PHYLOGENETIC AND GENETIC RELATIONSHIPS WITHIN *ASTRAGALUS* SECT. *INFLATI*, SUBSECT. *PRORIFERI*: POSITION OF PEIRSON'S MILKVETCH

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INTRODUCTION

In November 1979, Peirson's milkvetch [*Astragalus magdalenae* E.L. Greene var. *peirsonii* (Munz & McBurney) Barneby] was listed as an endangered species under the California Endangered Species Act. This little-known taxon was believed to occur only at the Algodones Dunes in Imperial County, and near Borrego Springs, in San Diego County, California. Due to increasing concern over impacts from recreational use of the Algodones Dunes, Peirson's milkvetch was also afforded protection as a threatened species, under the federal Endangered Species Act, on October 6, 1998. This protection has led to ongoing controversy over the land management at Algodones Dunes and the need for protection of Peirson's milkvetch. The Algodones Dunes have been, and remain, a very popular site of off-road recreation. While conservationists have pressed the BLM to prevent off-highway vehicle (OHV) impacts to Peirson's milkvetch and other sensitive species, organizations promoting OHV recreation and OHV enthusiasts seek to prevent Peirson's milkvetch from restricting access to the dunes. As a consequence, a series of lawsuits have been filed to enforce protection of *Astragalus magdalenae* var. *peirsonii*, as well as to delist (remove protection from) this taxon. However, throughout this controversy little attention has been paid to the taxonomy or distribution of genetic variation of Peirson's milkvetch. Currently, this taxon is treated as a variety of a much more widespread species; however, there is morphological evidence that species status may be more appropriate. Further, recreational activity on the dunes continues to have some impact upon specific populations of Peirson's milkvetch. How local extirpation influences genetic variation of Peirson's milkvetch is unknown. However, with background information regarding genetic diversity of Peirson's milkvetch and the distribution of this variation across the dunes, we may begin to assess these impacts.

Munz and McBurney (Munz 1932) discovered and described *Astragalus peirsonii* Munz and McBurney. Munz contrasted *Astragalus peirsonii* with *A. coulteri* M.E. Jones (now treated as a variety of *A lentiginosus* Dougl.). During research for his monograph on the North American members of *Astragalus*, Barneby (1958) noted similarities between *A. peirsonii* and *A. magdalenae*, reducing *A. peirsonii* and *A. niveus* Rydberg to the varieties of *A. magdalenae*. Barneby's taxonomic treatment is far from irrefutable and there is good reason to question the reduction in rank. Barneby placed Peirson's milkvetch within subsection *Proriferi* (a group of 7

species; Table 1), of section *Inflati* (Barneby 1964). In *Astragalus* section *Inflati*, the leaves all terminate in three leaflets, each with a small petiolule. *Astragalus magdalenae* var. *peirsonii* is the only member of this section that possesses an extended rachis leading to the terminal leaflet. This is referred to by Barneby (1964) as having the terminal leaflet confluent with the rachis. Indeed, this is the only instance in *Astragalus* where this feature varies within a species (see Barneby 1964). Peirson's milkvetch may not be as closely related to *A. magdalenae* as has previously been believed and may not be conspecific. It may represent a distinctive species, whose range and distribution has been misunderstood and confused.

When described, Peirson's milkvetch was known only from "sand dunes between Holtville and Yuma, Imperial County," California (Munz 1932). This location corresponds to the Algodones Dunes. The Algodones Dunes remained the only known occurrence until Barneby (1964) reported a collection of Peirson's milkvetch from the Borrego Valley in San Diego County. Unfortunately, Barneby did not identify the collector or herbarium where the voucher could be found. Repeated searches have failed to locate any collection(s) from the Borrego Valley, leaving the report somewhat dubious. Several other reported localities have proved erroneous. A report of Peirson's milkvetch from the Yuma Dunes, near Yuma, Arizona (Shreve and Wiggins 1964; Wiggins 1980) was based on a misidentified collection of *A. aridus* A. Gray (P. Jenkins pers. comm. 2004). Reports of Peirson's milkvetch from the dunes north of San Felipe, Baja California, Mexico (Shreve and Wiggins 1964) were based upon misidentified collections of *A. magdalenae* var. *niveus* (Porter, pers. obs., examination of specimens at JEPS, UC and RSA/POM). Two anecdotal reports (L. Olech, see CDFG Natural Diversity Data Base) of populations on the southwest margin of the Salton Sea are also dubious. No deep, unstabilized dunes presently occur at the cited locations and no populations are currently present (Porter, pers. obs.); but shallow barchan dunes occurred historically in this region (Smith 1978). The lack of recent collection at these localities does not necessarily mean that there are no other populations in California, but there is no evidence that natural populations, apart from those associated with the Algodones Dune system, occur in California.

Beyond the boarders of the United States, Peirson's milkvetch is known to occur in the Gran Desierto of Sonora, Mexico, based largely on field studies by Dr. Richard Felger (Felger 2000); however, the extent of the population(s) in Sonora, and morphological similarity to

populations in California have never been assessed, because Dr. Felger's specimens have not been accessioned into in any herbarium.

We report on research on the relationships among Peirson's milkvetch and other members of *Astragalus* sect. *Inflati*, subsect. *Proriferi*. Specifically, we have surveyed populations of *Astragalus magdalenae* and other members of *Astragalus* sect. *Inflati*, subsect. *Proriferi*, using ISSR (inter-sequence simple repeat) DNA markers. These data, in combination with phylogenetic analysis of chloroplast, noncoding DNA sequences, should allow us to determine if Peirson's milkvetch maintains high levels of genetic diversity. Peirson's milkvetch has been shown to possess self-incompatibility (SI), an inherited genetic mechanism for self-recognition and rejection of self-pollen. The SI system requires that successful mating will occur only if pollen from an unrelated individual sires the seed. Such obligate outcrossing species require high degrees of genetic diversity, at least at the SI genes, to allow populations to persist. In addition, these data will permit us to determine if Peirson's milkvetch is conspecific with *Astragalus magdalenae*. Our expectation is that, if Peirson's milkvetch is conspecific with *A. magdalenae*, then Peirson's milkvetch will: 1) coalesce (share most recent common ancestry) with *A. magdalenae*; 2) display no greater divergence than do other varieties of *A. magdalenae*; 3) show greatest DNA similarity with *A. magdalenae*, rather than other species; 4) be genetically similar to *A. magdalenae*, and 5) not possess unique and fixed alleles absent in *A. magdalenae*. Failure to realize these expectations, or most of them, constitutes substantial evidence that Peirson's milkvetch and *A. magdalenae* are not conspecific, and therefore represent different species. In addition, we assess the genetic similarity between populations of Peirson's milkvetch in Mexico and U.S.

MATERIALS AND METHODS

Plant Tissue—Specimens were collected by J. Mark Porter and vouchers are housed in the herbarium (RSA-POM) at Rancho Santa Ana Botanic Garden. Fresh leaf material was collected from at least 25 (preferably 30, if present) individuals and placed into 15 ml tubes with silica gel desiccant, as indicated in Table 2. Additional specimens from the Rancho Santa Ana Botanic Garden herbarium (RSA) were used to provide sufficient taxonomic sampling as described in the original proposal. A total of 12 samples were used. Any remaining leaf material for plants is

housed in the permanent silica gel tissue collection, and all DNA extractions are stored in the permanent freezer (-80ºC) collection of the molecular lab at RSA.

DNA Extraction—Total genomic DNA was extracted from 201 samples of *Astragalus magdalenae* and close relatives using standard CTAB extraction methods (Doyle and Doyle 1987). These DNAs represent six populations (N=[13-]25-31) used for DNA sequence and ISSR analyses and six individuals used for DNA sequence analyses (Table 2).

cpDNA Sequence Amplification—Initial amplification, sequencing, and analysis of the *trnL-trnF* intron and intergenic spacer (IGS) region resulted in a small number of variable characters for samples within *Astragalus magdalenae*. The results prompted the screening of five additional intron or IGS regions from the plastid genome (*trnG-trnS* IGS, *trnT-trnL* IGS, *trnC-rpoB* IGS, *trnT-trnD* IGS, and the *trn*f*M-trnS* IGS). All six regions were amplified and sequenced using standard methods as described in Prince and Kress (2006). Nucleic acid sequences for all primers are provided in Table 3. Only those regions with variable characters were pursued to completion, and include the *trnG-trnS* IGS, *trnT-trnL* IGS, and *trnT-trnL* IGS.

ISSR Amplification—Samples of 160 individuals were amplified using four ISSR primers: 807-Fam: AGA GAG AGA GAG AGA GT, 808-Vic: AGA GAG AGA GAG AGA GC, 814-Ned: CTC TCT CTC TCT CTC TA, and 821-Pet: GTG TGT GTG TGT GTG TT, using standard PCR amplification protocols. PCR products were diluted and run on an ABI 3130xl, Automated DNA Analyzer, using a specially designed, Liz1200, size standard. Samples were amplified and run at least in duplicate, to insure that results are repeatable.

DNA Sequence Analysis—Sequences were edited in Sequencher 3.1.1 (Gene Codes Corp., Ann Arbor, Michigan, USA) and aligned manually in Se-Al version 2.0a11 (Rambaut 1996). Data sets were analyzed separately under Fitch (Fitch 1971) maximum parsimony criteria using PAUP4.0*b10 (Swofford 2002). Analyses utilized the “branch and bound” search option which allows for an exhaustive search of the data set if there are fewer than 20 individuals being compared. This approach ensures that the best solution (most parsimonious) will be found and is

preferable to the more commonly used methods involving numerous random addition replicates with branch swapping.

Strength of relationships was evaluated using nonparametric bootstrap analysis (BS; Felsenstein 1985). Bootstrap analysis consisted of 1000 pseudoreplicates, each of 100 random addition sequences, TBR branch swapping, hold 2 trees, retaining a maximum of 100 trees per random addition replicate (Freudenstein et al. 2004). Results are summarized in Figure 1 and Table 4, for separate and combined analyses.

Maximum likelihood analyses were conducted on each data set independently. The model of evolution was specified based on results of the model selection program Modeltest (Posada and Crandall 1998) as shown in Table 4. Two selection criteria, the hierarchical likelihood ratio test (hLRT; Posada and Crandall 1998, 2001) and the Akaike Information Criterion (AIC; Akaike 1974), are evaluated by Modeltest. When different models were selected under the two criteria, analyses were conducted under each. Model parameters were specified in a "PAUP block" at the end of the nexus format data file, with all analyses conducted in PAUP using 10 random addition replicates, hold=2, TBR branch swapping, saving all shortest trees. Strength of relationships was evaluated using posterior probabilities (PP) generated by 2 concurrent 1 million generation analyses in MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2005). Burn-in times (Table 5) were specific to each data set and were determined using the new variance of splits feature of MrBayes. All early generations were discarded until the variance remained below 0.01. A combined analysis was also completed, using the simplest model identified for the individual data sets

ISSR Fragment Analysis—Although the ABI 3130xl uses software that estimates the length of fragments produced in the ISSR amplifications, we found error and miscalling of fragments. As a result, all runs were manually rechecked and each fragment was proofed as to presence or absence, and length. A binary matrix was generated and analyzed under the assumption of Hardy-Weinberg equilibrium. We calculated effective number of alleles (n_e, Kimura and Crow 1964), Nei's (1973) gene diversity (*h*), Shannon's Information index (*I*, Lewontin 1972), and percent polymorphic loci; number of polymorphic loci (*P*), using POPGENE version 1.32 (F. C. Yeh, et al. 2000; www.ualberta.ca/~fyeh/index.htm). Because there was concern over potential fungal contamination and its influence on genetic measures, all calculations were repeated with

potentially contaminated individuals removed. Nei's unbiased genetic identities (I_G) and unbiased genetic distances (D) were calculated, using POPGENE 1.32. Nei's unbiased genetic distances were also used to generate a similarity dendrogram, based on UPGMA, using POPGENE 1.32. In order to describe genetic structure and variability among and within populations and species, Analysis of Molecular Variance (AMOVA) was performed using GeneticStudio 2.0.1 (R. Dyer 2001; www.umsl.edu/~biology/Dyer/GeneticStudio).

RESULTS AND DISCUSSION

Previous examination of comparative DNA sequences of *Astragalus* (e.g., Liston 1992; Wojciechowski et al. 1993; Liston and Wheeler 1994) have revealed surprisingly little variation at the nucleotide level for this species rich group. Often within sections or subsections of the genus, there is no variation whatsoever. For any one of the chloroplast regions we have investigated, our results (Figs. 1 and 2) are consistent with previous studies. There are, in general, low degrees of variation in the *trnL-F*, *trnT-L*, and *trnS-G* regions. However, because these regions are linked and inherited as a single unit, combining them is not considered problematic. When combined, these three regions provide a substantial degree of divergence, particularly with respect to Peirson's milkvetch and *Astragalus magdalenae*.

The samples of Peirson's milkvetch coalesce in parsimony and likelihood analyses of *trnL-F*, *trnS-G*, and the combined regions (Figs. 1 and 2). This provides evidence for the common ancestry of California and Sonora, Mexico populations of Peirson's milkvetch. Populations of *Astragalus magdalenae* varieties *magdalenae* and *niveus* coalesce in parsimony and likelihood analyses of *trnS-G*, and the combined regions. Again, this provides support for the common ancestry of these two varieties from the Baja peninsula and Sonora.

There is some difference based upon the method of analysis concerning the coalescence between Peirson's milkvetch and the other *Astragalus magdalenae* varieties. Parsimony based methods applied to the combined sequences infer common ancestry among Peirson's milkvetch, *A. magdalenae*, *A. aridus*, and *A. fastidius*; however, the precise branching order is not clear. In contrast, maximum likelihood and Bayesian analyses of combined sequences do infer most recent common ancestry between Peirson's milkvetch, and *A. magdalenae*; however, this relationship lacks statistical support. Regardless of the possible close common ancestry of

Peirson's milkvetch and the other *Astragalus magdalenae* varieties, the mutational differences between these two are remarkably high given the overall low degree of variation found in *Astragalus* as a whole.

It is evident that Peirson's milkvetch and *Astragalus magdalenae* display significant differences in the chloroplast DNA sequences examined (Fig. 1). There are no fewer than 16 mutations (point mutations and insertion-deletion events), between these two taxa. This represents the greatest divergence level among all of the species in our sample. In fact, there are only 10 mutation differences between Peirson's milkvetch and *A. douglasii*, the assigned outgroup. In terms of the number of mutations, Peirson's milkvetch shows greater similarity to all other species sampled, rather than *A. magdalenae*.

Given the limitations of sampling, it appears that the amount of divergence between Peirson's milkvetch and *Astragalus magdalenae* is much higher than between either of them and *A. aridus, A. fastidius, A. palmeri, A. gruinus,* and *A. douglasii*. It also seems evident from the number fixed differences (mutations) separating these two taxa that they do not share the same gene pool. Although there is some evidence that Peirson's milkvetch and *Astragalus magdalenae* may share most recent common ancestry (Fig. 2), this also may be an artifact of rooting the tree using *A. douglasii*. It may be necessary to include species that are more distantly related to verify the rooting.

ISSR Survey—We have screened 160 individuals, representing six populations: three populations of Peirson's milkvetch, two populations of *Astragalus magdalenae* var. *magdalenae*, and one population of *A. magdalenae* var. *niveus*. In addition, all samples, including duplicate runs, have been proofed for four primers: 807-fam, 808-Vic, 814-Ned, and 821-Pet. In total, 111 alleles have been identified and 79 alleles were scored. The level of polymorphism is high: of the 79 bands, 74 were polymorphic (93.67%). At the population level, polymorphism ranged from 58.23% (*A. peirsonii* at Anza Borrego) to 83.45% (*A. peirsonii* at Gran Desierto) for all data (Table 6), and from 58.23% (*A. peirsonii* at Anza Borrego) to 83.45% (*A. magdalenae* var. *magdalenae* at Guerrero Negro, Baja California Sur) for data with potentially fungi contaminated individuals removed (Table 7).

In general, genetic diversity was highest at the Algodones Dunes and Gran Desierto populations of Peirson's milkvetch (Table 6), with Nei's gene diversity measures of 0.2373 and

0.2287, respectively. The lowest measures of gene diversity were for *A. magdalenae* var. *magdalenae* at Guerrero Negro, Baja California Sur and *A. magdalenae* var. *niveus* at Puerto Peñasco, Sonora, with Nei's gene diversity measures of 0.1517 and 0.1636, respectively. The low heterozygosity but high number of polymorphic loci in the population of *A. magdalenae* var. *magdalenae* at Guerrero Negro can be explained by the maintenance of a large, but isolated population with the presence of inbreeding. This could result in the maintenance of polymorphism, but largely in the homozygous condition.

Between the California populations of *Astragalus peirsonii* and the Mexican populations of the *A. magdalenae* varieties, Nei's unbiased genetic identity (Tables 8 and 9) ranges from 0.9151–0.9582(–0.9703). These values conform to those reported between different species of *Astragalus*, using allozymes, RAPDs, AFLPs and ISSRs (Liston 1992; Alexander et al. 2004), Genetic identities below 0.960 generally characterize interspecific contrasts. The extreme value of 0.9703 is between a small, extirpated, and presumed artificial population from the Anza Borrego area, California, and the *A. magdalenae* var. *niveus* population, near Puerto Peñasco, Sonora. This high value is possibly an error in the estimate: a function of the small sample size (N= 13), and high frequency of fixed alleles at population Amp-AB.

The population of *Astragalus peirsonii* in the Gran Desierto, Sonora, Mexico displays a strikingly different pattern. Between population Amp-14281 and the *A. magdalenae* varieties, Nei's unbiased genetic identity (Tables 8 and 9) ranges from 0.9700–0.9858. By contrast, between the California populations of *Astragalus peirsonii* and populations Ap-14281 these values range from 0.9433–0.9541. This would seem to be consistent with Ap-14281 being a species different from *A. peirsonii* in California, and likely conspecific with *A. magdalenae*. This is precisely what the UPGMA tree (Fig. 3) would indicate. However, this would seem to conflict with both the chloroplast data and the morphological evidence which aligns the Gran Desierto populations with *A. peirsonii*. We believe that the genetic similarities between Ap-14281 and *A. magdalenae* are a function of introgressive hybridization with *A. magdalenae* var. *niveus*. Felger (2000) suggested that *A. peirsonii* and *A. magdalenae* var. *niveus* were hybridizing, but he provided no evidence. The ISSR data convincingly provide confirmation that, at least at population Ap-14281, there is a history of hybridization that has altered the genetics of the population. There is some further morphological evidence that introgression has occurred, involving one of the most characteristic traits of Peirson's milkvetch, seed size. Barneby (1964)

noted that the seeds of Peirson's milkvetch are perhaps the largest of the genus. Peirson's milkvetch seeds are nearly twice the size of those of *A. magdalenae* (Fig. 4). Seeds of individuals from the Gran Desierto are significantly smaller than those from the Algodones Dunes population, and significantly larger than those of *A. magdalenae*. The intermediate seed size of the Gran Desierto population could be the result of introgression. The degree of introgression involving Peirson's milkvetch in the Gran Desierto is unknown, but could be extensive. Because of this hybridization, population Ap-14281 will be removed from some subsequent analyses.

AMOVA of all sampled populations (Table 10) reveals that most of the genetic variation is within populations; however, a significant proportion (P= 0.0010) is among populations (F_{ST}= 0.1680). This among population variation represents, in large part, a contrast between the varieties of *Astragalus magdalenae* and *A. peirsonii*. AMOVA contrasting *A. magdalenae* and *A. peirsonii* (with population Ap-14281 removed; Table 11) reveals that the between species variance component is slightly larger than between populations (F_{ST}= 0.1813; P= 0.0010).

CONCLUSION

We conclude that Peirson's milkvetch has diverged from other species of *Astragalus* sect. *Inflati*, subsect. *Proriferi*, including *Astragalus magdalenae*. The chloroplast phylogenies collectively support the interpretation that *A. peirsonii* is a lineage apart other members of subsec. *Proriferi*. Peirson's milkvetch may or may not be more closely related to *A. magdalenae* than it is to any other species of this subsection. Indeed, the two show the greatest difference, in terms of nucleotide substitutions and length mutations of chloroplast DNA of any of the taxa sampled. The degree of genetic divergence observed between *A. peirsonii* and *A. magdalenae* is the same degree that has been observed between different species of *Astragalus*. It appears that Barneby's (1964) reduction of *Astragalus peirsonii* Munz & McBurney to varietal status may have been in error. There is ample evidence to support the recognition of Peirson's milkvetch at the rank of species.

Astragalus peirsonii displays higher genetic diversity than *A. magdalenae*. This is consistent with the recognition that Peirson's milkvetch is a self-incompatible plant species, requiring outcrossing for reproductive success. At the same time, the populations of Peirson's milkvetch at Algodones Dunes and the Gran Desierto are genetically very different from one

another. In part, we believe, this difference is due to introgressive hybridization between *A. magdalenae* var. *niveus* and members of the Gran Desierto population of Peirson's milkvetch. As a result, these two regions, Algodones Dunes and the Gran Desierto, should be treated as different genetic units for conservation purposes. It may be desirable to recognize the Gran Desierto populations as a separate subspecies of *Astragalus peirsonii*, characterized in part by its smaller seed size. However, the extent of introgression is not known; and, until more is known regarding genetic diversity throughout the Gran Desierto, we are reluctant to make such a change.

LITERATURE CITED

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Autom. Contr.***19**: 716–723.

Barneby, R.C. 1958. Notes: Preliminary to an account of *Astragalus* in California. *Aliso* **4**: 131-137.

Barneby, R. C. 1964. Atlas of North American *Astragalus*. *Memoirs of the New York Botanical Garden* **13**: 858-863.

Cronn, R. C., Small, R. L., Haselkorn, T. and Wendel, J. F. 2002. Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany* **89**: 707–725.

Demesure, B., Sodzi, N. and Petit, R. J. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* **4**:129–131.

Doyle, J. J. and Doyle, J. L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochem Bulletin* **19**: 11–15.

Doyle, J. J., Davis, J. I, Soreng, R. J., Garvin, D., and Anderson, M. J. 1992. Chloroplast DNA inversions and the origin of the glass [grass] family (Poaceae). *PNAS* **89**: 7722–7726.

Felger, R S. 2000. Flora of the Gran Desierto and Rio Colorado of northwestern Mexico. The University of Arizona Press, Tucson, AZ. 673 p.

Felsenstein, J. 1985. Confidence intervals on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fitch, W. M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* **20**: 406416.

Freudenstein, J. V., van den Berg, C., Goldman, D. H., Kores, P. J., Molvray, M. & Chase, M. W. 2004. An expanded plastid DNA phylogeny of Orchidaceae and analysis of jackknife branch support strategy. *Amer. J. Bot.* **91**: 149–157.

Liston, A. 1992. Variation in the chloroplast genes RPOC1 and RPOC2 of the genus *Astragalus* (Fabaceae): evidence from restriction site mapping of a PCR-amplified fragment. *American Journal of Botany* 79: 953-961.

Liston, A., and J. A. Wheeler. 1994. The phylogenetic position of the genus *Astragalus* (Fabaceae): evidence from the chloroplast genes rpoC1 and rpoC2. *Biochemical Systematics and Ecology* 22: 377-388.

Munz, P.A. 1932. Southern California Plant Notes-4. *Bulletin Southern California Academy of Science* **31**: 61-70.

Ohsako T, and Ohnishi, O. 2000. Intra- and interspecific phylogeny of wild *Fagopyrum* (Polygonaceae) species based on nucleotide sequences of non-coding regions in chloroplast DNA. *American Journal of Botany* **87**: 573–582.

Posada, D., and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution, version 3.7. *Bioinformatics* **14**: 817–818.

Posada, D., and Crandall, K. A. 2001. Selecting the best-fit model of nucleotide substitution. *Syst. Biol.* **50**: 580–601.

Prince, L. M. and Kress, W. J. (2006). Phylogeny and biogeography of the prayer plant family: getting to the root problem in Marantaceae. Columbus, J. T., Friar, E. A., Hamilton, C. W., Porter, J. M., Prince, L. M., and Simpson, M. G. (eds.). *Monocots: Comparative Biology and Evolution.* Rancho Santa Ana Botanic Garden, Claremont, California, USA. *Aliso* **22**: 645–659.

Rambaut, A. 1996. *Se-Al (v2.0a11) Sequence Alignment Editor*. Available at http://evolve.zoo.ox.ac.uk/. University of Oxford, Oxford, UK.

Ronquist, F., and Huelsenbeck, J. P. 2005. MrBayes: Bayesian inference of phylogeny, version 3.1.2. *Bioinformatics* **17**: 754–755.

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, S., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., and Small, R. L. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *Amer. J. Bot.* **92**: 142–166.

Shreve, F. and I. L. Wiggins. 1964. Vegetation and Flora of the Sonoran Desert. Stanford Univ. Press, Stanford. 2 vol. 1740 p.

Smith, R. S. U., 1972. Barchan dunes in a seasonally-reversing wind regime, southeastern Imperial County, California: Geol. Soc. America, Abs. Programs, vol. 4, pp. 240-241.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4(b10). Sinauer Associates, Sunderland, Massachusetts, USA.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991 . Universal primers for amplification of three non–coding regions of chloroplast DNA. Plant Mol. Biol. **17**:1105–1109.

Wojciechowski, M. F., M. J. Sanderson, B. G. Baldwin, and others. 1993. Monophyly of aneuploid *Astragalus* (Fabaceae): evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* **80**: 711-712.

Table 1. Classification of *Astragalus* Sect. *Inflati*, and in particular, Subsection *Proriferi*, following Barneby (1964). Species in bold have been included in this study.

Astragalus Sect. *Inflati*
- Subsect. *Inflati*
  - ***A. douglasii* (Torr. & A. Gray) A. Gray**
  - *A. macrodon* (Hook. & Arn.) A. Gray
  - *A. oocarpus* A. Gray
  - *A. deanei* (Rydb.) R. Barneby
- Subsect. *Proriferi*
  - ***A. gruinus* R. Barneby**
  - ***A. palmeri* A. Gray**
  - *A. proifer* M. E. Jones
  - *A. idrietorum* R. Barneby in Shreve & Wiggins
  - *A. piscinus* (M. E. Jones) R. Barneby
  - ***A. fastidious* (Kellogg) M. E. Jones**
  - ***A. magdalenae* Greene**
  - ***A. peirsonii* Munz & J. McBurney in Munz**
- Subsect. *Aridi* (ca. 16 spp.)
  - *A. allocharis* A. Gray
  - *A. wootonii* Sheldon
  - *A. thurberi* A. Gray
  - *A. wardii* A. Gray
  - *A. aquilonius* (R. Barneby) R. Barneby
  - *A. cerussatus* Sheldon
  - *A. endopterus* (R. Barneby) R. Barneby
  - *A. serpens* M. E. Jones
  - *A. pubentissimus* Torr. & A. Gray
  - *A. pardalinus* (Rydb.) R. Barneby
  - *A. sabulonum* A. Gray
  - *A. nutans* M. E. Jones
  - *A. gilmanii* Tidestrom
  - *A. geyeri* A. Gray
  - *A. insularis* Kellogg
  - ***A. aridus* A. Gray**
- Subsect. *Sparsiflori* (ca. 3 spp.)
  - *A. wetherillii* M. E. Jones
  - *A. sparsiflorus* A. Gray
  - *A. diaphanus* Douglas ex Hook.

Subsect. *Horniani* (1 spp.)
- *A. hornii* A. Gray

Table 2. Specimens acquired for genetic analysis of *Astragalus magdalenae* var. *peirsonii*. Specimens used for DNA sequence analysis are indicated by bold typeface.

Taxon	Source	Accession # / DNA Extraction #	Brief Location Information
Astragalus magdalenae E. Greene var. *magdalenae*	Field collection, J. M. Porter 14247	14247-1 through 14247-25	MEXICO. BAJA CALIFORNIA: 28º53'21"N 113º29'37"W; Bahia de Los Angeles. 25 individuals sampled.
Astragalus magdalenae E. Greene var. *magdalenae*	Field collection, J. M. Porter 14252	14252-1 through 14252-30.	MEXICO. BAJA CALIFORNIA SUR: 27º57'08"N 114º03'14"W; Guerrero Negro. 30 individuals sampled.
Astragalus peirsonii Munz & J. McBurney	Field collection, J. M. Porter 13485	AD1 through AD31 (Sequenced AD27)	USA. CALIFORNIA: Imperial County. Algodones Dunes. 12 sites and a total of 66 individuals sampled.
Astragalus peirsonii Munz & J. McBurney	Field collection, G. Wallace & J. M. Porter s.n.	AB01 through AB13	USA. CALIFORNIA: San Diego County. Blow Sand Canyon, Anza Borrego State Park. 13 individuals sampled, entire population.
Astragalus peirsonii Munz & J. McBurney	Field collection, J. M. Porter 14281	14281-1 through 14281-30	MEXICO. SONORA: Gran Desierto, 31° 34' 18" N, 113° 45' 59" W. 30 individuals sampled.
Astragalus magdalenae E. Greene var. *niveus* (Rydb.) Barneby	Field collection, J. M. Porter 14280	14280-1 through 14280-31	MEXICO. SONORA: Puerto Peñasco, 31° 17' 31" N, 113° 29' 39" W. 31 individuals sampled.
Astragalus fastidius (Kell.) M. E. Jones	RSA Herbarium Specimen #355161	ML012	MEXICO. BAJA CALIFORNIA: Santa Rosalillita, 0.4 mi. W of the village. 10 ft. Sanders #6399, 29 Mar. 1986.
Astragalus gruinus Barneby	RSA Herbarium Specimen #345916	ML013	MEXICO. BAJA CALIFORNIA: Parque Nacional San Pedro Martir, Observatory Mtn. ~2840 m. Thorne #60900, 19 June 1985.

Astragalus palmeri A. Gray	RSA Herbarium Specimen #286552	ML011	USA. CALIFORNIA: Riverside County. SE end San Jacinto Mts. desert slope; South base of Sugarloaf Mtn. 4100 feet. Emmel #693, 12 Sept. 1979.
OUTGROUP TAXA			
Astragalus aridus M. E. Jones	RSA Herbarium Specimen #614898	ML015	USA. CALIFORNIA: San Bernardino County. Mojave Desert, S edge of Cadiz Cry lake. 600 feet. Helmkamp #3107, 04 Mar 1998
Astragalus douglasii (Torr. & A. Gray) A. Gray (var. *parishii)*	RSA Herbarium Specimen #594759	ML010	USA. CALIFORNIA: Riverside County. NW Palomar Mountains; Agua Tibia Wilderness Area. Elev. 1640'. Banks & Hannon #1001, 03 May 1996.

Table 3. Nucleic acid sequences for primers utilitized in an investigation of the relationships among populations of *Astragalus magdalenae* var. peirsonii from the USA and Mexico.

Region	Primer Name	Primer Sequence (5′ to 3′)	Publication Reference
trnL-trnF	c	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991.
	f	ATTTGAACTGGTGACACGAG	Taberlet et al. 1991.
trnG-trnS	3'trnG-UUC	GTAGCGGGAATCGAACCCGCATC	Shaw et al. 2004.
	5'trnG2G	GCGGGTATAGTTTAGTGGTAAAA	Shaw et al. 2004.
	5'trnG2S	TTTTACCACTAAACTATACCCGC	Shaw et al. 2004.
	trnS-GCU	AGATAGGGATTCGAACCCTCGGT	Shaw et al. 2004.
trnT-trnL	a	CATTACAAATGCGATGCTCT	Taberlet et al. 1991.
	A2	CAAATGCGATGCTCTAACCT	Cronn et al. 2002.
	b	TCTACCGATTTCGCCATATC	Taberlet et al. 1991.
	IF	TTCTAATAAATTGGATGGAT	Unpublished, designed by L.M. Prince.
	IR	GTTTCCTTCTATCATTGTTTAA	Unpublished, designed by L.M. Prince.
trnC-rpoB	trnC-GCA-R	CACCCRGATTYGAACTGGGG	Shaw et al. 2004; modification of primer designed by Ohsako & Ohnishi 2000.
	rpoB	CKACAAAAYCCYTCRAATTG	Shaw et al. 2004; modification of primer designed by Ohsako & Ohnishi 2000.
trnT-trnD	trnD-GUC-F	ACCAATTGAACTACAATCCC	Demesure et al. 1995.
	trnT-GGU	CTACCACTGAGTTAAAAGGG	Demesure et al. 1995; modification of primer designed by Doyle et al. 1992.
trnfM-trnS	trnfM-CAU	CATAACCTTGAGGTCACGGG	Demesure et al. 1995.
	trnS-UGA	GAGAGAGAGGGATTCGAACC	Demesure et al. 1995.

Table 4. Summary of data matrices and maximum parsimony analyses for *Astragalus magdalenae* and close relatives. Indices and tree lengths exclude parsimony uninformative characters.

Data Set	Number of Aligned Characters (bases, indels)	Number of Potentially Parsimony informative Characters (bases, indels)	Number of Most Parsimonious Trees	Tree Length	C.I.	R.I.	R.C.
trnC-rpoB	~1200,0^	0,0^	n.a.	n.a.	n.a.	n.a.	n.a.
trnT-trnD	~1100,3^	0,0^	n.a.	n.a.	n.a.	n.a.	n.a.
trnfM-trnS	~830,0^	0,0^	n.a.	n.a.	n.a.	n.a.	n.a.
trnL-trnF	738,2	2,2	1	4	1.00	1.00	1.00
trnG-trnS	1464,1	4,1	1	5	1.00	1.00	1.00
trnT-trnL	1134,3	1,2*	1	3	1.00	1.00	1.00
combined	3336,6	7,5*	6	13	0.92	0.97	0.89

^ Incomplete data set. *Astragalus magdalenae* sequences appear to be invariant.
* 485 characters excluded from analysis due to incomplete data. Additional lab work is required.

Table 5. Results of Modeltest evaluation of data matrices for selection of the model of evolution to be applied in subsequent likelihood analyses for estimation of relationships within *Astragalus magdalenae*. The number of generations (gen) required to reach a stable Bayesian posterior distribution, using Markov chain Monte Carlo methods is provided as the Burn-In.

Marker	hLTR	AIC	Burn-In	Model Parameters
trnL-trnF	F81	HKY	310,000 gen	hLTR: Base=(0.3655 0.1683 0.1670) Nst=1 Rates=equal Pinvar=0 AIC: Base=(0.3656 0.1682 0.1667) Nst=2 TRatio=3.0021 Rates=equal Pinvar=0
trnG-trnS	F81	TVM	94,000 gen	hLTR: Base=(0.3673 0.1463 0.1477) Nst=1 Rates=equal Pinvar=0 AIC: Base=(0.3682 0.1455 0.1468) Nst=6 Rmat=(0.9260 0.3201 0.0000 1.5557 0.3201) Rates=equal Pinvar=0
trnT-trnL	F81	K81uf	149,000 gen	hLTR: Base=(0.4269 0.0892 0.0786) Nst=1 Rates=equal Pinvar=0 AIC: Base=(0.4287 0.0868 0.0766) Nst=6 Rmat=(1.0000 0.0994 0.0000 0.0000 0.0994) Rates=equal Pinvar=0

Table 6. Genetic diversity statistics for populations of *Astragalus peirsonii*, *A. magdalenae* var. *magdalenae*, and *A. magdalenae* var. *niveus*. Statistics include observed number of alleles (n_a), effective number of alleles (n_e, Kimura and Crow 1964), Nei's (1973) gene diversity (h), Shannon's Information index (I, Lewontin 1972), and percent polymorphic loci; number of polymorphic loci (PPL). Standard deviations are provided parenthetically.

Population	N	n_a	n_e	h	I	PPL(%)
Amm14247	25	1.6835 (0.4681)	1.2927 (0.3088)	0.1863 (0.1728)	0.2930 (0.2499)	68.35; n= 54
Amm14252	30	1.7595 (0.4301)	1.2397 (0.3130)	0.1517 (0.1694)	0.2461 (0.2380)	75.95; n= 60
Amn14280	31	1.6582 (0.4773)	1.2565 (0.3060)	0.1636 (0.1715)	0.2592 (0.2489)	65.82; n= 52
Ap14281	29	1.8354 (0.3731)	1.3731 (0.3387)	0.2287 (0.1792)	0.3549 (0.2468)	83.54; n= 66
ApAB	13	1.5823 (0.4963)	1.3041 (0.3611)	0.1806 (0.1946)	0.2748 (0.2772)	58.23; n= 46
ApAD	32	1.6962 (0.4628)	1.4188 (0.4044)	0.2373 (0.2079)	0.3527 (0.2887)	69.62; n= 55
Mean	160	1.9873 (0.1125)	1.3484 (0.2910)	0.2266 (0.1511)	0.3658 (0.1986)	

Table 7. Genetic diversity statistics for populations of *Astragalus peirsonii*, *A. magdalenae* var. *magdalenae*, and *A. magdalenae* var. *niveus*. Statistics include observed number of alleles (n_a), effective number of alleles (n_e, Kimura and Crow 1964), Nei's (1973) gene diversity (h), Shannon's Information index (I, Lewontin 1972), and percent polymorphic loci; number of polymorphic loci (PPL). Standard deviations are provided parenthetically. Eighteen potentially fungus-contaminated DNAs have been removed

Population	N	n_a	n_e	h	I	PPL(%)
Amm_14247	21	1.6456 (0.4814)	1.2872 (0.3117)	0.1817 (0.1758)	0.2841 (0.2553)	64.56; n=51
Amm_14252	28	1.7342 (0.4446)	1.2340 (0.3104)	0.148 (0.1692)	0.2401 (0.2385)	73.42; n=58
Amn_14280	29	1.6582 (0.4773)	1.2639 (0.3151)	0.1665 (0.1747)	0.2627 (0.2521)	65.82; n=52
Ap_14281	19	1.6835 (0.4681)	1.2852 0.3242)	0.1780 (0.1787)	0.2796 (0.2544)	68.35; n=54
Ap_AB	13	1.5823 (0.4963)	1.3041 (0.3611)	0.1806 (0.1946)	0.2748 (0.2772)	58.23; n=46
Ap_AD	32	1.6962 (0.4628)	1.4188 (0.4044)	0.2373 (0.2079)	0.3527 (0.2887)	69.62; n=55
Mean	142	1.9114 (0.2860)	1.3375 (0.3066)	0.2156 (0.1647)	0.3436 (0.2253)	

Table 8. Nei's (standard) genetic identity (above diagonal) and genetic distance (below diagonal), based in ISSR variation, incorporating 160 sampled individuals.

pop ID	Amm_14247	Amm_14252	Amn_14280	Ap_14281	Ap_AB	Ap_AD
Amm_14247	****	0.9538	0.9706	0.9585	0.9379	0.9420
Amm_14252	0.0473	****	0.9704	0.9553	0.9280	0.9092
Amn_14280	0.0298	0.0300	****	0.9678	0.9634	0.9530
Amp_14281	0.0424	0.0457	0.0327	****	0.9383	0.9250
Amp_AB	0.0641	0.0747	0.0373	0.0637	****	0.9689
Amp_AD	0.0598	0.0952	0.0482	0.0780	0.0316	****

Table 9. Nei's (standard) genetic identity (above diagonal) and genetic distance (below diagonal), based in ISSR variation, using a subsample of 142 individuals, removing 18 individuals whose collections showed evidence of fungal growth.

pop ID	Amm_14247	Amm_14252	Amn_14280	Ap_14281	Ap_AB	Ap_AD
Amm_14247	****	0.9538	0.9711	0.9726	0.9386	0.9416
Amm_14252	0.0473	****	0.9702	0.965	0.9298	0.9114
Amn_14280	0.0294	0.0302	****	0.9812	0.9644	0.9541
Amp_14281	0.0277	0.0349	0.0189	****	0.9471	0.9382
Amp_AB	0.0634	0.0728	0.0363	0.0544	****	0.9689
Amp_AD	0.0601	0.0927	0.0470	0.0638	0.0316	****

Table 10. Analysis of molecular variance (AMOVA) results contrasting six populations of *Astragalus peirsonii* and varieties of *A. magdalenae*. We report sums of squares (SS), mean square (MS), variance proportion (Var.), and probability that variance is not different from 0 (P). The corresponding F_{ST} is 0.1680.

Source	df	SS	M S	Var.	P
Among pop	5	292.899	58.580	0.168	0.001
Within pop	154	1427.064	9.267	0.832	0.001
Total	159	1719.963		1.000	

Table 11. Analysis of molecular variance (AMOVA) results contrasting two species, *Astragalus peirsonii* and varieties of *A. magdalenae* (*A. peirsonii* population from the Gran Desierto removed). We report sums of squares (SS), mean square (MS), variance proportion (Var.), and probability that variance is not different from 0 (P). The corresponding F_{ST} is 0.1813.

Source	df	SS	M S	Var.	P
Among species	1	134.168	134.168	0.181	0.001
Within species	129	1228.611	9.524	0.819	0.001
Total	130	1362.779		1.000	

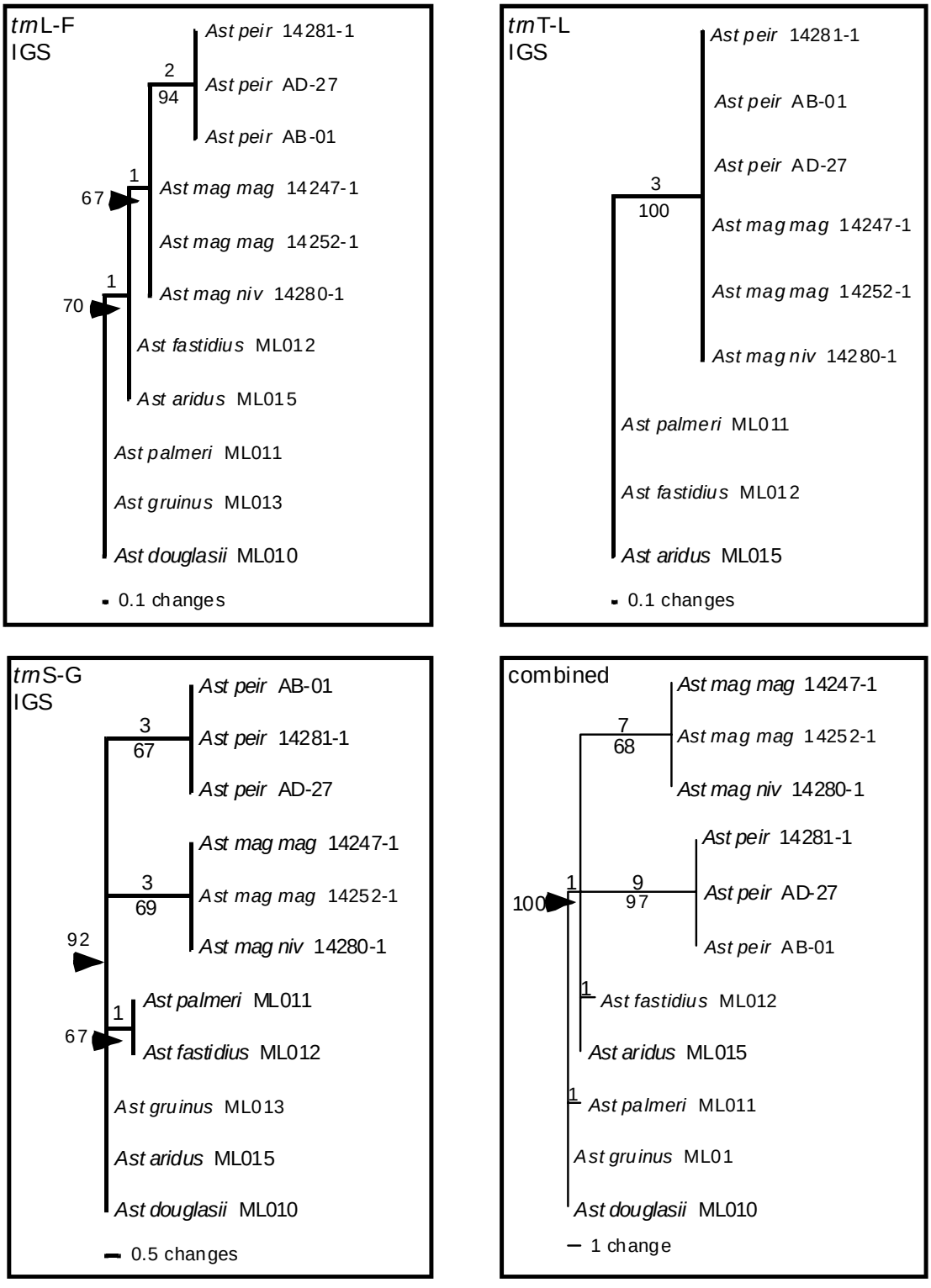

Figure 1. Single shortest tree generated during branch and bound maximum parsimony analyses for *Astragalus peirsonii* and close relatives. Combined data resulted in 6 shortest trees (arbitrarily selected tree shown here). The strict consensus of the combined data trees is identical. Numbers above branches are branch lengths, numbers below are bootstrap percentages.

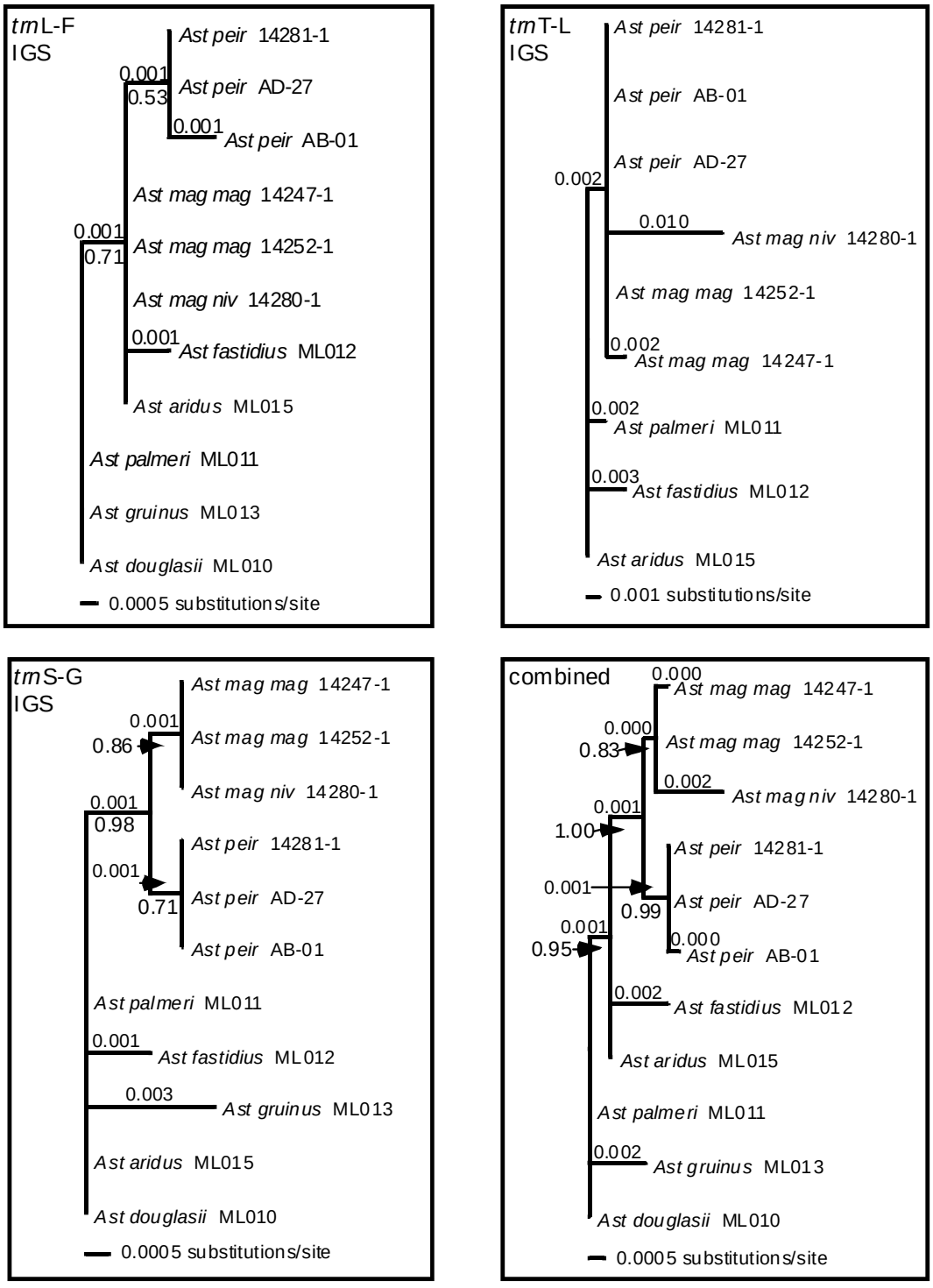

Figure 2. Single best tree obtained from 1000 random addition replicates of maximum likelihood analyses for *Astragalus peirsonii* and close relatives under both the hLRT and AIC criteria (results identical). Numbers above branches are branch lengths, numbers below are posterior probabilities based on MrBayes analyses described in the text.

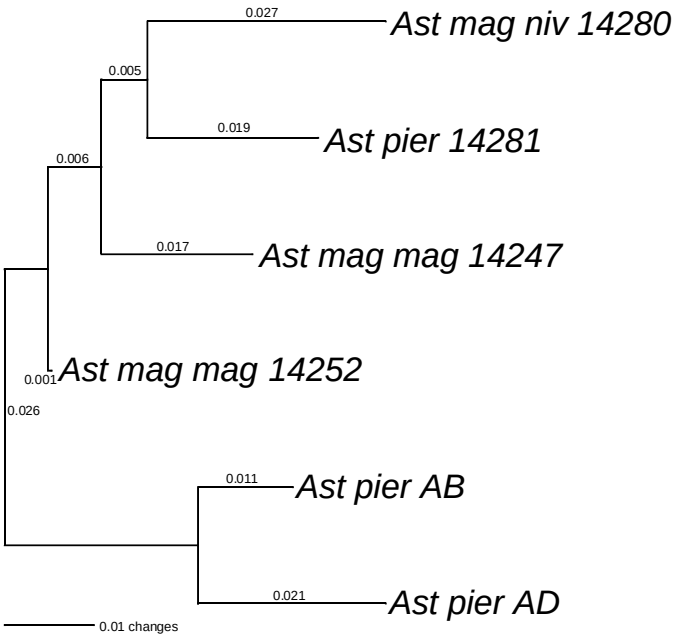

Figure 3. UPGMA dendrogram showing similarity relationships of populations of *Astragalus peirsonii* and *A. magdalenae* varieties *magdalenae* and *niveus*, using Nei's genetic distances of ISSR markers.

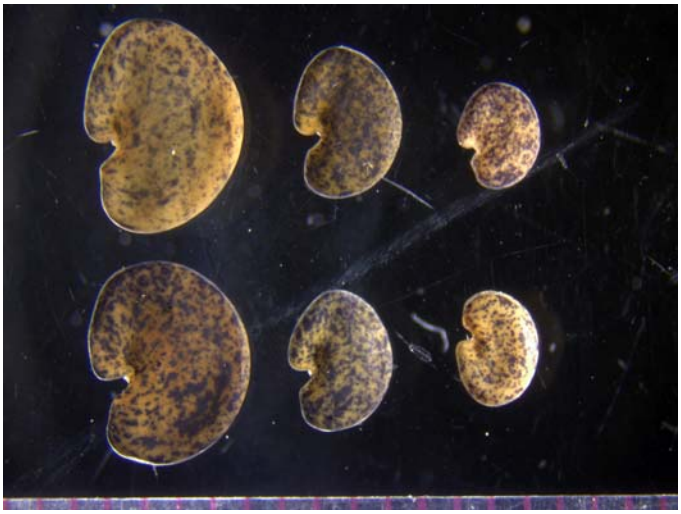

Figure 4. Typical seeds (scale in mm) of *Astragalus peirsonii* in California (left), *A. peirsonii* in Sonora, Mexico (center), and *A. magdalenae* var. *niveus* in Sonora, Mexico (right).