less if a parameter was excluded from the analysis, and overlays them using the weighted overlay tool. The tool was set to give each parameter equal weight. The result is an overlay that has values ranging from zero to five, or zero to the number of parameters being analyzed; five representing where the habitat is most likely located and zero representing where the habitat is least likely located.

Results and Discussion

The final model can be accessed as an ArcGIS Toolbox, labeled "Rare Plant Model" and is available on the JOTR Resource Division share drive. The model can be used for any species, provided geospatial data layers with known locations and at least one of the corresponding parameters are available.

Below are two examples of the automated model output. The first attempt (Figure C2) utilized all five data layers (elevation, aspect, slope, vegetation, and soil) without any modifications to the source data. As discussed above, the highest probability habitat is biased toward the single vegetation association and soil mapunit with the highest number of occurrences. For comparison, in order to represent *all* of the vegetation associations and soil mapunits with known localities, we modified the vegetation and soil source data layers for a second version of the model. This second version (Figure C3) also utilized all five parameters, but with the following modifications:

- 1) There are four vegetation associations represented by the presence point buffer layer. These were discussed above and were used to develop the preliminary habitat model. In order to

capture and equally weight these vegetation associations, we decided to combine all four of them into one common name and code, therefore forcing the script to recognize them all as one vegetation association.

- 2) We re-coded the three most common soil mapunits found within the presence point buffer layer, so that all would be included in the final overlay. Two of these soil mapunits were discussed above and were included in the preliminary model, and a third (Smithcanyon-Stubbespring-Rock outcrop complex) was added due to newly discovered populations found during the 2013 ground truthing surveys on that soil mapunit.

As expected, the second version of the automated model significantly expands the area of “highly probable habitat” within the Little San Bernardino Mountains (Figure C3). The second version seems to reflect probable habitat more accurately according to the authors experience and understanding for preferred habitat of *A. tricarinatus* in JOTR. In this second model, areas with newly discovered robust populations (for example, northwest of Eureka Peak) are highlighted as “very high probability” habitat, whereas in the first model (Figure C2) they are not.

As discussed earlier, the model will only select for the most frequent category of each parameter, such that adjusting the input layers to best reflect the known habitat preferences will likely improve results. Finally, it is important to keep in mind that some areas of JOTR have been surveyed more thoroughly than others and efforts to record data points have not been driven by the 15-m radius requirement of this model.

Future Suggestions

Future efforts will focus on the following items.

- 1) Field efforts to locate new populations will focus on high probability areas according to the map produced by version 2 of the current model (Figure C3).
- 2) Time permitting, geospatial data will be taken for each individual, unless there are multiple individuals within a 15-meter radius of the point.
- 3) New data will be added to the presence point buffer layer in a timely manner and the model will be updated accordingly.
- 4) Future models will consider the idea of utilizing absence points, as well as providing a means to weight the parameters differently.
- 5) Expand the geospatial scope of the model to include the entire range of the species; or at the very least incorporate habitat information from known localities outside the park boundary.
- 6) Additional trials will be done with the current model, to see if other modifications to the available data sources increase the accuracy of the output. For example, combining fields or removing parameters altogether. In addition, reducing the area being used in the model (e.g. to one watershed), may produce a more accurate prediction on a finer spatial scale.

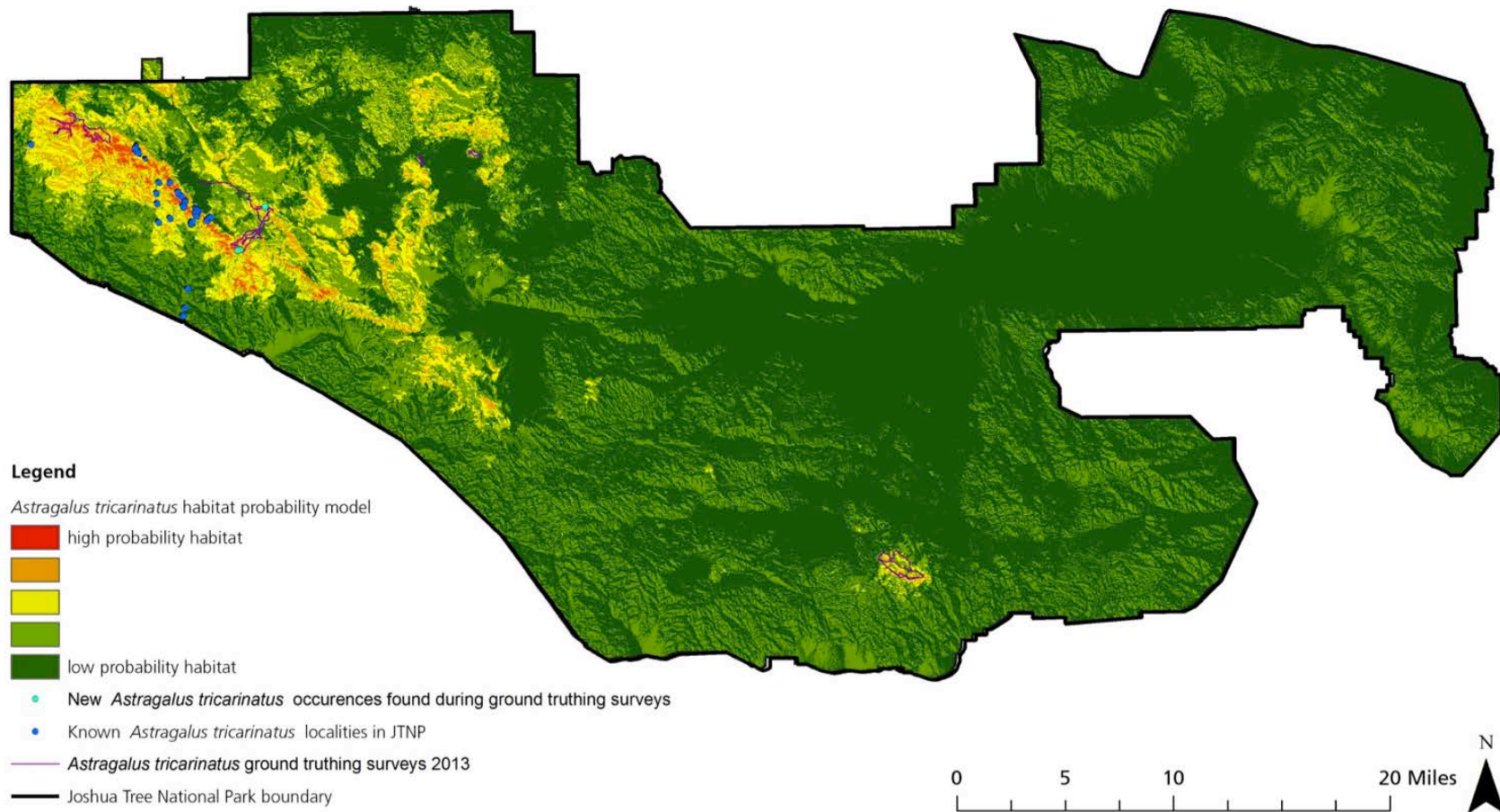


Figure C1. Preliminary *Astragalus tricarinatus* habitat probability model. This model reflects a weighted overlay of a manually defined range of values for elevation, slope, aspect, soil mapunits, and vegetation associations that are most commonly associated with the known *A. tricarinatus* localities within Joshua Tree National Park. Dark green represents the lowest probability habitat, while red represents areas of highest probability habitat. Ground truthing surveys were performed in spring and summer of 2013, yielding two new occurrences; one population of approximately 50 individuals south of Quail Mountain, and another estimated at over 50 individuals on the steep slopes of the Little San Bernardino Mountains.

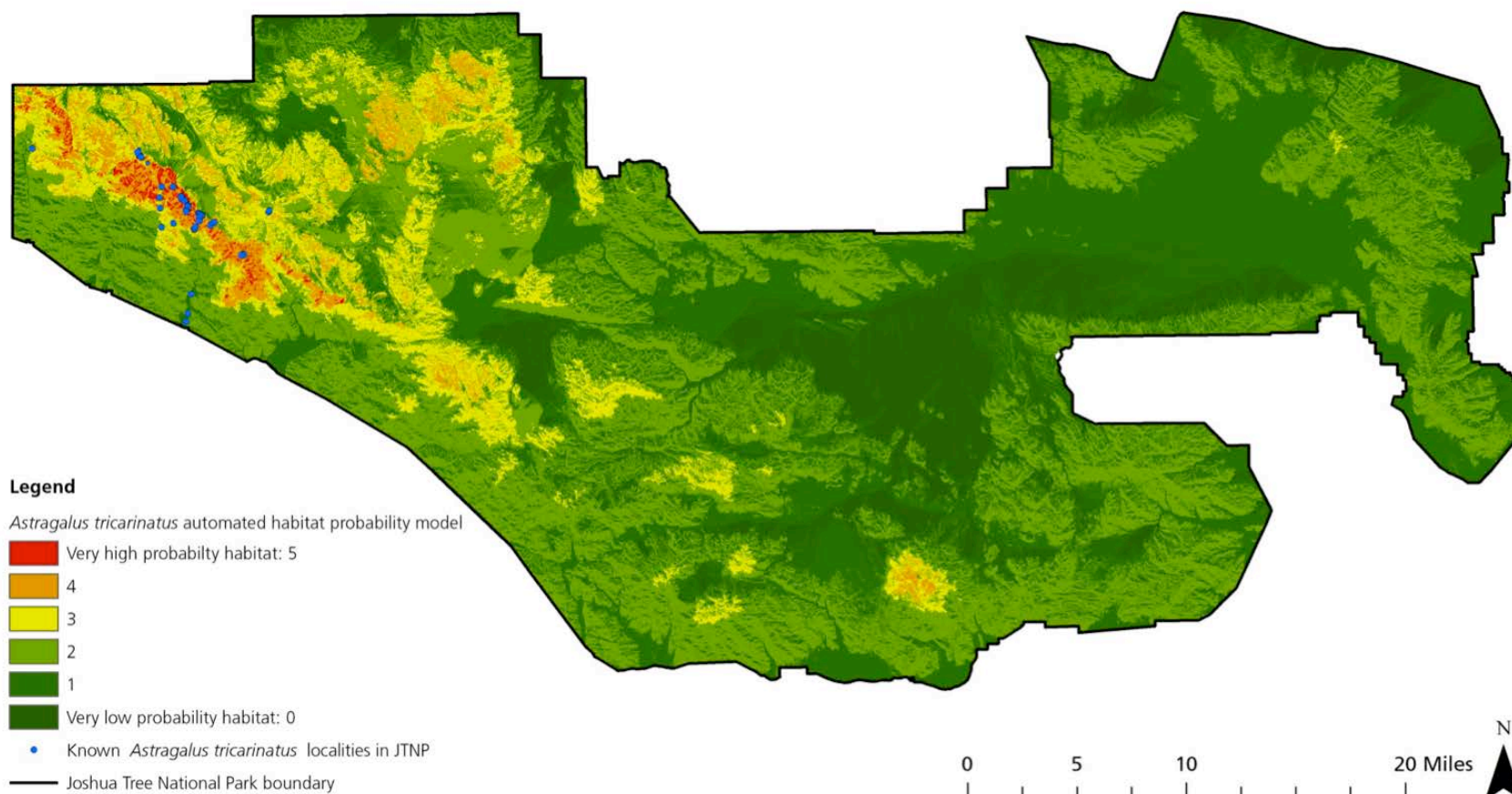


Figure C2. *Astragalus tricarinatus* habitat probability model output produced using the ArcGIS Toolbox automated script, Rare Plant Model. The model output reflects a weighted overlay of a statistically defined range of values for elevation, slope, and aspect representing values associated with 95% of the area in which the species occurs in Joshua Tree National Park. Soil mapunits and vegetation associations were restricted to the one type containing the highest frequency of occurrences. Areas falling within the target ranges of each parameter are assigned a value of "1", and areas falling beyond the range limitations are assigned a value of "0". Overlapping ranges are summed accordingly; five representing where the habitat is most likely located, and zero representing where the habitat is least likely located.

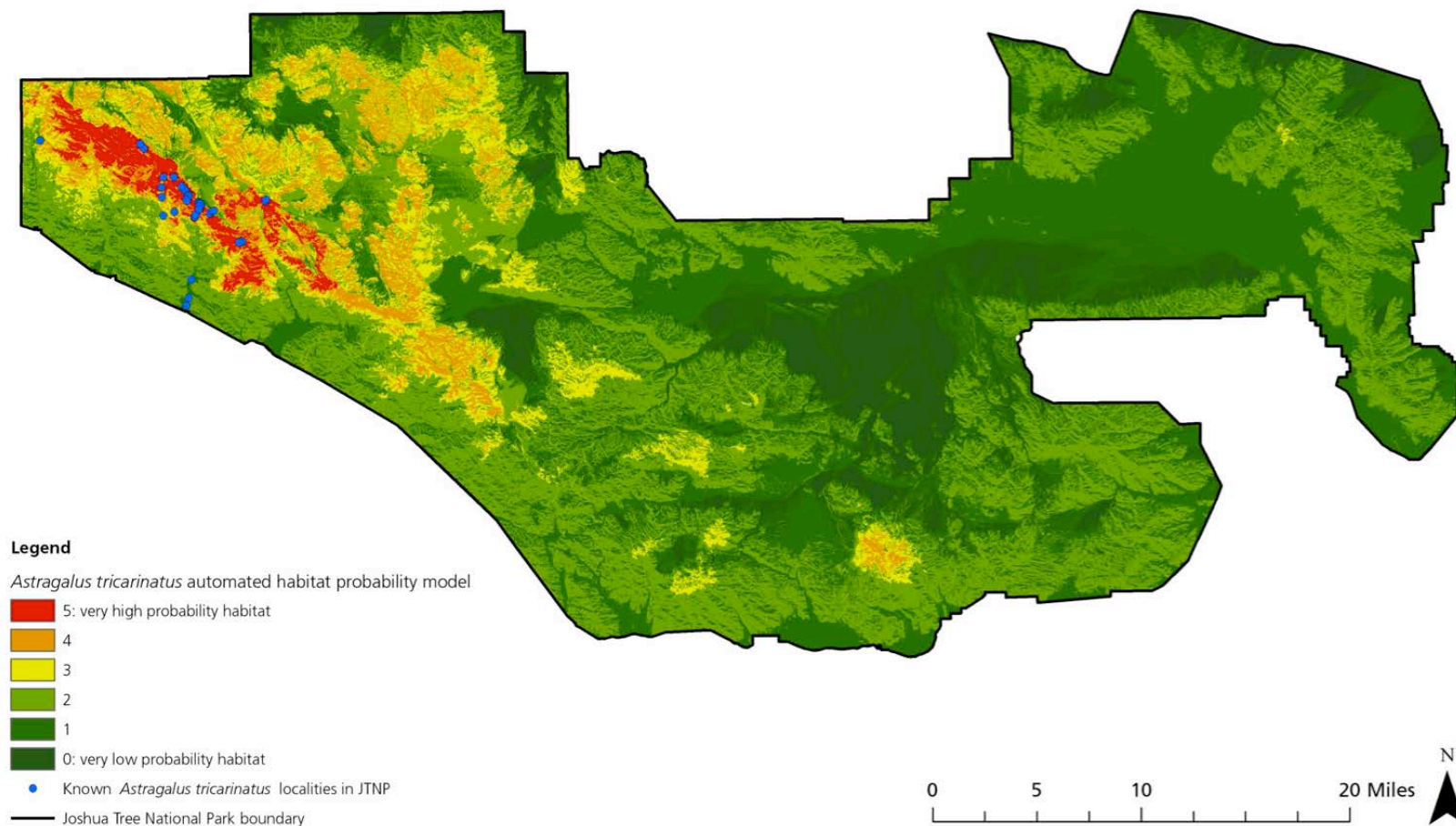


Figure C3. *Astragalus tricarlinatus* habitat probability model output produced using the ArcGIS Toolbox automated script, Rare Plant Model. The model output reflects a weighted overlay of a statistically defined range of values for elevation, slope, and aspect representing values associated with 95% of the area in which the species occurs in Joshua Tree National Park. Soil mapunit and vegetation association source data were edited to combine all types that contain occurrences of the species, resulting in four vegetation associations and three soil mapunits included in the analysis. Areas falling within the target ranges of each parameter are assigned a value of “1”, and areas falling beyond the range limitations are assigned a value of “0”. Overlapping ranges are summed accordingly; five representing where the habitat is most likely located, and zero representing where the habitat is least likely located.

Appendix D: Known occurrences for *Astragalus tricarinatus*

Table D1. List of 45 known occurrences of *Astragalus tricarinatus*. Sources CNDDDB, CCH 2014, JOTR. EO = Element Occurrence; ElmDate = Year species was last seen at occurrence; County = Riverside (RIV), San Bernardino (SBD); KeyQuad = USGS 7.5-minute quadrangle map. NOTE: EO#4 is now considered part of EO#5; EO#7 is now considered part of EO#3; EO#10 was deleted after specimen (Parish JUN 15 1895, UC1601949) was annotated to *A. bernardinus*; NA = EO# not yet assigned.

EO	ElmDate	County	KeyQuad	Elev (ft)	Location	General	OwnerMgt
1	2011	SBD	Morongo Valley	2500	BIG MORONGO CANYON, NEAR COVINGTON PARK IN TOWN OF MORONGO VALLEY, LITTLE SAN BERNARDINO MOUNTAINS.	2 PLANTS SEEN IN 1983. NO PLANTS WERE FOUND IN 1987, 1992, 1994, OR 2005. 1 PLANT OBSERVED IN EACH POLYGON IN 2011.	SBD COUNTY, BLM
2	2011	RIV, SBD	Morongo Valley	2200	WHERE BIG MORONGO CANYON CROSSES THE SAN BERNARDINO/RIVERSIDE COUNTY LINE, LITTLE SAN BERNARDINO MTNS	2 PLANTS OBSERVED IN 1986, 1 IN 1987, 40 IN 1992, 13 IN 1993, 5 IN 1994, 1 SEEN IN 2004 IN WESTERN POLYGON, NONE FOUND IN MAIN CANYON IN 2005. 2 PLANTS SEEN IN 2011 IN EASTERN POLYGON. INCLUDES FORMER OCCURRENCE #7.	BLM-BIG MORONGO CYN PRESERVE
3	1995	RIV	White Water	1800	WHITEWATER CANYON, NORTH OF WHITEWATER.	TYPE LOCALITY. NOT SEEN IN AREA IN 1987 (LOW RAINFALL). 1 IMMATURE PLANT FOUND IN 1995. COLLECTIONS FROM "WHITEWATER CANYON", "ATTRIBUTED TO THIS SITE. NEEDS FIELDWORK.	UNKNOWN
5	1946	RIV, SBD	Morongo Valley	1700	DRY MORONGO CANYON AND WASH.	INCLUDES FORMER OCCURRENCE #4. NEEDS FIELDWORK.	UNKNOWN
6	1994	RIV	Morongo Valley	2000	BIG MORONGO WASH, 1.9 MILES NORTH OF MOUTH, LITTLE SAN BERNARDINO MOUNTAINS.	8 PLANTS OBSERVED IN 1986, NONE FOUND IN 1987, SEVERAL OBSERVED IN 1993, 1 IN 1994, NONE FOUND IN 2005.	BLM-BIG MORONGO CYN PRESERVE
8	1985	RIV	Martinez Mtn.	1500	AGUA ALTA CANYON, ABOUT 1.6 MILES UPSTREAM FROM MARTINEZ CANYON, SANTA ROSA MTNS.	1 PLANT OBSERVED IN 1985.	BLM
9	2011	RIV	Catclaw Flat	2600	MISSION CREEK, CA. 0.5 MILE N OF CONFLUENCE WITH W. FORK MISSION CREEK, SAN BERNARDINO MTNS.	2 PLANTS OBSERVED IN 1997, 8 PLANTS SEEN IN 1998, AND 1 PLANT FOUND IN 2011.	BLM
11	1995	RIV	Desert Hot Springs	2100	MISSION CREEK CANYON, NEAR GATE ON MISSION CREEK ROAD, EAST OF THE RANCH.	ONLY SOURCE OF INFO FOR THIS OCCURRENCE IS A 1995 COLLECTION BY BALLMER. BALLMER LISTED THE PRESENCE OF THIS PLANT AS "SCARCE ALONG THE ROAD".	UNKNOWN
12	2010	RIV	White Water	2400	WHITEWATER CANYON, NEAR WHITEWATER TROUT FARM AND NNW OF TROUT FARM FOR ABOUT 1.2 MILES, SAN BERNARDINO MTNS	SITE BASED ON A 1987 COLLECTION BY PITZER AND A 2010 MISTRETTA ET AL. COLLECTION. NEED MAP DETAIL FOR THIS SITE.	BLM, UNKNOWN
13	1994	RIV	Morongo Valley	1800	BIG MORONGO CANYON, 4 ROAD MI BELOW THE CARETAKER'S HOUSE AT THE BIG MORONGO PRESERVE, LITTLE SAN BERNARDINO MTNS.	2 PLANTS OBSERVED IN 1994. NO PLANTS SEEN IN A 2005 SEARCH OF THE CANYON.	BLM-BIG MORONGO CYN PRESERVE

EO	ElmDate	County	KeyQuad	Elev (ft)	Location	General	OwnerMgt
14	2010	SBD	Catclaw Flat	3800	FROM WATHIER LANDING TO 1 MILE E. OF WATHIER LANDING, SAN BERNARDINO MTNS.	THOUSANDS OF PLANTS OBSERVED IN THE TWO WESTERNMOST POLYGONS IN 2010.	WILDLANDS CONSERVANCY, BLM
15	2011	RIV	Catclaw Flat	2500	MISSION CREEK CANYON, JUST S OF THE MOUTH OF W FORK MISSION CREEK, SAN BERNARDINO MTNS	ONE PLANT OBSERVED IN EACH OF THE 4 E. MOST POLYGONS IN 1998. 21 PLANTS OBSERVED IN THE W. MOST POLYGON BUT NO PLANTS SEEN IN WASH IN E. PART OF OCCURRENCE IN 2011.	THE WILDLANDS CONSERVANCY, BLM
16	UNK	RIV	Orocofia Canyon		OROCOPIA RANGE.	ONLY SOURCE FOR THIS SITE IS A SITE NAME MENTIONED IN BARNEBY (1964). NEEDS FIELDWORK.	UNKNOWN
17	1921	RIV	Myoma		THOUSAND PALMS CANYON, COLORADO DESERT.	ONLY SOURCES ARE A 1921 JAEGER COLLECTION FROM "THOUSAND PALMS" AND A 1921 SPENCER COLLECTION FROM "1000 PALMS CANYON". NEEDS FIELDWORK.	UNKNOWN
18	2009	RIV	White Water	2300	RIDGE E. OF WHITEWATER CANYON; ~2 MI N OF TOWN OF WHITE WATER (I-10), NEAR SUPER CREEK QUARRY, SAN BERNARDINO MTNS.	2 PLANTS WERE SEEN IN 2009.	BLM
19	2005	SBD	Morongo Valley	2400	BIG MORONGO CANYON, 0.8 MI S OF SE EDGE OF MORONGO VALLEY, LITTLE SAN BERNARDINO MTNS.	A SINGLE LARGE, REPRODUCTIVE PLANT WITH OVER 100 FLOWERING STEMS WAS OBSERVED IN 2005. NO PLANTS COULD BE RELOCATED IN 2011.	BLM-BIG MORONGO CYN PRESERVE
20	2011	SBD	Catclaw Flat	3400	E END OF CATCLAW FLAT, 2 MI E OF WATHIER LANDING, WEST OF MORONGO VALLEY.	>100 PLANTS (INCLUDING SEEDLINGS) WERE OBSERVED IN 2005. 96 PLANTS OBSERVED IN 2011.	THE WILDLANDS CONSERVANCY
21	2006	RIV	East Deception Canyon	1725	EAST DECEPTION CANYON, NEAR PARK BOUNDARY, ~0.3 MI N OF END OF E DECEPTION RD, LITTLE SAN BERNARDINO MTNS.	3 PLANTS WERE SEEN AND DESCRIBED AS WAIFS IN 2006. VOUCHERED BY T. LA DOUX (#1454).	NPS-JOSHUA TREE NP
22	2006	RIV	East Deception Canyon	1804	EAST DECEPTION CANYON, NEAR PARK BOUNDARY, ~0.7 MI N OF END OF E DECEPTION RD, LITTLE SAN BERNARDINO MTNS.	1 PLANT WAS SEEN IN 2006. VOUCHERED BY T. LA DOUX (#1452).	NPS-JOSHUA TREE NP
23	2006	SBD	Yucca Valley South	2748	LONG CANYON; STOP 2 IN SIDE CANYON EAST OF MAIN WASH, LITTLE SAN BERNARDINO MTNS.	1 PLANT, PROBABLY A WAIF, WAS FOUND IN 2006. VOUCHERED BY T. LA DOUX (#1446).	NPS-JOSHUA TREE NP
24	2011	SBD	Catclaw Flat	3200	MISSION CREEK; JUST NE OF THE PACIFIC CREST TRAIL, ABOUT 0.9 AIR MILE NORTH OF SBD / RIV COUNTY LINE.	IN 2011, THERE WERE 2 PLANTS GROWING ON THE WEST SIDE OF THE SIDE CANYON JUST BEFORE THE FIRST COTTONWOODS. EXTENSIVE SURVEY WAS NOT CONDUCTED; POSSIBLY MORE PLANTS IN THE AREA.	BLM
25	2011	RIV, SBD	Catclaw Flat	3000	CA. 1.5 MI N OF CONFLUENCE OF MISSION CREEK & W FORK MISSION CREEK, NEAR RIV/SBD CO LINE, SAN BERNARDINO MTNS.	1 PLANT OBSERVED IN 1998 IN CENTER POLYGON. 31 SEEN IN NORTHERN POLYGON AND 6 OBSERVED IN SOUTHERN POLYGON IN 2011.	BLM, UNKNOWN

EO	ElmDate	County	KeyQuad	Elev (ft)	Location	General	OwnerMgt
26	2010	RIV	East Deception Canyon	4600	2.2 AIR MILES SW OF COVINGTON SPRING, W OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	IN NORTHERN POLYGON: 20 PLANTS OBSERVED IN 2009, 21 ADDITIONAL PLANTS OBSERVED IN 2010. IN SOUTHERN POLYGON: 24 PLANTS OBSERVED IN 2010.	NPS-JOSHUA TREE NP
27	2009	RIV	East Deception Canyon	3650	3.3 AIR MI SW OF COVINGTON SPRING, W OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	5 PLANTS OBSERVED IN 2009, WAIFS GROWING IN WASH	NPS-JOSHUA TREE NP
28	2008	RIV	East Deception Canyon	4400	2.2-2.9 AIR MI SSW OF COVINGTON SPRING, S OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	2008 POPULATION NUMBERS OBSERVED IN POLYGONS FROM NORTH TO SOUTH: 54, 1, 3, AND 5 PLANTS. IN 2010, REVISITS TO THE TOP TWO POLYGONS OBSERVED 60 AND 3 PLANTS, RESPECTIVELY.	NPS-JOSHUA TREE NP
29	2008	RIV	East Deception Canyon	4700	2.3 AIR MI SSW OF COVINGTON SPRING, S OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	4 PLANTS OBSERVED IN NE POLYGON AND 2 PLANTS SEEN IN SW POLYGON IN 2008.	NPS-JOSHUA TREE NP
30	2010	RIV	East Deception Canyon	3600	3.7 AIR MI SW OF COVINGTON SPRING, SW OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	4 PLANTS OBSERVED IN 2010.	NPS-JOSHUA TREE NP
31	2006	RIV	East Deception Canyon	2100	BOTTOM OF DECEPTION CANYON, ABOUT 1.5 MI N OF CANYON MOUTH, LITTLE SAN BERNARDINO MTNS.	3 PLANTS OBSERVED IN 2006.	NPS-JOSHUA TREE NP
32	2010	RIV	Joshua Tree South	5000	0.4 AIR MILES SW OF EUREKA PEAK; NW OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTS.	175 PLANTS OBSERVED IN SE POLYGON IN 2010. 40 PLANTS OBSERVED IN NW POLYGON IN 2011.	NPS-JOSHUA TREE NP
33	2009	RIV	Joshua Tree South	4000	2.9 AIR MI WSW OF COVINGTON SPRING, W OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	1 PLANT OBSERVED IN 2009.	NPS-JOSHUA TREE NP
34	2009	RIV	Joshua Tree South	4500	2.4 AIR MI WSW OF COVINGTON SPRING, W OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	4 PLANTS OBSERVED IN 2009.	NPS-JOSHUA TREE NP
35	2009	RIV	Joshua Tree South	3800	3.1 AIR MI WSW OF COVINGTON SPRING, W OF UPPER COVINGTON FLAT, LITTLE SAN BERNARDINO MTNS.	3 PLANTS OBSERVED IN 2009. VOUCHERED BY T. LA DOUX (#1953).	NPS-JOSHUA TREE NP
36	2010	SBD	Catclaw Flat	4000	1 AIR MILE WNW OF WATHIER LANDING, SAN BERNARDINO MTNS.	300+ PLANTS OBSERVED IN EASTERN POLYGON AND 600+ OBSERVED IN WESTERN POLYGON IN 2010.	UNKNOWN
37	2011	SBD	Catclaw Flat	3400	RIDGELINE BETWEEN MISSION CREEK & W. FORK MISSION CREEK, CA. 2 MILES E. OF CATCLAW FLAT, SAN BERNARDINO MTNS	62 PLANTS OBSERVED IN 2011.	BLM

EO	ElmDate	County	KeyQuad	Elev (ft)	Location	General	OwnerMgt
38	2011	RIV	Catclaw Flat	2930	RIDGE AT SE END OF CATCLAW FLAT, 1.1 AIR MILES NNW OF RED DOME, NE OF WHITEWATER RIVER, SAN BERNARDINO MTNS	1 PLANT OBSERVED IN 2011.	BLM
39	2011	RIV	Catclaw Flat	3000	RIDGE BETWEEN W. FORK MISSION CREEK AND WHITEWATER RIVER, ABOUT 1 MI NE OF RED DOME, SAN BERNARDINO MTNS	51 PLANTS OBSERVED IN WESTERN POLYGON AND 26 SEEN IN EASTERN POLYGON IN 2011.	BLM
40	2011	RIV	Catclaw Flat	2700	0.6 AIR MILE NNW OF RED DOME, 0.3 MILE WEST OF PACIFIC CREST TRAIL, ALONG WHITEWATER RIVER, SAN BERNARDINO MTNS	1 PLANT OBSERVED IN 2010, 6 PLANTS SEEN IN 2011.	BLM
NA	1921	RIV	UNK	UNK	CHUCKWALLA MOUNTAINS	ONLY KNOWN FROM A VOUCHER COLLECTED BY SPENCER (#1770, GH366431)	BLM
NA	1975	RIV	UNK	1800	LONG CYN, 1.5 MI N OF JOSHUA TREE NM BOUNDARY; T2SR5ES22; LITTLE SAN BERNARDINO MTNS	COMMUNITY TYPE: ENCELIA, HYMENOCLEA, DALEA CALIFORNICA; ONLY KNOWN FROM VOUCHER COLLECTED BY PATRICK LEARY 5/3/1975 (UNLV9780). LIKELY A WAIF IN WASH.	NPS-JOSHUA TREE NP
NA	2011	RIV	Joshua Tree South	5000	APPROX. 0.75 AIR MILES SSE OF EUREKA PEAK, LITTLE SAN BERNARDINO MOUNTAINS	OBSERVED BY M. HARDING, ESTIMATED 70 INDIVIDUALS ON STEEP LOOSE SLOPES ABOVE WASH	NPS-JOSHUA TREE NP
NA	2010	RIV	Joshua Tree South	3800	3.2 AIRMILES SW OF COVINGTON SPRING, WEST OF UPPER COVINGTON FLAT, LITTLE SB MTNS	2 PLANTS OBSERVED IN 2010	NPS-JOSHUA TREE NP
NA	2013	RIV	East Deception Canyon	5000	1.3 AIRMILES SW OF QUAIL PEAK, WEST OF JUNIPER FLAT, LITTLE SB MTNS	APPROX. 80 PLANTS OBSERVED BY M. HARDING IN STEEP DRAINAGES AND SLOPES	NPS-JOSHUA TREE NP
NA	2013	RIV	East Deception Canyon	4700	2.2 AIRMILES W OF STUBBE SPRING, LITTLE SB MTNS	24 PLANTS OBSERVED ON STEEP, LOOSE SLOPES BELOW RIDGE, POP'N ESTIMATED AT >100	NPS-JOSHUA TREE NP

Appendix E: Pilot study for *Astragalus tricarlinatus* in Joshua Tree National Park

By Tasha La Doux and Mitzi Harding, Joshua Tree National Park

Introduction and Methods

In April of 2010, fifty *Astragalus tricarlinatus* individuals were tagged in the Little San Bernardino Mountains (Figure E1) with the intention of tracking these individuals over time for life expectancy and to assess a variety of life history and reproductive traits. It became very clear during the first visit that frequent observations of the plants would result in extreme destruction to their habitat and likely lead to high mortality rates, therefore data collection was kept to a minimum until better protocols and study sites could be established. Revisits to these plants in 2013 were conducted as a means to glean what we could from the data, however we do not recommend revisiting these plants again.

In 2010, all 50 plants were in flower with green vegetative growth. The following parameters were recorded for each plant: height and two widths, GPS location, percent slope, aspect, reproductive phenology, habitat, associated species, and elevation. We did revisit these plants in 2013, however due to the dry conditions that year, no reproductive activity was observed. In addition, very few plants had any new growth, so height and two widths were recorded for the dead material only.

Height was measured perpendicular to the slope of the ground. The longest axis of any plant material was recorded (dead or alive). During the 2010 season, new growth consistently exceeded the old growth so only one measurement (total growth) was recorded. Width measurements were taken parallel to the slope. The longest axis was measured first, then the second axis was measured perpendicular to the first axis (see Figures F2 and F3 in Appendix F).

Reproductive phenology for each individual was recorded as one of the following flowering stages:

- Vegetative = no reproductive activity
- Bud = No open flowers, head with green phyllaries and/or fresh buds
- Early Flowering = Flowers are open, no open flowers are senescing
- Late Flowering = some or all flowers are senescing or going to fruit
- Fruiting = fruit/seed development with no open flowers

At the time of initial tagging (April 2010), most known locations of *A. tricarlinatus* in the Park consisted of 1 to 5 individuals growing sporadically along steep canyon walls, therefore establishing a permanent plot with >10 individuals was not thought to be possible. In addition we were attempting to minimize our impact to any one site by tagging individuals throughout the canyon.

After 2010, several new, and quite large, populations of *A. tricarlinatus* were discovered in the Park (see discussion in main report), providing an opportunity to reconsider the monitoring protocol. In 2013, we revisited the 50 plants tagged in 2010 and established two permanent plots, one of which incorporates seven of the plants tagged in 2010. A new monitoring protocol was drafted and

employed on the new plots (see Appendix F), although we were not able to fully test efficiency and utility of the protocol due to the lack of vegetative growth and reproductive activity in 2013.

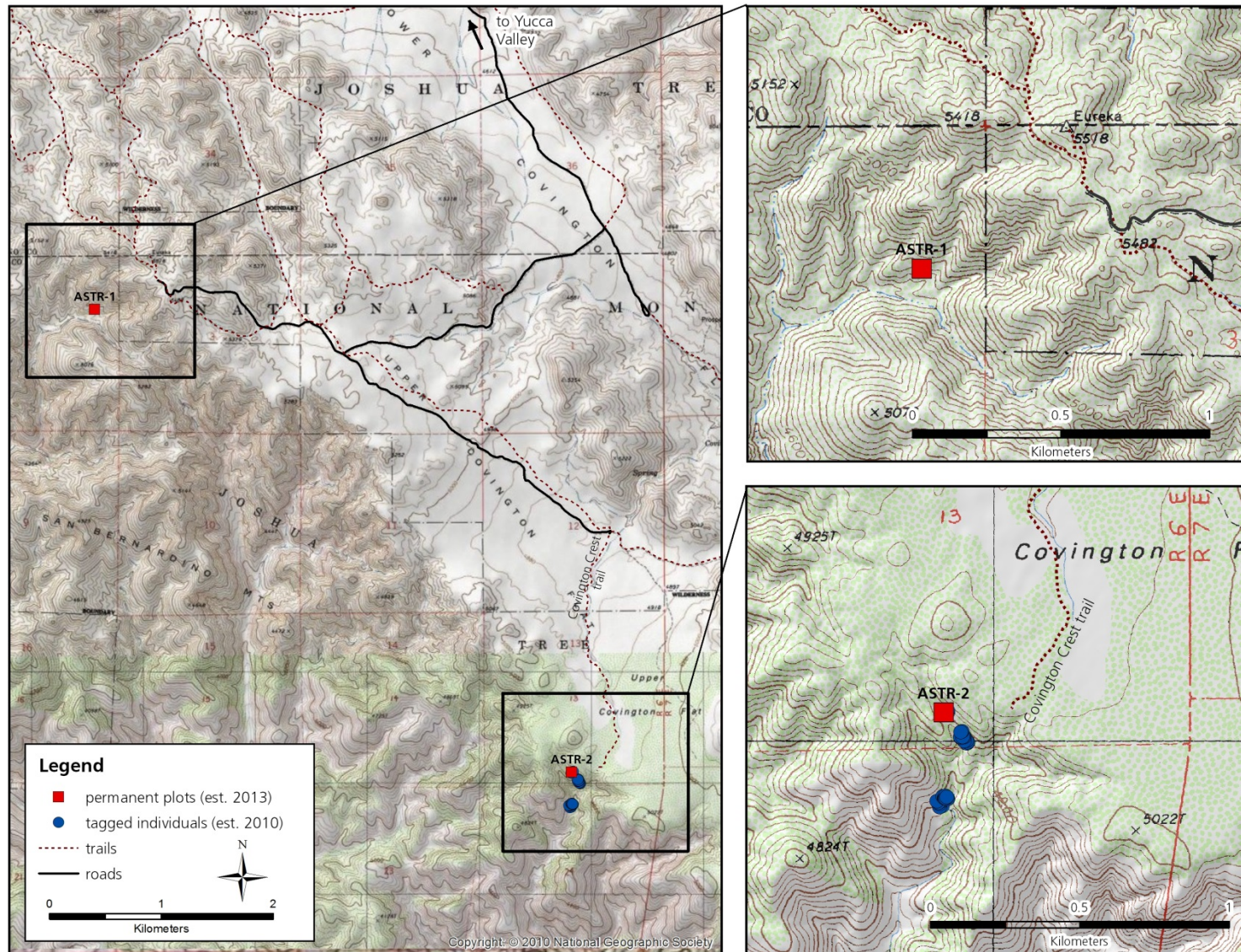


Figure E1. In 2010, fifty *Astragalus tricarlinatus* individuals were tagged (blue circles) and two permanent plots were established in 2013 (red squares) for the purpose of monitoring demographics and reproductive capacity.

The data collected each year will need to be tested and analyzed to assess whether adaptations need to be made before the following season. Ultimately, the objectives of the long-term monitoring protocol presented in this report are as follows:

1. To establish expected life span for individuals.
2. To assess longevity and long term viability of populations.
3. To monitor reproductive capacity.
4. To establish mortality rates and reproductive capacity for size classes.

In addition to these objectives, there are a number of questions (see also the Recommendations section below) that can be built into the monitoring efforts as time and staffing allow. At a minimum, we recommend calculating the ovule/seed ratio as well as collecting/observing any pollinators.

It is impossible to express in this document the difficulties encountered while attempting to establish monitoring plots for this species. Due to the steep slopes, loose soils, and shallow rooting system exhibited by this species, accessing the individual plants is precarious at best. Any person responsible for performing data collection for this species must be forewarned that they need to be prepared for extremely unstable conditions on the slope. This is not to say that the person is necessarily unsafe, but rather their presence on the slope places a high risk for mortality on a federally listed plant. For this reason, the value of the data should always far outweigh the negative impacts of collecting the data to the long-term viability of the species. And perhaps more importantly, only the most agile and experienced hikers will be asked to perform these tasks.

Results and Discussion

All 50 plants tagged in 2010 were flowering, 88% had fruit in addition to flowers (though six of these plants were almost entirely in fruit). All plants were found on SE- to SW-facing slopes with an average grade of 43° (median = 42°, minimum = 20°, maximum = 90°). Elevation ranged from 1339–1443 m (4393–4734 ft). Black aphids and ladybugs were observed on five separate individuals, a “honeybee” was observed visiting flowers on one individual. Insects were not captured.

Based on data from 2010, plants varied in height from 10–70 cm, whereas total volume ($H \times W_1 \times W_2$) varied from 7,500–2,361,528 cm³ (Median = 147,808 cm³). Plants were measured following the protocol described in Appendix F. Measurements were taken on live material, which exceeded any dead material present.

In 2013, two of the 50 tagged individuals were found dead in the wash below the slope; eight plants were not relocated and are therefore assumed to be dead (mortality rate = 20%). The 40 plants re-measured varied in height from 18–53 cm, and total volume varied 12,312–433,964 cm³ (median = 69,296 cm³). Total volume is based on measurements taken of dead material, as none of the plants exhibited any new growth. It is possible that many of the individuals measured were in fact dead, but until we have conditions that allow for comparing normal growth in healthy individuals to dead material, we felt it premature to make these calls. In particular, there were two individuals that

appeared to have a slightly grayer look to the stems (i.e. appeared to be dead), as compared to a yellow-brown present in the others, but because they were still rooted in the ground we took a conservative approach and assumed they were still alive.

Based on the experience from these two years of monitoring, it is clear that annual visits to the plants will be necessary to glean meaningful data. Tracking the response to seasonal precipitation will be very helpful in discerning mortality rates due to drought versus normal senescence. It will also allow for a better understanding of how well these plants can resprout from the base, how quickly they can respond to a rain, and how varying rainfall patterns/amounts affect reproductive capacity. Moreover, if, as indicated in previous reports (Amsberry and Meinke 2007, Sanders 1999, USFWS 2009), the lifespan is three to five years, capturing data annually for that time span will be essential.

Relocating plants in 2013 was extremely difficult and time consuming, in part because the tags did not always stay in the ground. The notes and photos from the previous monitoring season were helpful in trying to positively identify some plants. For this reason, we recommend taking photos and including as much detail in the monitoring notes as time permits.

Future monitoring

In 2013 two permanent belt transects were established in the Little San Bernardino Mountains, near Eureka Peak and Upper Covington Flats (Figure E1). Each is different in size and bearing (Table E1). The goal was to keep the survey area within each transect narrow enough that data could be collected with minimal impact to the site and still capture >10 individuals. More importantly, the plot area should be easily surveyed for new seedlings. The plot near Eureka Peak (ASTR-1) is 25 m long and 12 m wide; the X-axis starts at the top of the slope and runs exactly south (180°) for 25 m; 18 individuals were tagged and measured in May 2013. The plot below Upper Covington Flats (ASTR-2) is 15 m long and 12 m wide; the X-axis starts at the bottom of the slope and runs NNE for 15 m; 14 individuals were tagged and measured in June 2013. The endpoints for each belt transect are permanently marked with rebar.

Table E1. Detailed location and orientation of the two permanent *Astragalus tricarlinatus* monitoring plots located in the Little San Bernardino Mountains. All GPS data is recorded in NAD83 UTM, and bearings are recorded with a 12° east declination.

Plot ID	Origin Location			# of plants	Plot Size and Orientation	
	Zero point	UTM E	UTM N		X-axis	Y-axis
ASTR-1	North end is 0, runs downhill	559493	3765469	18	25m @ 180°	±6m on either side of line
ASTR-2	South end is 0, Runs uphill	~563753	~376129 2	14	15m @ 30°	±6m on either side of line

It was the intention of the authors to establish four permanent plots in 2013, however for a number of reasons this was not accomplished. The primary reasons for the shortcoming are due to a lack of sites with >10 plants easily accessible from a transect line, plus the poor conditions of that season (most plants were dormant). Although the plot west of Eureka Peak (ASTR-1) is fairly accessible without causing excessive soil damage while sampling, the other plot is much more difficult to traverse

without causing soil disturbance. If possible, additional sites will be established, though it might be wise to wait a year or two to see how these two plots withstand data collection as described in the current protocol (provided in Appendix F). It is recommended that data collection occur annually, accordingly. Each year the data needs be assessed as to whether the value of the data exceeds any risk or damage to the plants, as well as to the time and resources required to obtain that data. After careful evaluation any suggested changes to the protocol can be adopted.

Recommendations

- Conducting annual surveys is essential for building a robust long-term dataset that will allow meaningful conclusions about reproductive output and demographic trends. We recommend following the monitoring protocol provided in Appendix F annually for a minimum of 5 years, on the two permanent plots.
- Additional permanent plots will be established. Careful consideration must be given in choosing a site, as the slopes can often be much more difficult to traverse than expected upon first observation. Ideally the plot will be small enough that new seedlings will be easily observed, plants can be accessed without excessive soil disturbance, and a minimum of 10 individuals are found within 5 to 6 m of the transect line.
- We do not recommend revisiting the 50 tagged individuals from 2010.
- Establish a better understanding for dormancy in this species. Through annual monitoring of the conditions under which they can resprout from the base will become clearer.
- Determine meaningful demographic age and size classes.
- Determine rates of survivorship and reproductive output for age and size classes (if appropriate).
- Conduct analyses on how weather conditions affect demographics (survival rates for age/size classes) and reproductive biology (flowering period, seed production/viability) over time. Suggestions for important climate variables include: the amount and timing of rainfall, minimum precipitation per event or season, effect of summer rainfall, temperature extremes, and number of days under a certain temperature.
- Tracking the same 25 inflorescences (five stems on 5 individuals) with the goal of capturing the very beginning of flowering (buds) to when the plant has gone completely to seed will establish an expected duration for each reproductive stage, as well as the timing of anthesis and stigma receptivity.
- Study the waifs and transient populations for longevity, survivorship, and reproductive output to determine if they differ from individuals found in larger populations.

Appendix F: *Astragalus tricarlinatus* monitoring protocol

Ideally, each plot will be visited twice per season, targeting full flower and full fruit phenological stages (April to June). Two people are required for data collection and one day per plot per visit will be required for data collection. All measurements will be recorded in metric.

Things to bring with you in the field:

1. Data sheets from previous season and several blank ones
2. Measuring tape (minimum 25 m)
3. Additional stakes and tags
4. Compass
5. Foldable measuring stick (2 m minimum)
6. Clip boards, Sharpie pens, pencils,
7. GPS unit, extra batteries
8. Seed envelopes
9. Insect vials, net
10. Pin flags (~10 to 20)

Data collection:

1. The belt transects are 25 or 15 m in length (X-axis) and 12 m wide (Y-axis). The X-axis runs down the center of the belt creating a west and east side, 6 m on either side (Figure F1).
2. Establish transect by laying down one measuring tape with the 0 point starting from the origin and running to the established endpoint. One person walks to the endpoint with the tape measure reel, while the other person stands at the origin and guides them with the proper bearing. This will serve as the “X-axis” of the belt transect. The Y-axis is defined by the distance of a plant away from the X-axis, at a perpendicular angle (90°), in both the west and east directions within 6 m.
3. If conditions allow, pin flags can be placed along the x-axis every 2 m, or can be used to mark y-axis measurements. Remember that you will have to go back for them once you walk away, so it is best if they are not used unless they are easily retrieved.
4. One person will be the recorder, and a second person will be the observer calling out data for the recorder. The recorder should stay out of the plot as much as possible.
5. For each plant the following information will be recorded/verified at each visit:
 - o Location of plant within transect. The location represents the center of the plant, to the nearest 0.1 m. The Y coordinate will be accompanied by “E” or “W”, indicating which side of the X-axis the plant is located (Figure F1).
 - o Plant ID (metal tag should be in ground next to plant, or attached to plant)
 - o Height1, width1, width2 of the new growth or live material only, measurements will be to the nearest cm (see Figures F3 and F4).
 - o Height2, width3, width4 of total cover, including old or dead material, only if greater than the above measurements. Measurements will be to the nearest cm (see Figures F3 and F4).
 - o Number of inflorescences
 - o Flowers or Pods/Stem.

- o Reproductive status
 - o Tag location
 - o Notes
6. If the tag is missing, attempt to use the past coordinates to deduce the plant ID. Write down the old ID number in the notes and make sure this information stays on the datasheet. Assign a new ID and attach new tag to the plant or stake it to the ground (preferred). Always record the location of the tag on the datasheet.
 7. Be sure to look for and record any new seedlings or juvenile plants that were not previously recorded. Assign a new ID and tag, accordingly.
 8. On the first visit, randomly select five stems with flowers, use plastic tape to mark the stem by tying it to the base of the inflorescence. Count the number of flowers and circle “Flws” on the datasheet. If both flowers and pods are present on the first visit, count them separately and indicate how many of each by using the prefix “F” or “P”; for example F-8, P-6 would mean there are eight flowers and six pods on that stem.
 9. On the second visit, relocate the five stems with tape, count the number of pods on each stem, circle “Pod” on the datasheet.
 10. Collect one mature (but not dehiscent) fruit from two different stems, place them in separate envelopes, then circle the appropriate box on the datasheet (for example, if you collected a pod from the 1st and 3rd stem on your datasheet, circle the numbers in R1 and R3). Envelopes will be labeled with following: date, collector initials, plot ID, and Plant ID. Remove tape from stem.

Explanation of Fields:

Height: measure perpendicular to ground, from base of plant to highest point (see Figure F2)

Width1 or 3: measure longest distance, parallel to ground (see Figures F2 and F3)

Width2 or 4: measure longest distance perpendicular to width 1 or 3 (see Figures F2 and F3)

Number of inflorescences: count the number of stems with flowers or pods from the current growing season only

of Flws/stem: randomly choose five inflorescences, tie some tape around the stem at the base of inflorescence, then count the number of flowers on each inflorescence. Circle “Flws” on datasheet, or if both flws and pods are present apply a “F” or “P” as a prefix to the numbers, accordingly.

of Pods/stem: locate the five inflorescences with tape and count the number of pods on each inflorescence.

Reproductive Status: If there are no inflorescences from this season then call it vegetative (VEG), otherwise categorize as buds only (BUD), flowering (FLW), flowering and fruiting (FLW/FRT), fruiting (FRT), or post-fruiting (POST). Be sure to base this on the current season inflorescences only.

Tag location: Describe where the tag is located relative to the center of the plant, as well as whether it is attached to the plant or staked into the ground (e.g. on plant, SW)

Notes: Indicate anything about the plant that may be helpful or useful. For example, if it appears to be a juvenile or dying, location of the plant relative to other landmark, herbivory, etc.

Important Notes:

- X- and Y-axis readings are to the nearest 0.1 m.
- All plant measurements will be to the nearest cm.
- Never leave a field blank (except in Notes).
- Timing of first monitoring should target when the plants are in full flower, though if they have some early stage fruit that is fine. The idea for the first visit is to capture the maximum number of flowers per stem.
- Timing of second monitoring should target when the plants are in full fruit, if flowers are present, they are in late stages. The idea for the second visit is to capture the maximum number of mature fruits and therefore the maximum contribution to the seed bank.
- It is very important to minimize your impact to the area. For this reason, only one person should walk around inside the plot. Avoid stepping near, under, or above the base of the plant.
- Bring a copy of the previous years' data with you to ensure all individuals are revisited. This will ensure that any new seedlings or juveniles are recognized. Data can be found in the Botany Program folder within the JOTR Resource Division share drive.
- Blank datasheets (Figure F4) can also be found in the Botany Program folder within the JOTR Resource Division share drive, be sure to print out several copies for each field day.
- Data will be transcribed into an electronic version within a week of data collection. Hard copies of the datasheets will be archived.
- Do not remove the tape from a stem if doing so will damage the plant.
- If you cannot relocate the tape, pick a new stem.

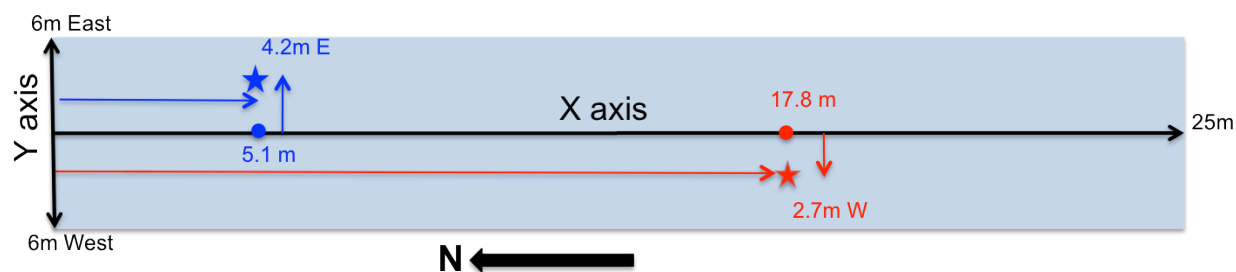


Figure F1. Plot diagram for belt transects, showing two examples of how to measure the x, y axis readings for the location of a plant within the plot. Measurements are taken to the nearest 0.1 m. The y-axis reading is given as the distance from the x-axis with a cardinal direction (e.g. west or east) from the x-axis. Blue star location: x-axis = 5.1 m, y-axis = 4.2 m East. Red Star location: x-axis = 17.8 m, y-axis = 2.7 m West.

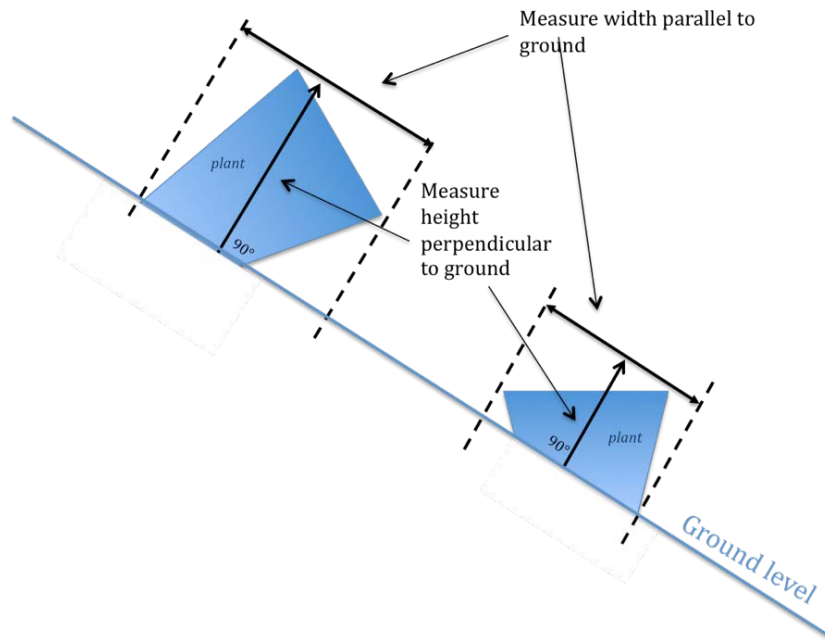


Figure F2. Side view of a hill slope with two plants. Solid black lines demonstrate proper method for measuring width and height.

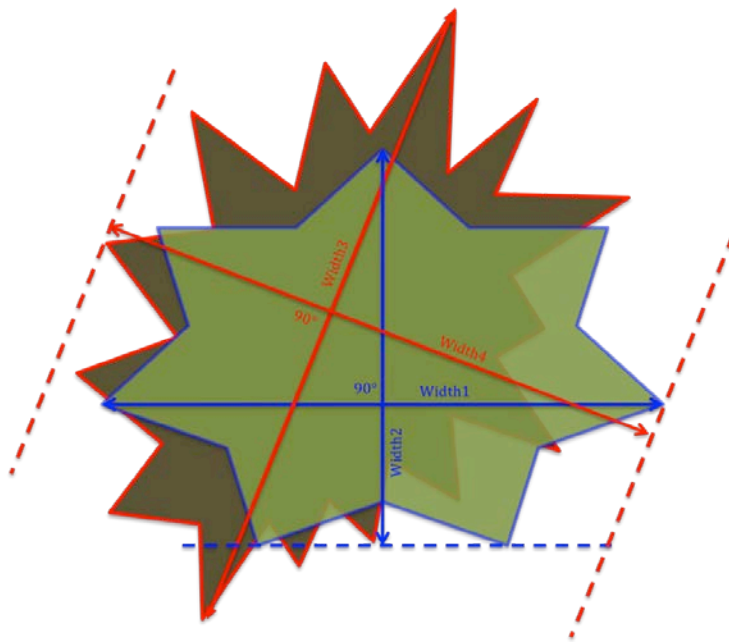


Figure F3. An aerial view of a plant; solid lines demonstrate where to take proper width measurements. Old or dead plant material is shown in brown with red outline, whereas new growth or live material is represented by green with blue outline. Width 1 and 2 represent live (green) material only (blue lines); width 3 and 4 represent total cover (red lines), which includes dead and live material. Width 1 and 3 represent the longest axis, width 2 and 4 are always perpendicular (90°) to their respective axis.

<i>ASTRAGALUS TRICARINATUS</i> MONITORING										Date:	
Observer(s): _____										X-axis bearing:	
Plot Location: _____										Plot size:	
GPS for 0/0: _____										Slope:	
Datum: _____										Elev:	
										Aspect:	
										# Pods Collected:	

Plant ID	Axis reading:		New growth only			Total cover (+old material)			# infl.	# Flws or Pods/stem					Reprod Status	Tag	Notes
	X	Y	H1	W1	W2	H2	W3	W4		R1	R2	R3	R4	R5			

Y-axis reading should include a cardinal direction. #infl = Count the # of stems that are reproductive; #flws or pods/stem = count # flws or pods on 5 separate (randomly chosen) reproductively active stems, circle Flw or Pod to indicate reproductive status or if both are present use F or P as a prefix, accordingly. If pods are collected, circle appropriate box. Categories for Repr status: veg, buds, flw, flw/frt, frt, post-frt. Tag – location of tag relative to center of plant. If tag is attached to plant, indicate this in the notes, otherwise assume it's staked in the ground. Notes should include info about health, age, herbivory, pollinators.

Plot Description (Soil, Habitat, Associated Species) and other Notes:

Figure F4. Example datasheet for *Astragalus tricarlinatus* monitoring.

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