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DIVERSIFICATION RATES IN A TEMPERATE LEGUME CLADE: ARE THERE "SO MANY SPECIES" OF *ASTRAGALUS* (FABACEAE)?[1]

MICHAEL J. SANDERSON[2,4] AND MARTIN F. WOJCIECHOWSKI[3]

[2]Section of Evolution and Ecology, University of California, Davis, California 95616; and
[3]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721

Astragalus, the largest genus of flowering plants, contains upwards of 2500 species. Explanations for this exceptional species diversity have pointed to unusual population structure or modes of speciation. Surprisingly, however, three different statistical analyses indicate that diversification rates in *Astragalus* are not exceptionally high compared to its closest relatives. Instead, rates are high throughout the "Astragalean clade," a much broader radiation distributed throughout the temperate zone. The increase in diversification rate is associated with the origin and divergence of this clade from common ancestors of it and several much less diverse and more narrowly distributed Asian genera. This suggests that causal factors in the shift toward higher rates of diversification must be due not to factors unique to *Astragalus,* but to characteristics common to the entire Astragalean clade. However, this larger clade has never been circumscribed in classifications based on morphological data. This raises the possibility that the causes of increased diversification may not be due to morphological innovation, but may instead be related to ecological factors or cryptic physiological or biochemical features.

Key words: *Astragalus*; diversification rate; Fabaceae; phylogeny; radiation.

As noted by Willis (1922), and numerous authors since then (reviewed in Dial and Marzluff, 1989), the distribution of species among higher taxa usually follows a so-called "hollow curve," characterized by a few species-rich groups and many species-poor or monotypic groups. Willis estimated that in angiosperms fully one-third of genera are monotypic and only a few are exceedingly large. *Astragalus, Carex, Senecio, Euphorbia,* and *Psychotria* each contain over 1000 species (Mabberley, 1993). The existence of hollow curves in disparate taxa and the persistence of large taxa despite the repeated efforts of taxonomists to dismember them suggests the action of an underlying biological process. However, the apportionment of species diversity among higher taxa is strongly dependent on taxonomic practice. For example, the 400 North American species of *Astragalus* were split into 28 genera earlier in this century (Rydberg, 1929), and *Eupatorium,* which formerly included 1200 species, now encompasses only 38, the remaining ones having been dispersed to other genera (Mabberley, 1993). In addition, differences in the absolute ages of taxa of the same rank confound matters by making it difficult to estimate absolute rates of diversification in the absence of a good fossil record.

Nonetheless, despite widespread acknowledgment of the convoluted taxonomic history of genera such as *Astragalus,* the capriciousness of rank, and the inadequacies of the fossil record, the question "why are there so many species of *Astragalus*?" continues to be raised (Polhill, 1981a; Liston, 1989), as do similar questions for other large genera (e.g., Cronquist, 1981, p. 740, regarding evolutionary success in *Euphorbia*). Recent methods that combine statistical analyses of diversification rates with explicit phylogenetic hypotheses hold promise to provide answers to these kinds of questions even in the absence of a good fossil record. However, this promise must be tempered by the inherent uncertainty of phylogenetic estimation. This paper analyzes diversification in *Astragalus,* considered by some to be the largest angiosperm genus, with at least 2500 species. Its goal is to assess whether increases in diversification rate coincided with the origin of *Astragalus.* This will allow meaningful discussion of hypotheses about evolutionary innovations that might have been responsible for shifts in diversification rate (Nitecki, 1990). Phylogenetic analysis will permit an assessment of the appropriateness of the hierarchical level of these hypotheses. Do putative "key" innovations evolve at about the same time as shifts occur, or do they occur earlier or later? Finally, all analyses will be put in the context of phylogenetic uncertainty associated with the data set used to estimate the tree.

Astragalus possesses several features that have been postulated to promote diversification rates in angiosperms, including geographic population structure consisting of local isolates with restricted gene flow (Niklas, Tiffney, and Knoll, 1985), the herbaceous habit (with associated reduced generation time; Doyle and Donoghue, 1993), exceptional chromosomal variability (Levin and Wilson, 1976), and a tendency toward parallelism and reversal associated with recurring ecological specializations (Barneby, 1964; Spellenberg, 1976). In addition the genus possesses morphological novelties that might be candidates for key innovations, such as the longitudinal septum primitively present in the pod, but these have never been suggested to be causal factors in rate change.

The analysis of diversification rates based on standing diversities (numbers of taxa) has undergone a renaissance in the last 15 yr (reviewed in Sanderson and Donoghue,

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[4] Author for correspondence.

1995; Mooers and Heard, in press). The recognition that a phylogeny can provide truly comparable taxa in the form of sister groups (Hennig, 1966; Vrba, 1984) set the stage for much later work. Statistical advances made it possible to detect differences in sister-group diversities (Slowinski and Guyer, 1989), and repeated co-occurrences of sister-group diversity differences and putative causal factors (Farrell, Dussourd, and Mitter, 1991; Wiegmann, Mitter, and Farrell, 1993). However, sister-group comparisons are "nondirectional" (Jensen, 1990; Brooks and McLennan, 1993; Sanderson and Bharathan, 1993), because they do not permit tests of the polarity of rate change. Recent maximum likelihood methods that rely on models of the diversification process overcome this limitation (Hey, 1992; Nee, Mooers, and Harvey, 1992; Nee et al., 1994a, b; Sanderson and Donoghue, 1994). These methods converge in some ways with techniques developed for groups with fossil records (Gilinsky and Good, 1992), but attempt to compensate for the lack of information about extinction by more efficiently utilizing information about phylogeny.

The methods now available differ in their assumptions, robustness, and statistical power. We therefore use a range of methods and extract conclusions that are invariant to the methods used. The first part of this paper presents results from phylogenetic studies on *Astragalus* and related genera based on nuclear ribosomal internal transcribed spacer (ITS) sequences that have proven useful across diverse angiosperm taxa (Baldwin et al., 1995). The second part of the paper uses information on standing diversity of the relevant groups within this phylogeny in combination with the suite of currently available statistical methods described above to study diversification rates. In addition to testing the long-standing prior hypothesis that the genus *Astragalus* has undergone a marked increase in diversification rate, we also search for significant rate changes in the large group comprising the relatives of *Astragalus*. Lastly we consider how robust these conclusions are to phylogenetic error.

Background: the temperate herbaceous clade—Although a considerable portion of the species diversity of the legume family (Fabaceae) is concentrated in tropical and subtropical regions, the family has undergone several radiations into the temperate zone. In fact, most temperate legumes belong to a single vast radiation, which involves six tribes, 45 genera, and some 4000 species in the subfamily Papilionoideae, herein referred to as the "temperate herbaceous clade" or THC. It includes many of the most important cultivated legumes, such as *Cicer* (chick pea), *Pisum* (garden pea), *Medicago* (alfalfa), and *Trifolium* (clover), in the tribes Cicereae, Vicieae, and Trifolieae. The remaining tribes are Carmichaelieae, Hedysareae, and Galegeae. Carmichaelieae comprises a small group of morphologically distinctive trees and shrubs endemic to New Zealand. Hedysareae contains seven genera, two of which, *Hedysarum* and *Onobrychis,* are relatively diverse. None of these tribes, however, approaches the diversity of Galegeae, with some 20 genera and 3000 species, including *Astragalus* (Polhill, 1981a).

The monophyly of the THC is supported by morphological evidence (Dormer, 1946; Polhill, 1981b), the loss of a 25-kb inverted repeat in the chloroplast genome (Lavin, Doyle, and Palmer, 1990), by chloroplast DNA restriction site data from the *rpo*C genes (Liston and Wheeler, 1994), DNA sequence data from a chloroplast group I intron (M.F. Wojciechowski and M.J. Sanderson, unpublished data), and nuclear ribosomal DNA ITS sequence data (see below). Representatives of disparate members of this clade were in place throughout the temperate zone by the Oligocene (Axelrod, 1992).

One genus, *Astragalus,* contains more than half the species diversity of this radiation. The genus is distributed in colder arid and semiarid parts of the Northern Hemisphere and South America, and to a lesser extent in Mediterranean regions. It is especially diverse in southwest Asia (1000–1500 spp.), the Sino-Himalayan region (500 spp.), western North America (400 spp.) and the Andes and Patagonia in South America (100 spp.). It is absent from temperate Southern Africa and Australia, where it is replaced by ecologically similar and closely related genera.

Astragalus forms the bulk of the tribe Galegeae. The tribe includes two monotypic subtribes, Galeginae (containing *Galega*) and Glycyrrhizinae (containing *Glycyrrhiza*), and two larger subtribes, Astragalinae and Coluteinae. Coluteinae includes genera that are morphologically and ecologically similar to *Astragalus* in most respects, differing mainly in floral adaptations to distinct pollinators (Polhill, 1981a) and correlated morphological novelties such as a style brush (Lavin and Delgado, 1990). Astragalinae includes *Astragalus,* the large genus *Oxytropis* (> 300 spp., generally considered the most closely related genus to *Astragalus*; Barneby, 1952), and several other genera, including *Caragana,* a spiny shrub or small tree, and *Alhagi,* a small subshrub with simple leaves.

METHODS I. PHYLOGENETIC ANALYSIS

Taxon sampling—Nucleotide sequence data from the nuclear ribosomal ITS region were obtained from accessions sampled from the legume temperate herbaceous clade (Appendix, Table 1). These included 20 accessions from within *Astragalus,* three from *Oxytropis,* 21 species in 17 other genera in Galegeae, including representatives of all four subtribes, and 14 species from nine genera in the remainder of the THC. Two genera from Millettieae, *Wisteria* and *Tephrosia,* were included as outgroups. The nearest outgroups of the THC are most likely found among those Millettieae that also have lost the inverted repeat (now known to include *Wisteria, Callerya,* and some species of *Millettia*: Liston, 1995). *Tephrosia* is thought be even further removed because it possesses both copies of the chloroplast DNA inverted repeat. Two other potential outgroups, the tribes Loteae and Coronilleae, share the morphological syndrome that the THC possesses but lack the inverted repeat deletion (Chappill, 1995); ITS sequences from two representatives of these tribes, *Lotus* and *Coronilla,* were highly diverged from all of the sequences cited above and very difficult to align except in a few regions (data not shown). We take this as confirmatory evidence that these two tribes really are phylogenetically excluded from the THC (see also Liston, 1995).

Sampling from *Astragalus* included representatives of eight of the nine Old World subgenera (Bunge, 1868, 1869), including representatives of the segregate Asiatic genus *Astracantha* (Podlech, 1986) and several representatives of circumboreally distributed North American sections with Old World relatives. A North American species, *Astragalus bodini* Sheldon, was used as a placeholder for the large New World aneuploid clade, thought to be monophyletic based on previous work (Sanderson, 1991; Liston, 1992; Sanderson and Doyle, 1993; Wojcie-

TABLE 1. Taxa sampled from the temperate herbaceous clade (THC) and outgroups in the tribe Millettieae.[a]

Phylogenetic analysis I	Phylogenetic analysis II	Genus and tribe	Number of species	Distribution
		Galegeae (Bronn) T. & G.		
1	1	*Clianthus* Sol. ex. Lindl.	1	Australia, New Zealand
1	1	*Swainsona* Salisb.	85	Australia, New Zealand
1	1	*Sutherlandia* R. Br.	6	S. Africa
1	1	*Lessertia* DC.	50	S. Africa
1	1	*Colutea* L.	28	Mediterranean–China, Africa
		Oreophysa (Bge ex Boiss.) Bornm.	1	Southwest Asia
1	1	*Sphaerophysa* DC.	2	Southwest Asia–China
1	1	*Smirnowia* Bunge	1	Central Asia
1	1	*Eremosparton* Fisch. et Mey.	3	Central Asia
1		*Halimodendron* Fisch. & Mey.	1	S. W. and Central Asia
2	1	*Caragana* Fabr.	80	Eastern Europe, Asia
1		*Calophaca* Fisch.	5	Central Asia
1	1	*Chesneya* Lindl. ex Endl.	20	Southwest Asia–Mongolia
1	20	*Astragalus* L.	2500	North Temperate, South America
1	3	*Oxytropis* DC.	300	Asia, North America
1	1	*Biserrula* L.	1	Mediterranean–East Africa
1		*Gueldenstaedtia* Fisch.	10	Siberia–Himalayas
2	1	*Alhagi* Adans.	3	Mediterranean–Nepal
2		*Galega* L.	6	Eurasia–East Africa
2		*Glycyrrhiza* L.	20	Eurasia, Australia, North America, South America
			Σ = 3123	
		Carmichaelieae Hutch.		
		Streblorrhiza Endl.	1	New Zealand
		Notospartium Hook. f.	3	New Zealand
		Chordospartium Cheesm	1	New Zealand
		Corallospartium J. B. Armst	1	New Zealand
1	1	*Carmichaelia* R. Br.	41	New Zealand, Lord Howe Island
			Σ = 47	
		Hedysareae DC.		
		Eversmannia Bunge	1	Asia
1	1	*Hedysarum* L.	100	North Temperate
		Taverniera DC.	10	Africa, Southern Asia
		Stracheya Benth.	1	Himalayas
		Sartoria Boiss. & Heldr	1	Turkey
1	1	*Onobrychis* Mill.	130	Europe, Himalayas
		Ebenus L.	20	Mediterranean
			Σ = 265	
		Vicieae (Adans.) DC.		
2		*Vicia* L.	140	North Temperate, South America, E. Africa
		Lathyrus L.	150	North Temperate, South America, E. Africa
		Lens Mill.	5	Mediterranean
1		*Pisum* L.	2	Mediterranean
		Vavilovia A. Federov	1	Southwest Asia
			Σ = 298	
		Cicereae Alefeld		
2		*Cicer* L.	40	Mediterranean, Asia
		Trifolieae (Bronn) Benth.		
		Ononis L.	75	Mediterranean, S. W. Asia, N. Africa
		Parochetus Buch.-Ham ex D. Don.	1	Asia, Africa
2		*Melilotus* Mill.	20	Europe, Asia, N. Africa
		Trigonella L.	80	Europe, Central Asia, Africa, Australia
		Factorovskya Eig.	1	E. Mediterranean–S. W. Asia
2		*Medicago* L.	50	Cosmopolitan
2		*Trifolium* L.	250	Cosmopolitan
			Σ = 477	
			Σ = 4250	(for entire THC)
		Millettieae Hutch.		
2		*Tephrosia* Pers.	400	pantropical
2		*Wisteria* Nutt.	6	North America, China, Japan
41	38	Totals		

[a] Number preceding a genus indicates the number of species sampled from that genus for analysis I and II. Species diversities were obtained from Mabberley (1993), except that of *Astragalus,* which was newly estimated from more recent sources.

chowski et al., 1993; M.F. Wojciechowski and M.J. Sanderson, unpublished data).

An initial set of phylogenetic analyses was undertaken using the entire sample of taxa. However, the computational burden imposed by this large data set precluded the degree of exploration of the data and its robustness (see below) that was desirable for the present study. We therefore used the preliminary results from the global analysis to partition the data set into two overlapping subsets of taxa: sample "I," which emphasizes generic level relationships, and sample "II," which focuses on *Astragalus* and its closest relatives only. This two-tiered approach was feasible because of the extraordinary robustness of a clade that was found in both analyses, which provides an "anchor" between the two. This so-called "Astragalean clade" features *Astragalus* and a subset of the tribe Galegeae (Sanderson and Liston, 1995). A much more detailed exploration of an expanded version of the second data set will be reported elsewhere (M.F. Wojciechowski and M.J. Sanderson, unpublished data).

Molecular data—Total genomic DNA was isolated from field-collected or greenhouse-grown leaf material using the 2X CTAB procedure (Doyle and Doyle, 1987) as described previously (Wojciechowski et al., 1993). About one-third of the samples listed in Table 1 relied on preparation of genomic DNA from herbarium specimens. This was achieved using a scaled down 2X CTAB procedure followed by PCR amplification in the buffer described in Pääbo (1990). DNA's suitable for PCR and sequencing were obtained from 0.05 to 0.2 g of dried herbarium leaf tissues from samples as old as 60 yr. PCR amplification of the nuclear rDNA–ITS regions followed procedures described in detail by Baldwin (1992) and subsequently modified by us (Wojciechowski et al., 1993). PCR products were then analyzed by gel electrophoresis, purified by differential filtration in Millipore Ultrafree-MC tubes, and sequenced by dideoxy methods (Wojciechowski et al., 1993; M.F. Wojciechowski and M.J. Sanderson, unpublished data) according to reaction conditions specified by the manufacturers. To prevent base compressions, 7-deaza-dGTP was routinely substituted for dGTP. Samples were electrophoresed in 5–6% polyacrylamide-8 mol/L urea gels, the gels were fixed in 5% methanol/5% glacial acetic acid, vacuum dried, and exposed to autoradiographic film.

Some sequences included the highly conserved 5.8 S gene between the two ITS spacers, but this provided little phylogenetic information. Sequences and voucher information for all taxa shown have been deposited in GenBank (see the Appendix for accession numbers) and the complete aligned data set can be obtained from the phylogenetic database TreeBASE at the World Wide Web URL "http://phylogeny.harvard.edu/treebase/" by searching for either author's name in the bibliographic database.

Phylogeny reconstruction—Sequences were aligned manually. Phylogenetic analyses were undertaken using parsimony and maximum likelihood. Parsimony methods were implemented using PAUP 3.1 (Swofford, 1993) with a set of heuristic search options that included several addition sequences (random, simple, closest, with various values of the HOLD parameter), TBR branch swapping, and a MAXTREE limit of 5000. Maximum likelihood analyses used the program fastDNAml (Olsen et al., 1994). Multiple runs examined taxon order, various regimes of branch swapping, and a range of transition/transversion ratios from 1.0 to 6.0.

Confidence estimation—Confidence levels for parsimony trees were estimated using the bootstrap (Felsenstein, 1985; Sanderson, 1989). We generated our own bootstrap replicates using a short C program to calculate character weights, running the heuristic searches, and then saving a strict consensus tree of the resulting trees. This is more conservative than the procedure used in PAUP (Swofford, 1993), which fractionally weights different trees found among the set of equally parsimonious trees on any one replicate. For example, in PAUP a clade that is found in 90% of the equally parsimonious trees in each replicate, but never found in all of the trees of any replicate, is assigned a confidence level of 90%, whereas under our method, it would be assigned a confidence level of zero. Effectively, this is a bootstrap majority rule tree of the strict consensus tree resulting from each replicate.

Bootstrapping of the maximum likelihood data was computationally prohibitive because each replicate required on the order of 10–100 h of workstation time. Therefore all confidence limits refer to the parsimony trees only. However, congruence of the results from the two algorithms is indicated to show the extent to which results are sensitive to the choice of algorithm.

METHODS II. DIVERSIFICATION RATES

Identification of shifts in diversification rate—Diversification rate is defined as the difference between speciation and extinction rate, $D = S - E$ (Stanley, 1979). The literature on methods for estimating diversification rates is extensive (reviewed in Sanderson and Donoghue, 1995), much of it focused on cases in which data from the fossil record is available so that both the speciation and extinction components can be estimated. When fossil evidence is poor or nonexistent, the assumption of a constant or phylogenetically unbiased extinction rate is usually invoked, sometimes implicitly (Cracraft, 1984) to permit estimation of differential diversification rates on the basis of standing diversity alone.

One model has been widely used to study extant diversity patterns, the stochastic Yule or "pure-birth" model (Yule, 1924; Harris, 1964; Raup, 1985). This model is a continuous-time, discrete-state, markov process, in which the number of splitting events along any path in a tree follows a Poisson distribution. This means that the time between splitting events is exponentially distributed. Poisson models are the simplest models for discrete processes in continuous time. The Yule model is the diversification analog of essentially all of the discrete character evolution models in wide use in molecular evolutionary studies. Although the simplicity of a model always opens it to criticism, the Yule markov model has been difficult to reject for diversification patterns observed in many real taxa (Savage, 1983; but see Guyer and Slowinski, 1993, for counterexamples). Thus, it is likely to provide a good first approximation for diversification estimates, one that can be discarded in favor of more complicated models when necessary (Sanderson and Donoghue, 1994).

Three methods are used to identify shifts in diversification, covering a range in statistical power, robustness, and kind of inference that can be made.

Method 1. Sister-group method—Null model methods can test the adequacy of a Yule model of homogeneous diversification rate. For two sister groups in which the diversity of the smaller group is r and the larger group is s, the null probability (p) of observing this or a greater difference in diversity if the null model is true is

$$p(r,s) = 2r/(r + s - 1) \quad (1)$$

(Slowinski and Guyer, 1989). In practice, one sister group must be 40 times more species rich than the other for this null model to be rejected at the 0.05 level. Although the simplicity of this test is appealing, it is not a statistically powerful test because the number of observations on the diversification process used by the method is only two. Moreover, any method based solely on sister-group comparisons is intrinsically "nondirectional" (Jensen, 1990; Brooks and McLennan, 1993; Sanderson and Bharathan, 1993) in that one has no way of knowing if a taxon owes its higher diversity to an increase in rate or if its sister group suffered a decline in rate.

Method 2. Maximum likelihood model fitting—As a remedy for the lack of polarity in sister-group methods, Sanderson and Donoghue (1994) proposed a directional maximum likelihood method that can simultaneously permit rejection of the model of homogeneous branch-

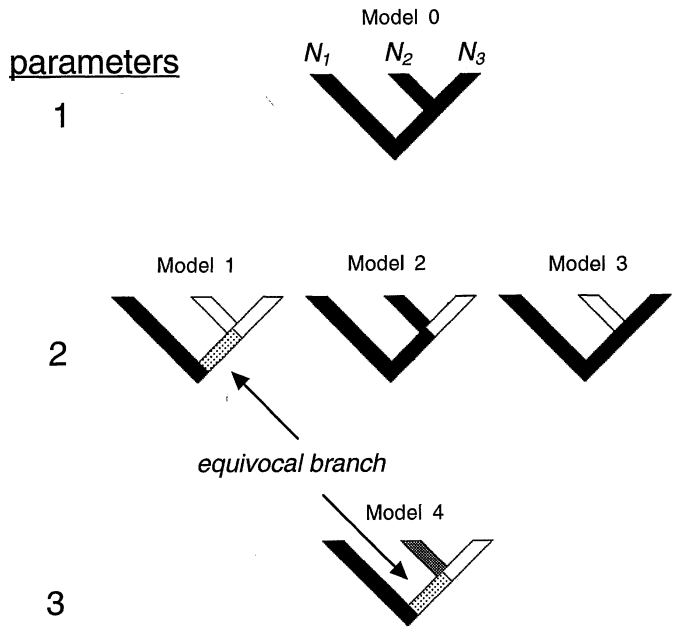

Fig. 1. Illustration of sequential diversity test modified from Sanderson and Donoghue (1994). Species diversities are indicated by N_i. Three taxa are compared, an outgroup and the basal two ingroups. Each tree represents a different model for the diversification process. The models vary in the number of rate parameters they include. One one-parameter and three two-parameter models are compared (via a likelihood ratio) to the best-fit three-parameter model (the lone internal internode is ignored, unlike in Sanderson and Donoghue, 1994). The model with the fewest parameters is chosen as long as it provides an adequate fit to the best-fit model (values of fit < 0.95 are accepted; lower values correspond to highest fit). Model numbers above trees are used for reference to Table 2. Significance of fit was assessed by Monte Carlo simulation (1000 replicates).

ing and identify shifts (i.e., polarity) in diversification rate in three-taxon comparisons. It relies on the Yule model of diversification but considers a set of progressively more complex combinations of Yule models with one or more rate parameters in different parts of the phylogeny (Fig. 1). Constraints on the relative timing of the internal branch point can be imposed based on fossil evidence, but in many cases the data are such that robust conclusions emerge even if the timing is left almost completely unconstrained, which is the case of interest when fossil information is lacking. Generalizations of this method to more than three-taxon statements are possible along the lines described in Sanderson and Bharathan (1993), but little appears to be gained by doing this in the absence of some constraints on branching points from the fossil record. This method exploits the properties of the Yule model by using it to impose a prior probability on the internal branch time. A copy of the program to perform this analysis is available by FTP from M.J. Sanderson (contact author for more information).

Method 3. Maximum likelihood with branch time information—If estimates of times of splitting events are available, methods with significantly more power to discriminate among alternative hypotheses are possible (Hey, 1992; Nee, Mooers, and Harvey, 1992; Sanderson and Donoghue, 1995; Mooers and Heard, in press). Previous authors have used estimates of branch times based on UPGMA-estimated phylogenies, but this algorithm is known to have a narrow range of statistical consistency (Huelsenbeck and Hillis, 1993) because of its reliance on the assumption of clock-like rates of evolution. The advantage to this approach, however, is that sample size is increased in proportion to the number of branches included in the analysis, which leads to less biased estimates of rate and more powerful tests (Sanderson and Donoghue, 1995). We attempted to improve on this approach despite its shortcomings, by using maximum likelihood methods to reconstruct the branching times, rather than ultrametric distance methods such as UPGMA. The program DNAMLK in PHYLIP (Felsenstein, 1993) was used to reconstruct these times under the assumption of a molecular clock. The validity of the molecular clock assumption was tested in a likelihood ratio test against a nonclock model used in DNAML (as described in the PHYLIP documentation; Felsenstein, 1993).

Hey's (1992) method for estimating diversification rates given times of branching was then generalized to permit the inclusion of incompletely sampled higher taxa of high diversity. Consider a phylogeny in which the T terminal clades have species diversities of $\{N_1, \ldots N_T\}$, at the ends of branches of duration $\{d_1, \ldots d_T\}$. Let the durations of the B internal branches be $\{d_{T+1}, \ldots, d_{T+B}\}$. Then the likelihood of the data under a Yule model with parameter, λ, is

$$L(\lambda) = \prod_{k=1}^{T} e^{-\lambda d_k}(1 - e^{-\lambda d_k})^{N_k - 1} \prod_{j=T+1}^{T+B} \lambda e^{-\lambda d_j}, \quad (2)$$

which can be maximized numerically (Press et al., 1988) to estimate the branching rate. The first product accounts for the diversity in the terminal taxa. The second product adds information based on the "observed" waiting times between splits (Sanderson and Bharathan, 1993).

This likelihood formulation can be used to identify shifts in diversification rates by testing a two-rate-parameter model in which a change occurs at some place in the tree against a null one-rate-parameter model in which no rate change occurs (see Fig. 7; see also Sanderson, 1994).

Overview of analyses of Astragalus and the Astragalean clade—The three statistical analyses of diversification rates were repeated in each of the two phylogenetic analyses based on the two-taxon sampling schemes outlined in Table 1. Each of these phylogenies can be reduced to three- or four-taxon statements regarding the nearest relatives of the relevant taxa (Table 2). The design was to begin with the Astragalean clade, test for rate homogeneity in it by application of the three diversity tests, and if rate homogeneity could not be rejected, move on to the higher level phylogenetic analysis and test for rate homogeneity there. There is a danger of erroneous rejections of homogeneity owing to multiple test problems if this process is repeated ad infinitum. However, the number of repeated tests here is limited. Analyses that relied on estimates of branching times used the results of molecular clock reconstructions as described above (Table 3).

Confidence sets of trees—Most studies that use phylogenies to estimate evolutionary parameters fail to consider the uncertainty in those phylogenetic estimates (although see Debry, 1992). We generated a confidence *set* of trees and then repeated the sister-group diversity tests on every tree in the confidence set, in a search for patterns common to all those trees. Worst case (lowest) P values among these trees were taken as the estimated P value for the significance of any rate estimate (Fig. 2).

The confidence "set" of trees was generated by bootstrapping the data matrix and examining the strict consensus trees of the resulting replicates. Trees were rank-ordered according to the relative bootstrap support of a focal clade and its various sister groups. For example in the higher level analysis of the THC (sampling scheme I), we examined the Astragalean clade and its sister groups, obtained the bootstrap support for the group consisting of the Astragalean clade plus each of the sister groups that emerged among various replicates in turn, and ranked these alternatives (Table 4). The confidence set comprised the largest collection of trees that suggested at least 5% support for a relationship of some sister taxon to the Astragalean clade. This corresponded to a confidence "interval" of at least 70–80% of the trees in most cases. Larger confidence sets of trees could not be constructed without including a large number of very poorly supported sets of relationships.

TABLE 2. Statistical analyses of diversification rates in the temperate herbaceous clade. Node designations refer to nodes labeled in Fig. 6.

Test	*Astragalus* vs. relatives (sampling scheme II)[a]	Astragalean clade vs. relatives (sampling scheme I)
I. Sister-group analysis[b]		
Node	c	b
Diversities	2496/520	30/3025
P value	0.34 (NS)	0.02
II. Three-taxon maximum likelihood analysis[c]		
Outgroup, ingroup nodes	c, d	a, b
Outgroup diversity	2496	85
Ingroup 1 diversity	300	30
Ingroup 2 diversity	229	3025
Best fit model (see Fig. 1)	0	2
Fit	0.35	0.00
III. Maximum likelihood analysis w/branch times[d]		
Nodes covered by one- vs. two-parameter models	{c, d} vs. {c}, {d}	{a, b, c, d} vs. {a, b}, {c, d}
One-parameter log likelihood	−23.56	−41.02
Two-parameter likelihood	−22.32	−27.30
−2 log likelihood ratio	2.48 (NS)	27.43 ($P \ll 0.05$)

[a] Refer to Table 1 and/or Figs. 3–5 for taxa included in these analyses.
[b] Test of the null hypothesis of a common homogeneous rate of branching across both sister groups.
[c] Test modified from the maximum likelihood model-fitting procedure described in Sanderson and Donoghue (1994). See Fig. 1.
[d] Test generalized from the maximum likelihood method of Hey (1992). A null hypothesis of homogeneous branching (one-rate parameter) was tested against an alternative two-rate model in which the rates differ in accordance with the stated contrast (e.g., one rate in *Astragalus*; another rate in its sister group). See Fig. 7 for further description of the models. Branch times were estimated based on the assumption of a molecular clock (see Table 3 for further information). The clock was rejected ($P < 0.005$) in every test for the ITS data, suggesting that the estimated times may not be reliable. Therefore diversification tests based on these times are the least robust of the three analyses. The function −2 log likelihood ratio is asymptotically χ^2 with 1 df. Monte Carlo simulation (1000 replicates) was used to check this approximation.

RESULTS

Sequence divergence—Kimura two-parameter distances between all pairs of taxa indicated a tremendous range of sequence divergence across the two analyses. Divergence values ranged from <6% within the larger genera (including *Astragalus*) to up to 35% between the outgroups of the higher level analyses (Millettieae) and some of the ingroup taxa in the THC. Divergence was usually <10% within the well-supported larger clades described below, and 10–30% between these clades.

Phylogeny—In the broader analysis (taxon sampling scheme "I") the temperate herbaceous group is monophyletic in all parsimony and maximum likelihood analyses (Figs. 3, 4), supporting previous work (cited above). Bootstrap levels in the parsimony analyses were 89%, perhaps not as high as expected because of alignment ambiguities among taxa at this high level of divergence. Many relationships within the THC are well resolved. Three large clades, which together comprise 97% of the species diversity of the THC, are each supported at better than the 95% level in bootstrap analyses, and are present in all maximum likelihood runs. The first has been termed the Astragalean clade (Sanderson and Liston, 1995; labeled "Agl" in Fig. 3) and comprises *Astragalus,* all of Galegeae subtribe Coluteinae, *Biserrula,* and the New Zealand endemic tribe Carmichaelieae, which is evidently closely related to the Australian genus *Swainsona.* The Astragalean clade includes 71% of the species diversity of the THC. The Vicioid clade ("V" in Fig. 3) is the second large clade within the THC. Collectively this clade contributes 19% of the species diversity of the THC, due to the large genera *Cicer, Trifolium, Medicago, Vicia, Lathyrus, Ononis,* and *Trigonella.* The Hedysarioid clade ("H" in Fig. 3) consists of the tribe Hedysareae plus *Alhagi* from Galegeae (with which it shares a similar fruit morphology) and contributes substantial diversity (≈ 263 spp.), mainly because of the two large genera, *Hedysarum* and *Onobrychis.* This clade accounts for 6% of the species diversity of the THC.

The relationships among these three large clades and the remaining genera in Galegeae, *Glycyrrhiza, Caragana, Halimodendron, Calophaca, Gueldenstaedtia,* and *Chesneya,* are partly resolved by this analysis. Parsimony

TABLE 3. Tests of clock-like evolution of ITS sequences in the two phylogenetic analyses described in text. Unconstrained likelihoods are estimated using the program DNAML (Felsenstein, 1993), which permits rate variation across branches. Constrained likelihoods were estimated by DNAMLK (Felsenstein, 1993), which assumes a molecular clock. The quantity −2 log likelihood ratio is expected to be distributed as a χ^2 with $n - 2$ df, where n is the number of taxa (Felsenstein, 1993). The closest approximation (smallest value of −2 log likelihood ratio) to a clock obtains in the Astragalean analysis, but it is still rejected with a highly significant probability.

Analysis	Unconstrained log L	Clock log likelihood	−2 log likelihood ratio	df	Reject clock? (significance)
THC (Taxon Sample I)[a]	−6725	−6856	266	36	Yes, $P < 0.005$
Astragalean (Sample II)	−3462	−3493	62	31	Yes, $P < 0.005$

[a] Refer to Table 1 and/or Figs. 3–5 for taxa included in these analyses.

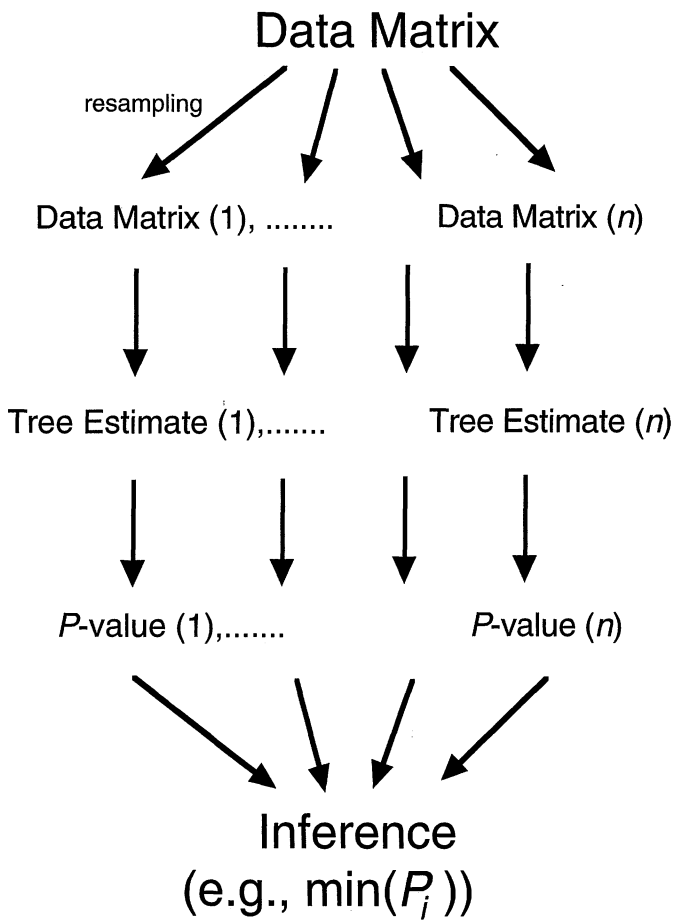

Fig. 2. Methodology for studying evolutionary processes in the context of phylogenetic uncertainty. A total of n replicate data matrices are generated by bootstrapping (or even some other resampling scheme), and evolutionary rate tests are repeated on each tree that results. Inferences are then made based on the n significance values obtained (P_i), across a confidence set of the 95% of trees that have the highest support.

and maximum likelihood analyses both place *Chesneya* and *Gueldenstaedtia* as the sister group of the Astragalean clade. It is somewhat less parsimonious to have either the Hedysarioid clade, the Vicioid clade plus the Hedysarioid clade, or *Caragana* and its relatives (*Halimodendron* and *Calophaca*) as the sister group of the Astragalean clade. The bootstrap consensus tree indicates *Chesneya* and *Gueldenstaedtia* as the sister group, but although this is consistent with the rest of the tree, it is not supported at the 50% level.

The tribe Galegeae is paraphyletic. One subtribe, Glycyrrhizinae (*Glycyrrhiza*) is the sister group to the rest of the THC. Another subtribe, Galeginae (*Galega*), is the sister group to the Vicioid clade. The genus *Alhagi* (Galegeae subtribe Astragalinae) is more closely related to tribe Hedysareae than to other members of its own tribe. Given the anomalous morphologies of *Galega, Glycyrrhiza,* and *Alhagi,* these results are not altogether unexpected (Polhill, 1981a).

Results using a more intensive taxon sampling scheme within the Astragalean clade (taxon sampling scheme "II"; Fig. 5) suggest that the Astragalean clade itself is comprised of four somewhat less well-supported clades. The first is a monophyletic genus *Oxytropis* (300 spp.) supported at the 100% bootstrap level. The second is a clade that includes all of subtribe Coluteinae, the tribe Carmichaelieae and three "outlier" *Astragalus* species (66% bootstrap support; ≈ 230 spp.). These *Astragalus* are oddballs. For example, *Astragalus complanatus* shares several morphological synapomorphies with Coluteinae including a pubescent style and lack of interlocking bosses on the keel and sockets on the wing petals; see Barneby, 1964, p. 1164, and further details in Sanderson and Liston, 1995). Third, a pair of species, *Biserrula pelecina* and *Astragalus epiglottis,* is united with high bootstrap support. On the optimal trees they are the sister group to the Coluteoid clade, but support for this is weak. Finally, the fourth clade comprises the vast majority of the genus *Astragalus* ("*Astragalus* sensu stric-

TABLE 4. Sister-group diversity tests across a confidence set of trees. Each row is a different phylogenetic relationship found among the bootstrap replicates. Rows are sorted in descending order based on their relative bootstrap support. Bootstrap support levels refer to a focal clade and its various sister groups. In the first analysis, the focal clade is the Astragalean clade; in the second it is the genus *Astragalus.* Sister group significance levels <0.05 suggest a departure from homogeneous diversification.

Contrast	Diversity(s)[a] of sister group(s)	Bootstrap support[b]	Cumulative bootstrap[c]	Diversity test significance
Astragalean clade vs. relatives	3025, 30	0.28	0.28	0.02
(Taxon Sampling Scheme II)	3025, 266	0.18	0.46	0.16
	3025, 115	0.15	0.59	0.07
	3025, 10	0.10	0.69	0.007
	3025, 1170	0.10	0.79	0.56
Astragalus vs. relatives[d]	2496, 520	0.43	0.43	0.35
(Taxon Sampling Scheme I)	2496, 300, 229	0.26	0.69	0.21, 0.16
	2496, 300	0.19	0.88	0.21
	2496, 229	0.09	0.97	0.16
	2496, 330, 125	0.02	0.99	0.23, 0.09

[a] Diversities of all taxa descended from node. A polytomy is indicated by three or more diversities. In a polytomous node, diversity tests were performed on all resolutions of the polytomy actually observed among the set of equally parsimonious trees.

[b] Based on 100 replicates of parsimony analyses with heuristic search options as indicated in Fig. 1. Bootstrap values are based on a strict consensus of trees found in each replicate. This leads to more conservative (smaller) values of support than reported in PAUP.

[c] This gives the size of the "confidence set" of trees consisting of this set of trees and all above it in the table.

[d] Based on taxon sample II. Bootstrap support for the monophyly of *Astragalus* was 77%. Values reported in the table exclude bootstrap replicates in which *Astragalus* is not monophyletic, because it is not clear which taxa are relevant to the hypothesis about diversification in *Astragalus* in that case.

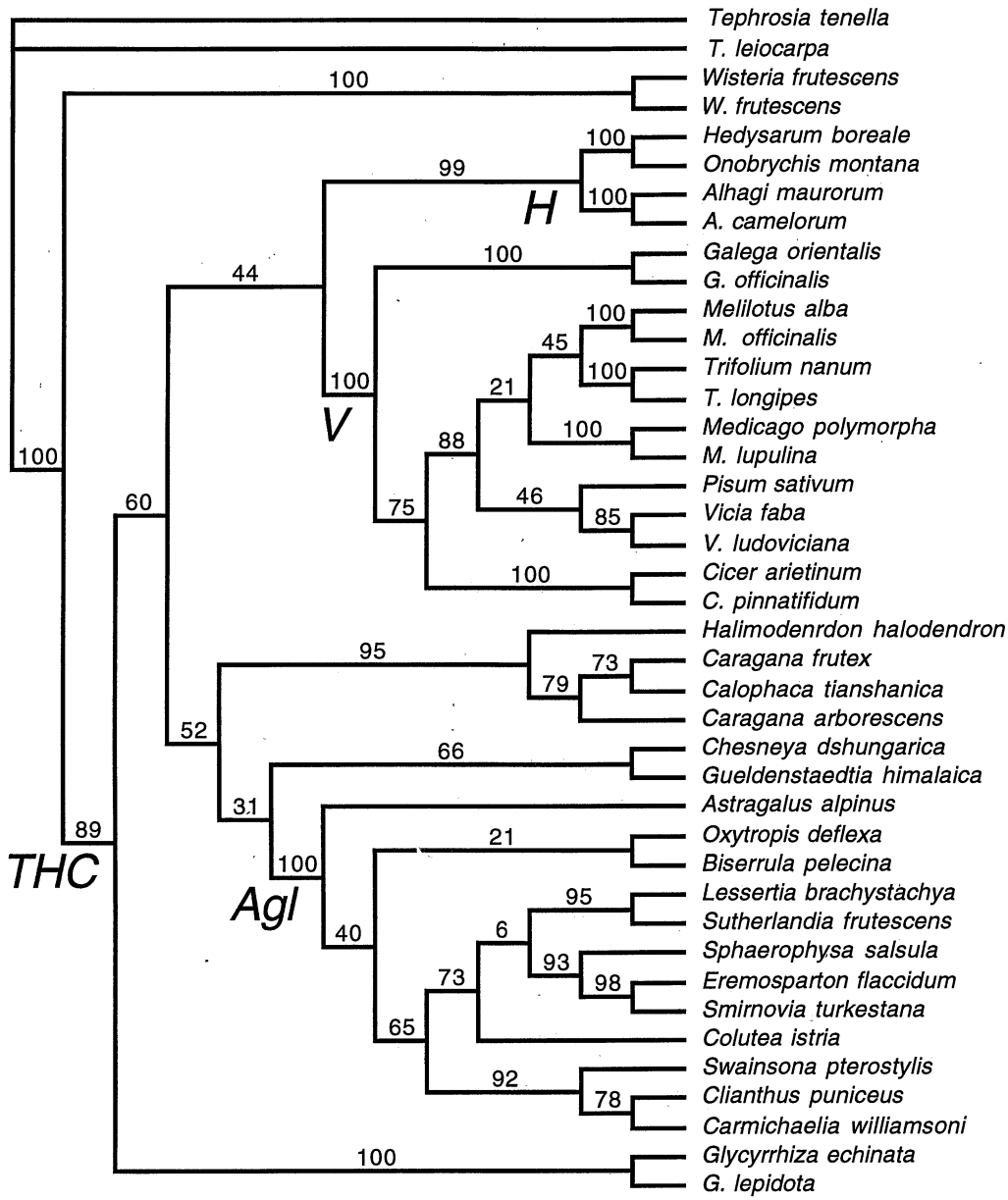

Fig. 3. Parsimony analysis of legume temperate herbaceous clade (THC) based on ITS sequence data from taxa sampled according to sampling scheme I (see Table 1). Tree is a bootstrap consensus tree showing estimated confidence levels for each clade. H = Hedysarioid clade; V = Vicioid clade; Agl = Astragalean clade.

to"), including the segregate genus *Astracantha,* and is supported at the 80% level. The most parsimonious tree and the maximum likelihood tree agree that the sister group of *Astragalus* is a group consisting of *Oxytropis* plus the Coluteoid clade. However, no relationship among the four clades is supported by bootstrap levels > 50% (Fig. 5).

Diversification rates on the optimal trees—Figure 6 shows the species diversities of major clades within the temperate legume radiation, showing substantial heterogeneity in taxonomic diversity. Results of the diversity analyses described above are reported in Tables 2 and 3. The sister-group comparisons show that there is no significant difference in diversity between *Astragalus* and its sister group. However, a sister-group comparison at the next most recent common ancestor does suggest a significant difference in diversity. Application of the maximum likelihood model fitting procedure to these two nodes along with the Astragalean clade confirms that diversification increases in the branch leading to the Astragalean clade (Table 2, part II), but does not shift *within* the Astragalean clade.

Maximum likelihood tests that rely on inferred branch times are indicated in Fig. 7 and Table 2, part III. A likelihood ratio test of the goodness of fit of the molecular clock was performed along the lines suggested in Felsenstein (1993) and Bruns and Szaro (1992). The

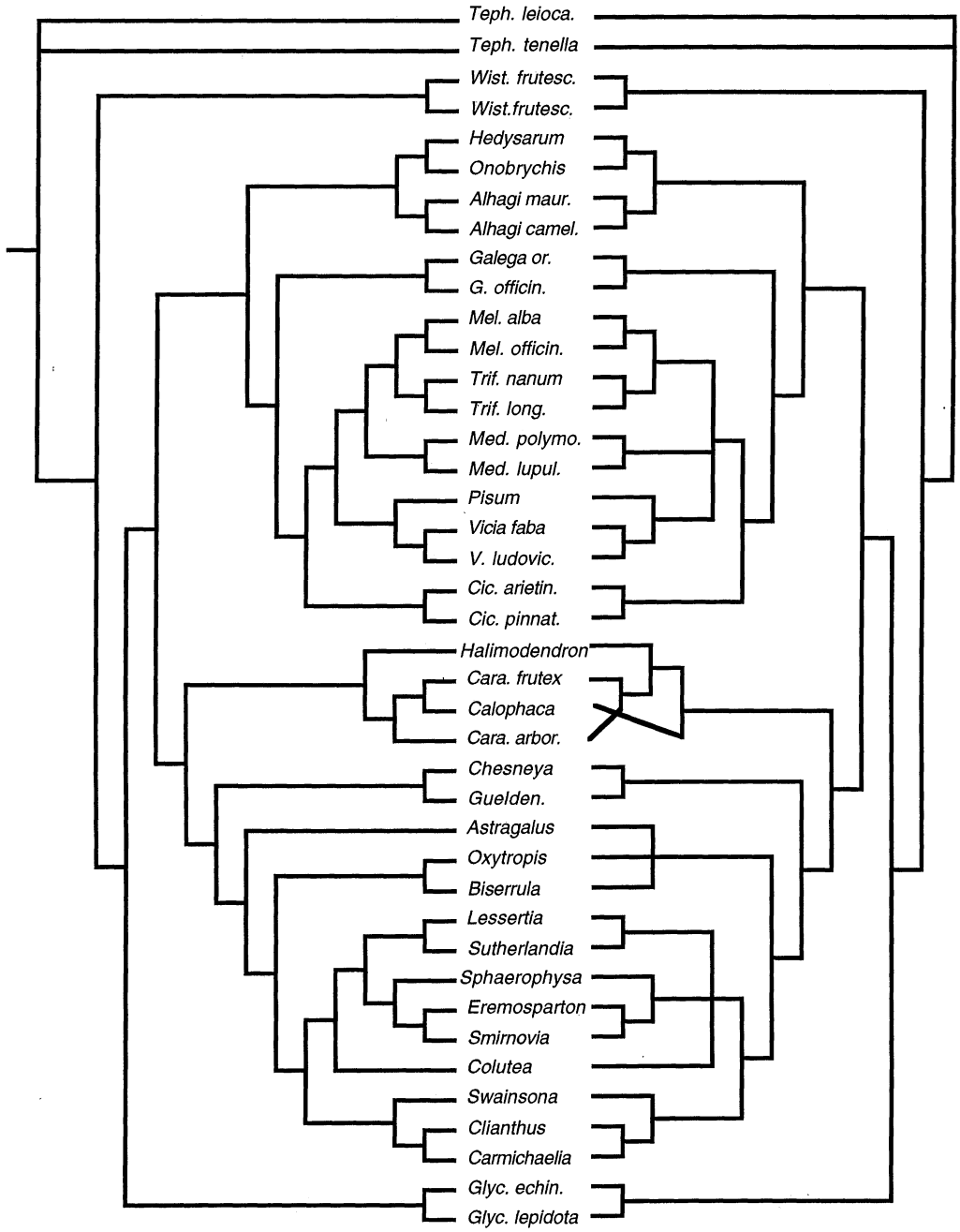

Fig. 4. Congruence between bootstrap parsimony tree (left) and maximum likelihood tree (right) for taxa sampled in sampling scheme I. Likelihood of tree is −6929.8 (transition/transversion ratio of 2:1; global swapping). Branch lengths that are not significantly greater than zero in the maximum likelihood tree are collapsed.

model does not provide an "adequate fit" relative to an unconstrained estimation of branch variation in rate (Table 3). Nonetheless, the estimated branch times were used in tests at two hierarchical levels: first to test for significant differences in rate in the genus *Astragalus*; second to test for significant differences in the Astragalean clade relative to its first two outgroups (Table 2). No significant difference in rate was detected between *Astragalus* and its relatives, but a strongly significant difference was found when the Astragalean clade was compared to its relatives, *Caragana, Chesneya,* and *Gueldenstaedtia.*

Diversification rates on the confidence set of trees—An ≈ 80% confidence set of phylogenetic trees using taxon sampling scheme "II" includes a number of sister-group relationships for the genus *Astragalus* in addition to the one found in the optimal trees (both parsimony and ML) (Table 4), although that relationship is the best-supported one. It also includes the possibility (albeit with relatively little support) that *Astragalus* is not monophyletic, although a large subset of it would be. In those trees the rest of the Astragalean group is closer to the core *Astragalus* than it is to another subset of *Astragalus.*

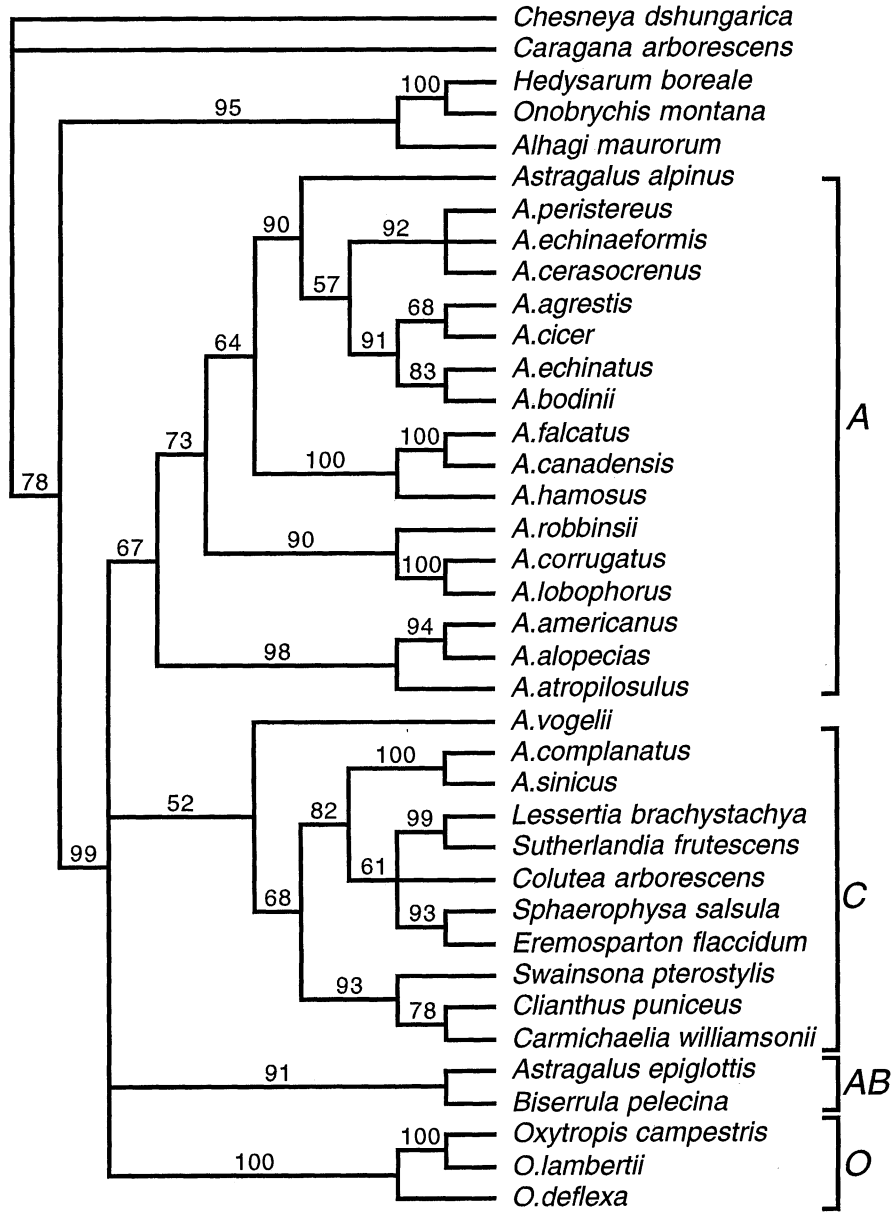

Fig. 5. Parsimony analysis of legume temperate herbaceous clade (THC) according to sampling scheme II (see Table 1), emphasizing the genus *Astragalus* and the Astragalean clade. Tree is a bootstrap consensus tree showing estimated confidence levels for each clade. A = *Astragalus* s. s.; C = Coluteoid clade; AB = *Astragalus epiglottis* and *Biserrula pelecina*; O = *Oxytropis*.

While phylogenetically interesting, none of the relationships in the confidence set suggest that *Astragalus* is significantly more diverse than its sister group. All sister-group tests suggest homogeneous diversification rates (Table 4).

In the broader analysis (sampling scheme "I"), results are more ambiguous. The confidence set of trees includes a variety of possible sister groups for the Astragalean clade, including several that are more diverse than the *Chesneya/Gueldenstaedtia* clade found on the optimal trees. Although an $\approx$ 70% confidence set of trees suggests that there is always a difference in diversity significant at the 0.16 level or lower, another component of the confidence set has the sister group being the entire remainder of the temperate radiation (some 1170 species, including the large Vicioid clade). Thus, to construct an 80% confidence set or broader we would be forced to include a set of trees that does not show any diversity differences between the Astragalean clade and its nearest relatives.

DISCUSSION

The results derived from all three methods agree on several points. First, *Astragalus* is not experiencing rates of diversification that are statistically significantly higher than its relatives in the Astragalean clade. Other clades in that group are themselves diverse. *Oxytropis* has 300 species and a clade of genera including *Colutea* and its relatives have over 200 species. These differences are not

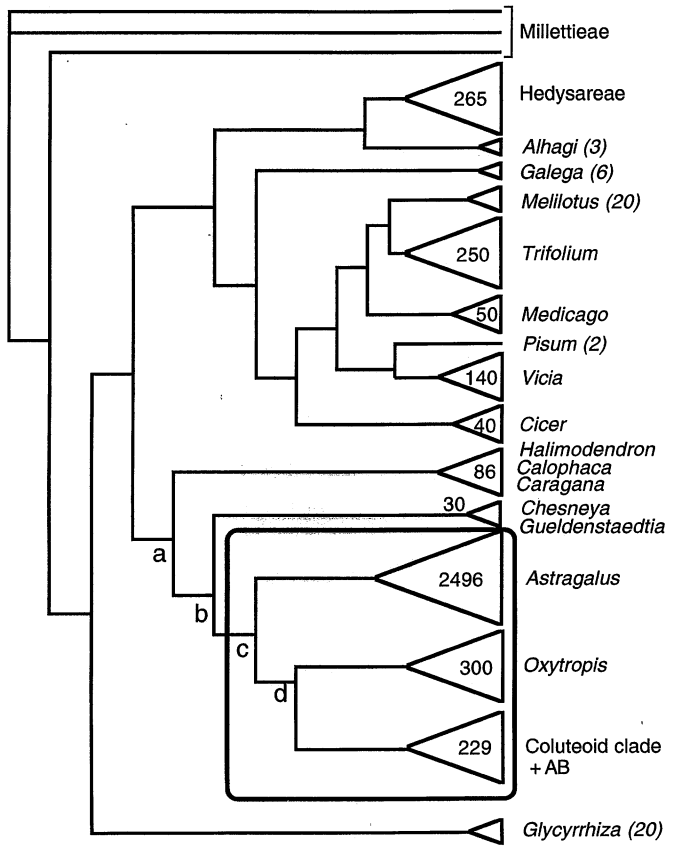

Fig. 6. Illustration of species diversities across the temperate herbaceous clade. Tree is based on Figs. 3, 5, and the optimal tree from sampling scheme II (not shown). Triangle sizes are roughly proportional to log of species diversity. Species diversities of small taxa are indicated in parentheses. Nodes labeled with lowercase letters are used in tests of Table 2. Circled clade is the Astragalean clade. AB = *Astragalus epiglottis* and *Biserrula pelecina*

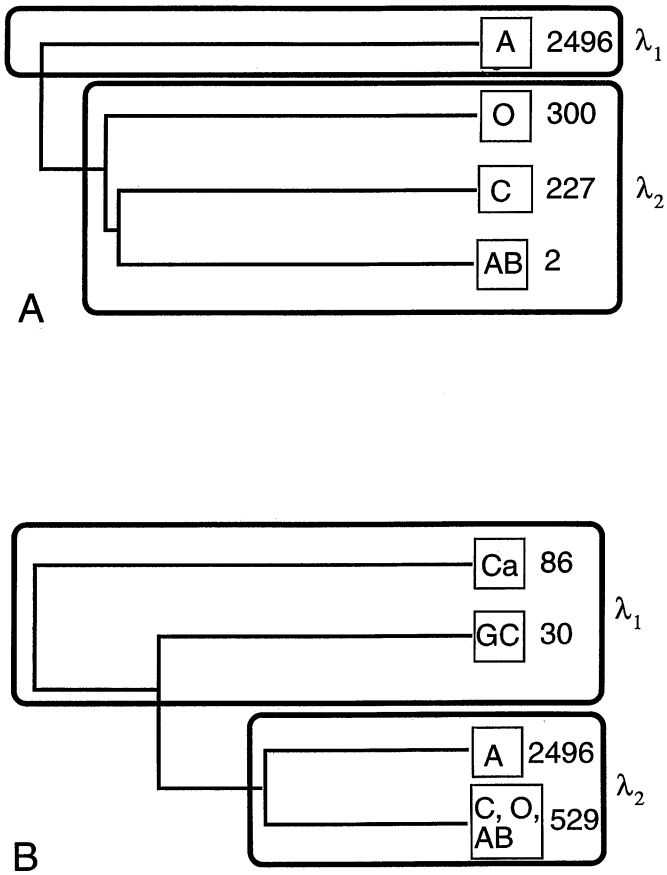

Fig. 7. Alternative hypotheses tested in Method III using estimates of branching time based on a molecular clock. Trees are based on Fig. 6. Branch lengths are proportional to time. In each tree a one-parameter model is tested against the alternative of two parameters, λ_1 and λ_2, where those parameters are in effect in the circled parts of the trees. Results of tests are given in Table 2. (A) Test within the Astragalean clade. (B) Test between Astragalean clade and its relatives. Abbreviations are: A = *Astragalus* s. s.; C = Coluteoid clade; AB = *Astragalus epiglottis* and *Biserrula pelecina*; O = *Oxytropis*. Ca = *Caragana, Halimodendron,* and *Calophaca*; and GC = *Gueldenstaedtia* and *Chesneya.*

statistically significant. The second conclusion common to all analyses (based on the optimal trees) is that the Astragalean clade as a whole *is* significantly more diverse than its closest relatives, which include the Asian genera *Caragana, Halimodendron, Calophaca, Chesneya,* and *Gueldenstaedtia.* The maximum likelihood tests not only confirm that the differences in diversity are significant, but also that the direction of diversification rate change is toward higher rates in the Astragalean clade, rather than toward lower rates in species-poor relatives. This supports the contention that some factor associated with the Astragalean clade may be responsible for an increase in diversification rate.

An examination of confidence sets of trees (Table 4), that is, a space of reasonable tree estimates generated by bootstrapping, indicates how robust these conclusions are to the uncertainty in phylogenetic relationships. Sister-group differences between *Astragalus* and its relatives are never significant anywhere in the confidence set, whereas sister-group diversities between the Astragalean clade and its relatives are always significant at the 0.16 level or better over a confidence set comprising some 70% of the trees. This confidence set is broader than the conventional 0.05 level, but this is mitigated by two observations that might increase the probability that the relationships in the confidence set are reasonable. First, the chloroplast DNA phylogeny of Liston and Wheeler (1994) agrees with ours that the sister group of the Astragalean clade is a species-poor Eurasian clade consisting of the genus *Caragana* and relatives (their analysis did not sample *Chesneya* or *Gueldenstaedtia*). Second, the alternatives to this arrangement found among the bootstrap replicates require rather striking convergences in morphology, because they postulate removing these Eurasian Galegeae to a much more distant relationship to the Astragalean clade. However, in the context of the present data, it is clear that the "cost" of considering phylogenetic uncertainty is likely to be diminished confidence in the evolutionary inferences based on those phylogenies.

Key innovations?—Evidently it is not necessary to seek explanations for the "exceptional" diversity of *Astragalus.* It is not exceptionally diverse. It is not necessary to speculate why unique morphological features of the genus (such as its unusual legume morphology) have led to high

rates of speciation or low rates of extinction. They have not had such an effect. Likewise, features associated with its chromosomal evolution (e.g., the striking New World aneuploid series), population structure, or supposed high rates of parallel morphological evolution (Barneby, 1964) are apparently of little relevance to its diversification rates. It may not be productive to continue to search for explanations for the unusual diversity of *Astragalus* or to postulate key innovations leading to its radiation.

On the other hand, there is evidence that diversification rates increased at about the time of the origin of the entire Astragalean clade. Perhaps a search for explanations of this event will be more revealing about the evolution of diversity in temperate legumes. The Astragalean clade is composed of 12 genera from Galegeae and five genera from the New Zealand tribe Carmichaelieae. These genera from Galegeae are all similar to *Astragalus* in ecology. They are generally xerophytic, predominantly herbaceous perennials (shrubs and trees in *Colutea* and *Eremosparton*), and often exhibit high levels of endemism. Perhaps the most remarkable aspect of the Astragalean radiation is the extent to which similar morphological adaptations to extreme environmental conditions have evolved countless times in parallel. Its 3000–3500 species represent endless variations on an essentially constant ground plan (Polhill, 1981a). Vegetative features involving leaf morphology and plant growth habit show the most extreme cases of parallelism. For example, the small endemic Asian genus *Eremosparton* has converged in its rush-like leaf morphology on numerous North American species of *Astragalus,* such as section *Lonchocarpi.* Even biochemical adaptations against herbivory, such as the synthesis of toxic alkaloids, have evolved in parallel in several genera (Molyneux, James, and Panter, 1985).

The New Zealand members of the Astragalean clade are very different. The Carmichaelieae are evidently the product of a classical island adaptive radiation (Carlquist, 1974). They are secondarily woody, show high levels of hybridization, and are found in an exceptionally diverse array of mesic and xeric habitats from sea level to subalpine zones. Molecular (ITS and *rpo*C data), morphological (possession of a style brush; P. Heenan, personal communication) and cytogenetic (polyploid chromosome number of $2n = 32$; see Goldblatt, 1981) evidence support the idea that the Carmichaelieae are closely related to the Australasian Galegeae in the Astragalean clade.

However, despite the ecological similarities among most of the genera in the Astragalean clade, excluding the New Zealand Carmichaelieae, and the existence of several known shared-derived characters that unite the Carmichaelieae with the rest of the Astragalean clade, no distinguishing morphological features are known for this clade as a whole. The subtribe Astragalinae recognized by Polhill (1981a) is split in two by the circumscription of the Astragalean clade; *Caragana, Calophaca, Chesneya, Gueldenstaedtia,* and *Halimodendron* are outside the Astragalean clade; *Astragalus, Oxytropis,* and *Biserrula* are within it. Subtribe Coluteinae, which is marked by the possession of a style brush (and perhaps ought now include the Carmichaelieae), is completely contained within the Astragalean clade (Fig. 8). Morphological novelties that have come to the attention of taxonomists of these groups do not coincide with the origin of the Astragalean clade or with the apparent increase in species diversity of that group. If there are features of the Astragalean clade that led to this episode of rapid diversification, they may be so cryptic that they have escaped the attention of systematists. On the other hand, renewed examination of the morphology of *Astragalus* and the other genera in the Astragalean clade may reveal shared novelties that have been overlooked before. Generic circumscriptions in Galegeae have been notoriously fluid (Barneby, 1964, p. 1164) presumably in part because of a lack of clear morphological diversification patterns. It is also possible that evolutionary processes related to diversification rates may be influenced by features other than the kinds of morphological novelties to which they have often been linked (Mayr, 1960; Heard and Hauser, 1995). In either case, rephrasing the problem at the appropriate hierarchical level may ultimately guide the search for credible explanations for shifts in diversification among these legumes.

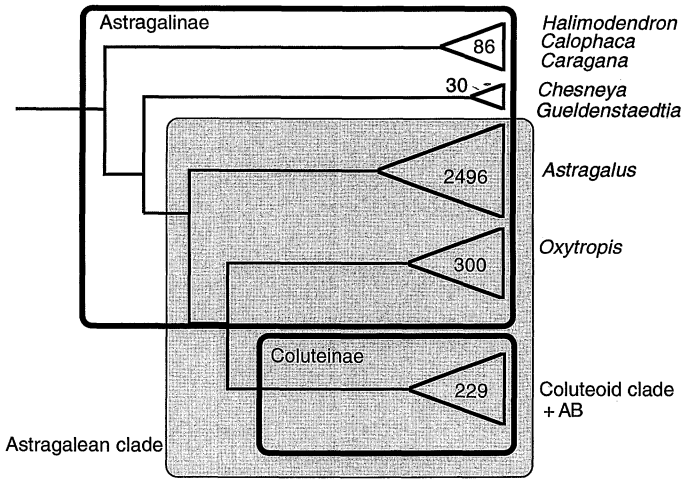

Fig. 8. Detail of the Astragalean clade, according to Fig. 6. Astragalean clade is shaded and the subtribes Coluteinae and Astragalinae of Galegeae are shown. Note that morphological, molecular, and cytogenetic evidence support the placement of the New Zealand Carmichaelieae *within* subtribe Coluteinae.

Diversification of the temperate herbaceous clade—The idea that *Astragalus* is diversifying unusually rapidly has been strongly rejected by our analyses. The alternative that the Astragalean clade is diversifying unusually rapidly receives strong support in the context of the optimal trees, but bootstrap estimates of phylogenetic uncertainty suggests that overall support for this idea is not quite at the conventional 0.05 significance level (see above). Perhaps other hypotheses should be considered, at least for discussion purposes.

One additional alternative is that the entire temperate herbaceous clade is diversifying unusually rapidly. The diversification of the THC superficially resembles the pattern noted for tropical–temperate angiosperm family pairs (Judd, Sanders, and Donoghue, 1994): a diverse clade of herbaceous plants appears to be derived from an assemblage of less diverse tropical woody groups. Unfortunately it was not possible to perform the diversification tests described above on the THC as a whole, because our sampling of taxa outside of the THC was too

limited. However, the conditions under which our conclusions might be overturned in favor of the alternative that the entire THC is diversifying unusually fast *can* be specified. These conditions require that future evidence about the phylogeny of the THC and its relatives provide strong support for all of the following. (1) The sister group to the THC must be a fairly species-poor clade. This would probably mean that the large Millettioid genera, *Tephrosia* (≈ 400 spp.), *Millettia* (≈ 100 spp.), and *Lonchocarpus* (≈ 100 spp.), could not be close relatives. Note that at least some species of *Callerya,* a segregate of *Millettia,* are known to have lost the inverted repeat in the chloroplast genome, suggesting a closer link to the THC. (2) The less species-rich clades within the THC, especially the smaller genera *Glycyrrhiza, Galega,* and perhaps *Chesneya, Gueldenstaedtia, Caragana,* and their relatives, cannot be arranged in a ladder-like (paraphyletic) arrangement at the base of the THC. If this occurred it would argue for a shift to higher rates within the more diverse Astragalean and Vicioid clades. However, if these less species-rich groups formed a monophyletic group that was the sister to the bulk of diversity within the THC, it would be possible to regard their lower diversity as a reversal to lower rates from a primitively high rate of diversification. Although there is some uncertainty about the relationships of *Caragana* and its relatives, the position of *Glycyrrhiza* and *Galega* in these phylogenies has been highly consistent and well supported in bootstrap confidence tests. They are also congruent with results from independent molecular data from the chloroplast genome (Liston and Wheeler, 1994). Thus, it seems fairly unlikely that both conditions outlined above will be met in such a way as to be consistent with the idea that the entire THC is radiating at a high rate. Instead, the THC appears to be another example of a diverse clade in which the bulk of its species diversity stems from events that occurred in the evolutionary history of a *subset* of its taxa. Whether this subset is the Astragalean clade, the Vicioid clade, or some combination of the two will only be resolved when phylogenetic relationships at the base of the legume temperate herbaceous clade become better understood.

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APPENDIX. Genbank accession numbers and voucher information for sequence data reported in text (accession numbers are listed in order for ITS 1 and 2 sequences).

Species	Accession[a]	Genbank accession
Alhagi camelorum Fischer	Adams 19-88 (ARIZ)[b]	U50756, U50757
Alhagi maurorum Medikus	USDA 502281	U50486, U50487
Astragalus agrestis Dougl. ex G. Don.	Sanderson 917	L10758, L10759
Astragalus alopecias Pallas	USDA 440154	U50508, U50509
Astragalus alpinus L.	USDA, 232536, Wojciechowski and Sanderson 183	L10760, L10761
Astragalus americanus (Hook.) Jones	Nelson 6870 (RM)[b]	U50492, U50493
Astragalus atropilosulus var. *venosus* (Hochst.) Gillett	USDA 193735, Wojciechowski and Sanderson 301	U50504, U50505
Astragalus bodini Sheldon	Welsh, Isely and Moore 6467 (COLO)[b]	U50592, U50593
Astragalus canadensis L. var. *brevidens* (Gand.) Barneby	Wojciechowski and Sanderson 302	U50496, U50497
Astragalus cerasocrenus Bunge (=*Astracantha cerasocrena* (Bge.) Podl.)	DELEP 880043	U50514, U50515
Astragalus cicer L.	USDA 206405, Wojciechowski and Sanderson 160	L10772, L10773
Astragalus complanatus R. Br.	DELEP 900279	U50500, U50501
Astragalus corrugatus Bertol.	USDA 227441, Wojciechowski and Sanderson 164	L10774, L10775
Astragalus echidnaeformis Sirj. (=*Astracantha echidnaeformis* (Sirj.) Podl.)	DELEP 880044, Wojciechowski and Sanderson 411	U50512, U50513
Astragalus echinatus Murray (=*A. pentaglottis* L.)	USDA 516498, Wojciechowski and Sanderson 407	U50510, U50511
Astragalus epiglottis L.	Wojciechowski and Sanderson 301 (A. Liston)	U50506, U50507
Astragalus falcatus Lam.	Weber 15359 (COLO)[b]	U50488, U50489
Astragalus hamosus L.	USDA 226627, Wojciechowski and Sanderson 166	L10778, L10779
Astragalus lobophorus Boiss.	USDA 330696, Wojciechowski and Sanderson 170	L10782, L10783
Astragalus peristereus Boiss. & Hausskn. (=*Astracantha peristerea* (Boiss. & Hausskn.) Podl.)	DELEP 880051	U50494, U50495
Astragalus robbinsii var. minor (Hook.) Barneby	Holmgren and Holmgren 9065 (RM)[b]	U50490, U50491
Astragalus sinicus L.	USDA 150557, Wojciechowski and Sanderson 408	U50502, U50503
Astragalus vogelii (Webb.) Bornm.	Shmida 3.2.80 (A. Liston)	U50498, U50499
Biserrula pelecinus L.	USDA 186284, Wojciechowski and Sanderson 294	U50518, U50519
Calophaca tianschanica (Fedtsch.) Boriss.	A. K. Skvortsov (A)[b]	U51220, U51221
Caragana arborescens Lam.	USDA 310390	L10798, L10799
Caragana frutex (L.) Koch	Sanderson 1575	U56001, U56002
Carmichaelia williamsonii Kirk	Sanderson 1550	U50520, U50521
Chesnya dshungarica Gobsk.	Goboskokov 5.29.55 (US)[b]	U50350, U50351
Cicer arietinum L.	Wojciechowski and Sanderson 189	U56003, U56004
Cicer pinnatifidum Jaub. & Spach.	USDA 458555, Wojciechowski and Sanderson 409	U50867, U50868
Clianthus puniceus (G. Don.) Lindley	T&M 7140 (A. Liston)	L10800, L10801
Colutea arborescens L.	USDA 369222, Wojciechowski and Sanderson 406	U56009, U56010
Colutea istria Miller	DELEP 890385	U69544, U69545
Eremosparton flaccidum Litw.	Leontief 10.5.35 (US)[b]	U56013, U56014
Galega officinalis L.	M. Smejkal (A)[b]	U50760, U50761
Galega orientalis L.	USDA 325341	U56015, U56016
Glycyrrhiza echinata L.	Liston 258	U55999, U56000
Glycyrrhiza lepidota (Nutt.) Pursh	Toolin 1572 (ARIZ)[b]	U50758, U50759
Gueldenstaedtia himalaica Baker	(A. Liston)	Not in GenBank
Halimodendron halodendron (Pallas) Voss.	Stevens 2394 (US)[b]	U56019, U56020
Hedysarum boreale Nutt.	Wojciechowski and Sanderson 131	U50482, U50483
Lessertia brachystachya DC.	USDA 208172	U56005, U56006
Medicago lupulina L.	Baker 9447 (ARIZ)[b]	U50865, U50866
Medicago polymorpha L.	Jenkins 91-8 (ARIZ)[b]	U50863, U50864
Melilotus alba Medikus	Wojciechowski 308	U50762, U50763
Melilotus officinalis (L.) Pallas	Wojciechowski 309	U50764, U50765
Onobrychis montana DC.	Mason and Mason 3773 (ARIZ)[b]	U50484, U50485
Oxytropis campestris var. *johannensis* (L.) DC.	USDA 504535, Wojciechowski and Sanderson 174	L10802, L10803
Oxytropis deflexa (Pall.) DC. var. *sericea* Torr. & Gray	Wojciechowski and Sanderson 132	L10804, L10805
Oxytropis lambertii Pursh	Wojciechowski 155	L10807, L10806
Pisum sativum L. (cv. Little Marvel)	Wojciechowski 398	U50861, U50862
Smirnowia turkestana Bge.	(A)[b]	U51218, U51219
Sphaerophysa salsula (Pallas) DC.	Yoder-Williams 78-120A-1 (RENO)[b]	U56011, U56012
Sutherlandia frutescens L.	Wojciechowski and Sanderson 266	U50516, U50517
Swainsona pterostylis (DC.) Bakh. f.	DELEP 900185, Wojciechowski and Sanderson 296	U56007, U56008
Tephrosia leiocarpa A. Gray	DELEP 880028	U50752, U50753
Tephrosia tenella A. Gray	Jenkins 88-1 (ARIZ)[b]	U50754, U50755
Trifolium longipes Nutt. var. *neurophyllum* (Greene) Martin ex Isely	McLaughlin 4284 (ARIZ)[b]	U56017, U56018
Trifolium nanum Torr.	University of Colorado, EPOB 4520 (1992) collection (ARIZ)[b]	U50859, U50860
Vicia ludoviciana Nutt.	McLaughlin and Bowers 3185 (ARIZ)[b]	U51216, U51217
Wisteria frutescens (L.) Poiret	Moldenke and Moldenke 29243 (ARIZ)[b]	U50750, U50751
Wisteria frutescens var. *macrostachya* (L.) Poiret	USDA 2774	U55997, U55998

[a] Abbreviations used in accession identifications: DELEP, Desert Legume Program (Boyce Thompson Southwestern Arboretum and University of Arizona), Tucson, AZ; USDA, U. S. Department of Agriculture Plant Introduction accession numbers.

[b] Designates samples taken from herbarium specimens; herbarium abbreviation given in parenthesis: ARIZ, University of Arizona, Tucson; COLO, University of Colorado, Boulder; A, Arnold Arboretum, Harvard University Herbaria, Cambridge; RENO, University of Nevada, Reno; RM, Rocky Mountain, University of Wyoming, Laramie; US, National Museum of Natural History, Smithsonian Institution, Washington, DC.