

# GEOGRAPHY AND PHYLOGENY OF SEXUAL DIPLOID *ERIGERON STRIGOSUS* (ASTERACEAE) IN ARKANSAS AND MISSOURI

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## ABSTRACT

*Erigeron strigosus* (Asteraceae) is a complex species consisting of a diversity of sexual diploid and apomictic polyploid populations. Previous work has established the geographic distribution and phylogenetic relationships among sexual diploid populations in the extreme southeastern United States. In this work we investigated disjunct sexual diploid populations in Arkansas and Missouri. We evaluated pollen quality and mapped geographic locations for 496 unique herbarium collections to determine that sexual populations are relatively common in Arkansas (37 out of 286 collections; 12.9%), especially in upland habitats in the western 2/3 of the state, but rare in Missouri (1 out of 210 collections; 0.5%). Eight rDNA (ITS plus ETS) sequences for seven populations reveal that sexual diploid *E. strigosus* from Arkansas is not genetically differentiated from other populations that have been studied. However, two genetic lineages were detected; those from the Ozark Highlands correspond to previously described rDNA haplotype II while samples from more southerly ecoregions correspond to previously described rDNA haplotype III. This work fills in a large gap in the knowledge of the distribution of genetic variation and lends insight into the phylogeography of the complex.

## RESUMEN

*Erigeron strigosus* (Asteraceae) es un complejo de especies que consiste en una diversidad de poblaciones sexuales diploides y apomícticas poliploides. Trabajos previos han establecido la distribución geográfica y las relaciones filogenéticas entre las poblaciones sexuales diploides del extremo sureste de los Estados Unidos. En este trabajo investigamos las poblaciones sexuales diploides disyuntas de Arkansas y Missouri. Hemos evaluado la calidad del polen y mapeado las localidades geográficas de 496 colecciones de herbario únicas para determinar que las poblaciones sexuales son relativamente comunes en Arkansas (37 de 286 colecciones; 12.9%), especialmente en los hábitats de las tierras altas de los 2/3 occidentales del estado, pero son raras en Missouri (1 de 210 colecciones; 0.5%). Ocho secuencias rDNA (ITS y ETS) de siete poblaciones revelaron que el diploide sexual *E. strigosus* de Arkansas no se diferencia genéticamente de otras poblaciones que se han estudiado. Sin embargo, se detectaron dos linajes genéticos; los de Ozark Highlands que corresponden al haplotipo II de rDNA previamente descrito mientras que muestras de ecorregiones más al sur corresponden al haplotipo III de rDNA previamente descrito. Este trabajo llena un gran hueco en el conocimiento de la distribución de la variación genética y permite una percepción de la filogeografía del complejo.

## INTRODUCTION

Polyploid complexes in plants present numerous challenges to evolutionary biologists especially as regards understanding the relationships between diploid progenitors and interpreting the origins of polyploids (Adams & Wendel 2005; Buggs et al. 2011). These challenges are augmented in apomictic polyploid complexes where extensive reticulation in combination with asexual reproduction yields an array of microspecies that may conceal the morphological and genetic diversity maintained by the progenitor diploid entities (Asker & Jerling 1992). Despite these challenges, noteworthy progress has been made recently in understanding the structure of apomictic complexes for a few groups using a diversity of molecular approaches (e.g., Schranz et al. 2005 [*Boechea*]; Grusz et al. 2009 [*Cheilanthes*]; Fehrer et al. 2009 [*Hieracium*]; Cosendai et al. 2011 [*Ranunculus*]).

For incompletely or poorly known complexes however, the initial step must be to comprehensively characterize diploid populations that may be progenitors of the polyploids (Stebbins 1950; Grant 1981). One such complex is the *Erigeron* sect. *Phalacrolooma* (Cass.) Torr. & A. Gray (Asteraceae) apomictic complex of North America. This complex includes *Erigeron strigosus*, the prairie fleabane, which is abundant throughout eastern North America west to the Great Plains, and then occurs sporadically in suitable mesic habitats to the west coast. This species is a late spring to early summer annual to weak perennial herb from a short rhizome that

produces one to a few erect stems ranging from ~0.4 to 1.0 m bearing terminal cymes of several to many capitula (Nesom 2006). The taxon consists of diploid ( $2n=18$ ) populations that reproduce sexually and polyploid (triploid and tetraploid,  $2n=27, 36$ ) populations that reproduce asexually via apomixis (Noyes 2007).

Several investigations have aimed to understand the geographic distribution and evolutionary relationships among and between sexual diploid and polyploid apomictic *Erigeron strigosus* (Noyes 2006, 2007; Noyes & Allison 2005; Noyes et al. 2006). These studies show that sexual diploid populations occur predominantly in the South Central Plains Ecoregion of east Texas and adjacent Louisiana, Southeastern Plains Ecoregion from Alabama to South Carolina south to northern Florida, and in the Central Basin of Tennessee (ecoregions according to EPA 2011). Apomicts are sympatric and parapatric to sexual plants in these regions but represent the only condition found in the northern and western part of the species' range. This overall pattern, with geographically restricted sexual diploids, and widespread apomicts extending the range of the species is typical for apomictic groups (Bierzuchudek 1985).

Studies to date show that the sexual diploid populations of *Erigeron strigosus* are morphologically and genetically diverse, constituting three subspecific taxa: *E. strigosus* var. *dolomiticola* J. Allison is restricted to a single dolomite glade complex in central Alabama; *E. strigosus* var. *callicola* J. Allison occurs on limestone glades in the Central Basin of Tennessee and adjacent southern Alabama; *E. strigosus* var. *strigosus* applies to the remainder of sexual diploid plants that range from Texas to South Carolina across the southeast. The name *E. strigosus* var. *traversii* (Shinners) Noyes has been applied to sexual diploid plants in Texas and adjacent Louisiana (Noyes et al. 2006), but it now appears likely that these plants are genetically similar to other *E. strigosus* var. *strigosus* populations in the eastern part of the range (Noyes & Groff, unpublished data).

A phylogeny for sexual diploids in the group shows that *Erigeron strigosus* var. *callicola* and *E. strigosus* var. *dolomiticola* are genetically distinct from *E. strigosus* var. *strigosus* (Noyes 2007). The typical variety is sister to *E. strigosus* var. *dolomiticola*, monophyletic, and genetically diverse, comprising three main rDNA lineages (haplotypes I, II, III) that are found individually and in combination in hybrids throughout the Southeast. These haplotypes are hypothesized to have evolved in isolation and then to have recombined upon range expansion into the Southeastern Plains. Hypothetical ancestral ranges of the haplotypes have not been identified.

Previous mapping and phylogenetic work for *Erigeron strigosus* has focused on the center of diversity of plants in the complex namely in Florida, Georgia, Alabama, South Carolina, Tennessee, and Texas. In the course of that work, analysis of pollen for a sample of herbarium specimens collected in Arkansas indicated that the Interior Highlands, including the Ozark Plateau and Ouachita Mountains, also likely harbored sexual diploid populations (Noyes 2007). Because of the isolation of this area from other regions where sexual diploids are found and because the Interior Highlands is an important center of endemism and biodiversity (Thorn & Wilson 1980; USDA 1999; Ouachita Ecoregional Assessment Team 2003; Robison et al. 2008), we undertook a study to determine the geographic distribution and evolutionary affinities of sexual diploid *Erigeron strigosus* in Arkansas and Missouri. We used pollen analysis from herbarium specimens to identify sexual diploid populations, mapped the populations using GIS methods, and relocated populations in the field from which DNA was isolated for phylogenetic assessment.

Our principal objectives were to determine the distribution of sexual diploid populations of *Erigeron strigosus* in Arkansas and Missouri and to evaluate the genetic relationship of these disjuncts to other sexual diploid *E. strigosus*.

#### METHODS

A total of 398 herbarium specimens of Arkansas and Missouri plants was borrowed from the Missouri Botanical Garden (MO; 219 specimens), the University of Arkansas at Fayetteville (UARK; 141 specimens), the University of Central Arkansas at Conway (UCAC; 22 specimens), and from the herbarium of the Arkansas Natural Heritage Commission in Little Rock (ANHC; 16 specimens). Data for these plants was appended to a database that included data gathered for 171 Arkansas and Missouri specimens studied previously (Noyes

2007) from diverse herbaria (BRIT: 49; FLAS: 1; FSU: 3; NCU: 33; TENN: 16; TEX: 29; UGA: 12; UNA: 1; USCH: 3; VDB@BRIT: 22; VSC: 2) thus yielding a total of 569 specimens examined for this study.

Pollen from herbarium specimens was evaluated to estimate mode of reproduction. For each specimen, pollen from mature but unopened florets was stained in Cotton Blue in lactophenol (Stanley & Linskens 1974, p. 307) and evaluated at 400× on an Olympus BX51 compound scope using bright field microscopy. A pollen sample was identified as being produced by a sexual diploid plant if the grains were uniformly small (ca. 12–15 μm diam.), with high viability (darkly stained), and few aborted grains (Noyes & Allison 2005). These samples are easily distinguished from polyploid apomicts which produce large pollen grains (>17 μm diam.) with high levels of pollen abortion and also usually with frequent pollen micrograins. Measurements of grains as required were performed using AnalySIS (v. 3.1; soft Imaging System, GmbH 1989–2001) on pollen images captured with an Olympus FV12 monochrome CCD camera.

The geographic coordinates for each specimen were estimated from label locality information using TOPO USA (version 5.0, DeLorme, Yarmouth, ME). The data was mapped using ESRI ArcInfo (v. 9.2, copyright 2004–2007; <http://www.esri.com>). Duplicate specimens were eliminated so that only unique collections were mapped. The base map providing a county boundary layer (tl\_2009\_US\_county.shp) was obtained from the US Census Bureau, Geographic Products Branch (<http://www2.census.gov/cgi-bin/shapefiles2009/national-files>). As a rough check of the accuracy of our plotted data, we verified that extracted county names from plotted data points matched input county names. To evaluate the distribution of plants, we employed Level III and Level IV Ecoregion Maps (EPA 2011) for Missouri and Arkansas and determined Ecoregion for plotted points using “Join Data” in ArcInfo.

To investigate the genetic relationship of sexual diploid *Erigeron strigosus*, sexual diploid plants were collected from seven populations in Arkansas; two sites from the Ozark Highlands, three sites from the Ouachita Mountains, and two populations from northern edge of the South Central Plains (Table 1; Fig 1). Plants were evaluated in the field for pollen quality and if consistent with the sexual diploid condition brought into cultivation at the University of Central Arkansas, Conway. Chromosome counts were made to verify the diploid condition for plants using aceto-carmin root-tip squashes. Total DNA was isolated for each plant using the DNeasy Plant Mini kit (Qiagen, Valencia, California, USA). For phylogenetic analysis, rDNA ITS (ITS1, 5.8S, ITS2) and ETS regions were amplified and sequenced separately but then concatenated into a common sequence. The primers and amplification conditions were identical to those used previously (Noyes 2006). Sequencing was performed at the University of Missouri, Columbia, DNA Core Facility.

Phylogenetic analysis was conducted by adding the eight new sequences (Table 1) to a base data set consisting of sequences obtained previously for 18 plants including three samples of *Erigeron strigosus* var. *calcicola* (Ca1543.AL, Ca1540.TN, Ca1557.TN), three samples of *E. strigosus* var. *dolomiticola* (Do1545.AL, Do1546.AL, Do1632.AL), and 12 samples representing *E. strigosus* var. *strigosus* with each of the three previously recognized haplotypes represented by four samples (haplotype I: I.1225.AL, I.1628.GA, I.1316.GA, I.1631.SC; haplotype II: II.1608.AL, II.1616.GA, II.1617.GA, II.1610.FL; haplotype III: III.1612.GA, III.1614.GA, III.1618.GA, III.1630.SC). Sample designations are the same as used previously (Noyes 2006); voucher information and GenBank Accession numbers are also reported there. Pairwise distance measures (uncorrected “p”) were obtained using PAUP\* (version 4.0b.10; Swofford 2002) as a measure of overall sequence similarities.

Three different approaches were used for tree construction. The first tree was constructed using weighted parsimony in PAUP\* with the empirical transition/transversion ratio (Ti/Tv) = 3.68. The second was a maximum likelihood analysis executed in PAUP\* using a TrN+I model of evolution selected by ModelTest 3.7 (Posada 1998) based on the Akaike Information Criterion (AIC). For each of these two analyses, statistical support was obtained via bootstrap analysis conducted in PAUP\* with 100 replicates with random addition and maximum number of trees per replicate set at 1000. Lastly, a Bayesian analysis was also executed with a GTR+I model of evolution, selected by MrModelTest 2.3 (Nylander 2004), with 2000K generations and a 50% burn-in. This eliminated all sample trees with an average standard deviation of split frequencies > 0.0055.

TABLE 1. Sexual diploid *Erigeron strigosus* sampled from Arkansas, USA for phylogenetic analysis. Sample locations plotted in Figure 1.

Sample	Location	Voucher	GenBank #s [ITS,ETS]
1. ARCR63.1	AR: Izard Co.; Limestone glade 6.0 km N of Calico Rock	<i>RDN 1654</i> (UCAC)	JF937682, JF937690
2. ARCR63.2	AR: Izard Co.; Limestone glade 6.0 km N of Calico Rock	<i>RDN 1655</i> (UCAC)	JF937683, JF937691
3. ARCR64.0	AR: Stone Co.; Limestone glade 4.0 km SW of Calico Rock	<i>RDN 1656</i> (UCAC)	JF937684, JF937692
4. ARBC1	AR: Perry Co.; Big Cove Creek, disturbed forest trail; 8 km S of Nimrod Lake	<i>RDN 1657</i> (UCAC)	JF937685, JF937693
5. ARLCR1	AR: Saline Co.; Dry Lost Creek, Igneous Glade vicinity; 3.2 km S of Bauxite	<i>RDN 1667</i> (UCAC)	JF937686, JF937694
6. ARLCM1	AR: Saline Co.; Dry Lost Creek, Igneous Glade; 3.2 km S of Bauxite	<i>RDN 1668</i> (UCAC)	JF937687, JF937695
7. ARGD1	AR: Howard Co; Disturbed slopes of Gillham Lake Dam	<i>RDN 1661</i> (UCAC)	JF937688, JF937696
8. ARGDE1	AR: Howard Co; Forested roadside, 0.8 km E of Gillham Lake Dam	<i>RDN 1666</i> (UCAC)	JF937689, JF937697

## RESULTS

Mode of reproduction was able to be estimated for 564 of 569 herbarium specimens. Consideration of duplicates reduced the sample to 496 unique collections. Of 287 specimens from Arkansas, 37 (12.9%) were determined to be sexual diploid and 250 (87.1%) were determined to be apomictic polyploid. Of 209 unique specimens from Missouri, the sexual diploid condition was indicated for only a single specimen (0.5%). The data pooled for both states yields 92.3% apomictic polyploid and 7.7% sexual diploid specimens (Table 2).

The map generated for the data (Fig. 1) shows that apomictic plants occur in all ten of the ecoregions recognized for the two states. Sexual diploid plants, in contrast, are restricted to six ecoregions. The lone Missouri sexual diploid collection (*E.J. Palmer s.n.*, Stone Co., MO, 19 May 1914) is from the Ozark Highlands Ecoregion in the vicinity of Galena close to the Arkansas border (Fig. 1; Table 2). In Arkansas, sexual diploid plants are present in the Ozark Highlands, Boston Mountains, Arkansas River Valley, Ouachita Mountains, South Central Plains, and Crowley's Ridge of the Mississippi Loess Plains (one collection), and completely absent from the broad Mississippi Alluvial Plain in eastern Arkansas (Fig. 1; Table 2). In the six Ecoregions of Arkansas where they occur, sexual plants are relatively frequent, representing ~11.1 percent of collections present in herbaria (Table 2). In general the sexual populations are widely distributed within Ecoregions occurring sporadically from the Oklahoma border to the edge of the Mississippi Alluvial Plain.

There are two aggregates of sexual collections, the first in north central Arkansas near the Missouri border within the Ozark Highlands (eight localities), and the second in west central Arkansas approaching the Oklahoma border in the Ouachita Mountain Ecoregion (eight localities). Most sexual populations apparently occur in close proximity to apomicts. Thus even though sexual plants are mostly restricted to the western 2/3 of Arkansas, there are no clearly defined areas where sexual plants occur exclusively. In addition, while sexual diploid plants are fairly frequent in the Ozark region of Arkansas, most of the Missouri Ozarks are devoid of sexual populations. The natural boundary for the northern extent of sexually reproducing *Erigeron strigosus* in the region thus appears to fall in extreme southern Missouri.

Herbarium specimen label data show that there is a tendency for sexual plants to be associated with specialized habitats. For instance, out of 26 collections with informative habitat label data, ten indicate 'glade' habitat, or either 'shale' or 'limestone' substrates. The other 16 collections do not indicate special edaphic habitat but are instead a diverse mixture of 'roadside', 'meadow', 'creek bed', 'clear-cut/disturbed forest', and 'rocky

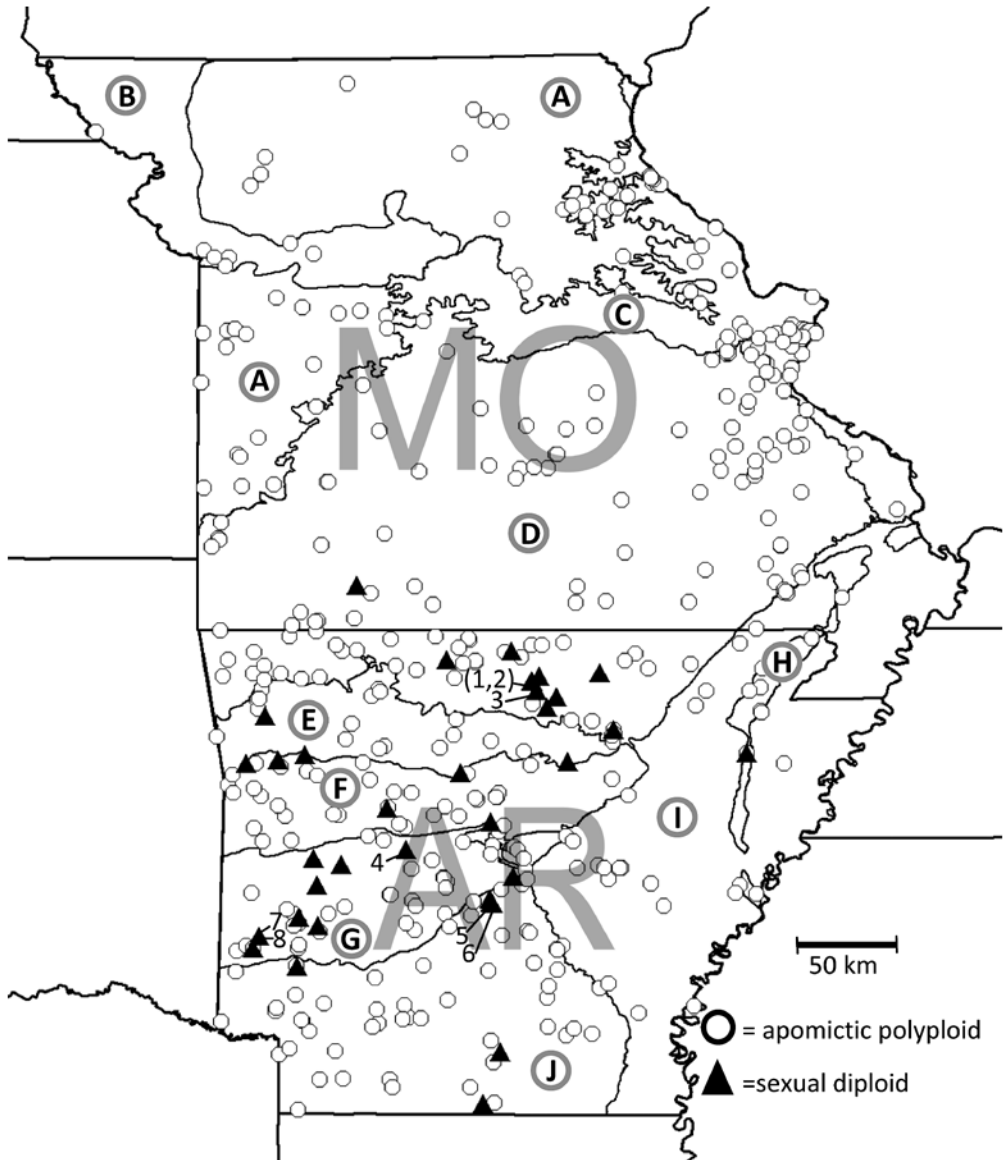


FIG. 1. Distribution of sexual diploid and apomictic polyploid *Erigeron strigosus* in Missouri and Arkansas. Mode of reproduction estimated from quality of herbarium specimen pollen. Level III Ecoregions A-J: (EPA 2011). A) Central Irregular Plains; B) Western Corn Belt Plains; C) Interior River Lowland; D) Ozark Highlands; E) Boston Mountains; F) Arkansas River Valley; G) Ouachita Mountains; H) Mississippi Loess Plains (Crowley's Ridge); I) Mississippi Alluvial Plain; J) South Central Plains. Plotted numbers (1–8) refer to approximate localities of samples obtained for phylogenetic analysis (Table 1).

ridge'. Thus it appears that sexual plants may occur on but are not restricted to specialized edaphic habitats. Among this small sample of herbarium specimens of sexual diploid plants, there is also conspicuous morphological variation in leaf shape (narrowly linear to oblanceolate, entire to toothed basally) and pubescence (leaves and stem sparsely rough strigose to densely soft villous). However, the sexual diploids do not, based on overall morphology, fall into obvious groupings that would warrant taxonomic recognition without further study.

TABLE 2. Incidence of sexual and apomictic *Erigeron strigosus* in Arkansas and Missouri by Ecoregion (EPA 2011). Regions correspond to those depicted in Figure 1.

Region	# Apomictic Specimens	# Sexual Specimens
A. Central Irregular Plains	33 (100%)	0 (0%)
B. Western Corn Belt Plains	8 (100%)	0 (0%)
C. Interior River Lowland	58 (100%)	0 (0%)
D. Ozark Highlands	151 (92.1%)	13 (7.9%)
E. Boston Mountains	24 (88.9%)	3 (11.1%)
F. Arkansas River Valley	44 (88.0%)	6 (12.0%)
G. Ouachita Mountains	45 (83.3%)	9 (16.7%)
H. Mississippi Loess Plains (Crowley's Ridge)	9 (90.0%)	1 (10.0%)
I. Mississippi Alluvial Plain	25 (100%)	0 (0%)
J. South Central Plains	61 (91.0%)	6 (9.0%)
<b>Total</b>	<b>458 (92.3%)</b>	<b>38 (7.7%)</b>

Chromosome counts for all eight *Erigeron strigosus* samples selected for phylogenetic analysis showed the diploid number of  $2n=18$ . The eight rDNA sequences for these plants were all of identical length and similar in length to previous *E. strigosus* var. *strigosus* sequences analyzed (Noyes 2006). Alignment necessitated the addition of a single base gap in all eight sequences to accommodate the sequences of *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola*. The eight new sequences exhibited very few DNA polymorphisms (14 total out of 9784 total bases; 0.14%) and all but two polymorphisms occurred in parsimony uninformative positions. It was thus inferred that the plants possessed a single predominant rDNA haplotype and were not hybrids containing two highly divergent types as had been found for plants elsewhere in the range of the species (Noyes 2006). The total aligned database for concatenated ITS (626 bp) and ETS (597 bp) was 1223 bp.

The three analytical methods resulted in the same overall phylogenetic pattern with similar levels of statistical support. The expected topology of Noyes (2006) was recovered (Fig. 2) showing *Erigeron strigosus* var. *calcicola* in a basal position, *E. strigosus* var. *dolomiticola* diverging next, and then the three clades representing the three haplotypes of *E. strigosus* var. *strigosus* with haplotype I diverging first, and haplotypes II and III as derived sisters. Each of these groups receives moderate to strong statistical support consistent with Noyes (2006). The parsimony analysis yielded 20 shortest trees the consensus of which was identical to the single trees resulting from maximum likelihood and Bayesian analyses except that the parsimony tree lacked support for one internal group within the type II clade.

All three analyses show that the Arkansas sequences fall into two groups. The three sequences from the Ozark region of northern Arkansas (ARCR63.1, ARCR63.2, ARCR64.0) unambiguously occur with type II haplotypes, the type II clade receiving bootstrap support values of 99%, 100%, and 1.0 clade credibility (Fig. 2). Further, the three Ozark sequences do not form a distinct subclade and instead occur intermixed with the other four type II sequences. The three Ozark sequences are very similar to type II sequences obtained from sexual diploid plants occurring in Florida, Georgia, and Alabama, differing by only an average of 3.8bp per sequence (average uncorrected pairwise "p" of 0.0030825). Only two base positions out of 1223 separate ARCR63.1, ARCR63.2, and ARCR64.0 from the II.1608.AL sequence from northern Alabama.

The five sequences from the southern part of the state (Ouachita Mountains and South Central Plains Ecoregions) all occur within the haplotype III clade (Fig. 2). The type III clade is moderately supported with bootstrap values for parsimony analysis and maximum likelihood of 74% and 66% respectively, and clade credibility value of 0.75 for Bayesian analysis. The five Arkansas sequences are very similar to each other as ARBC1, ARGDE1, ARLCM1, ARLCR1 are identical in sequence and differ from ARGD1 by only a single base position. The Arkansas sequences are similar to the other clade III sequences from Georgia and South Carolina yielding an average uncorrected "p" of 0.000574, an average pairwise difference of only 0.7 bases per sequence. Further, ARBC1, ARGDE1, ARLCM1, and ARLCR1 are identical to the sequences III.GA1272 and III.GA1402

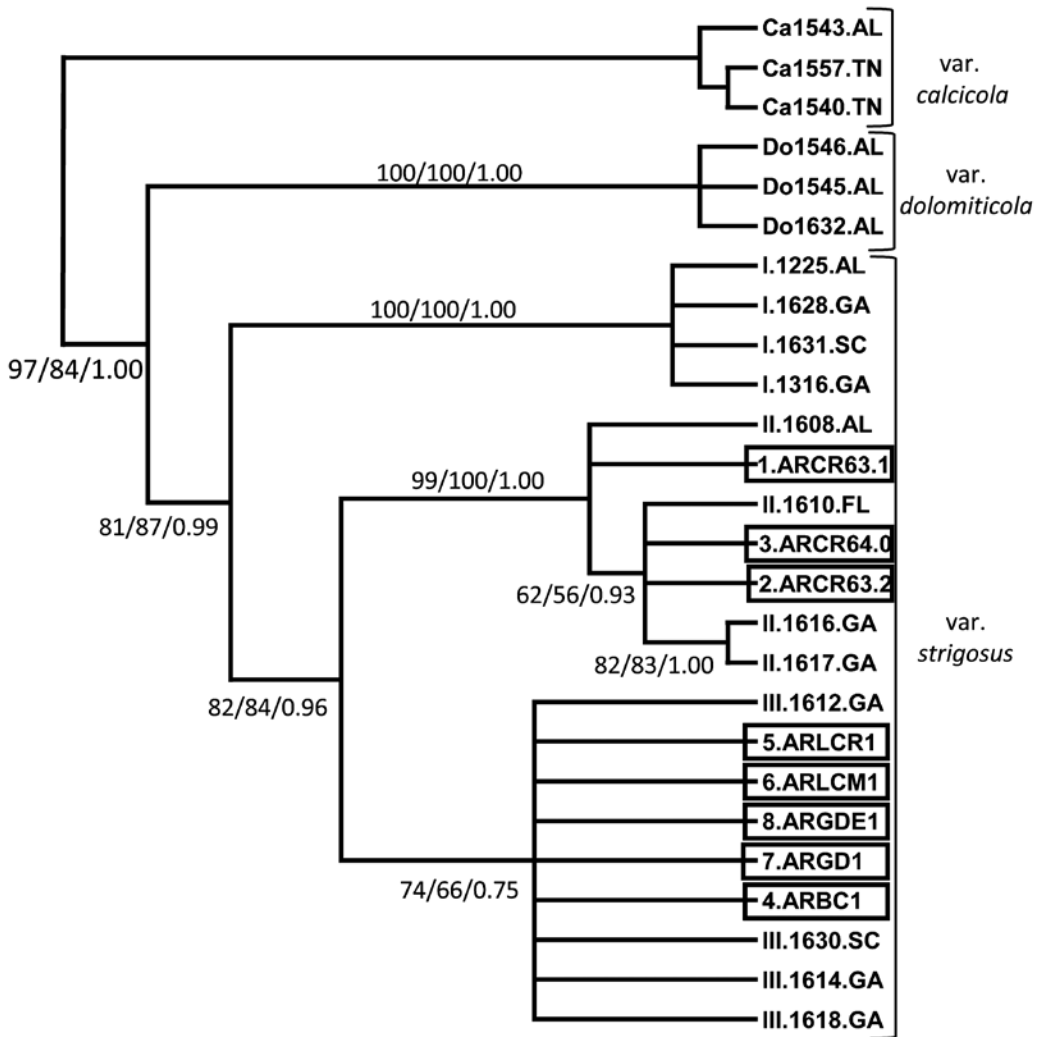


FIG. 2. Phylogenetic relationship of sexual diploid *Erigeron strigosus* from Missouri and Arkansas based on ITS plus ETS rDNA. Labels of sequences generated for this analysis are boxed. Base phylogeny consists of 18 sequences previously analyzed (Noyes 2006) including three sequences of *E. strigosus* var. *calcicola*, three sequences of *E. strigosus* var. *dolomiticola*, and 12 sequences of *E. strigosus* var. *strigosus* (4 sequences representing each of rDNA haplotypes I, II, and III). Topology is single most likely tree resulting from maximum likelihood analysis. Values associated with nodes reflect bootstrap values for weighted parsimony (Ti/Tv = 3.68), bootstrap values for maximum likelihood analysis based on TrN+I model of evolution, and clade compatibility values for Bayesian analysis based on GTR+I model of evolution.

from sexual diploid plants in Georgia. In contrast, the average pairwise uncorrected “p” distance comparing Arkansas clade II plants (ARCR63.1, ARCR63.2, ARCR64.0) with Arkansas clade III plants (ARBC1, ARGD1, ARGDE1, ARLCM1, ARLCR) is 0.0081 corresponding to about 10.0 bp.

#### DISCUSSION

The results of this work show that sexual diploid *Erigeron strigosus* var. *strigosus* is fairly common in Arkansas west of the Mississippi Alluvial Plain, representing about 13% of collections in herbaria from the state (Fig. 1). This extends considerably the documented range of sexual diploids from the Plains ecoregions of the Southeast

unambiguously into the four main ecoregions of the Interior Highlands: the Ozark Highlands, the Boston Mountains, the Arkansas River Valley, and the Ouachita Mountains, as well as Crowley's Ridge.

Although found in a diversity of habitats, sexual diploid plants in the region are often associated with glades or barrens. This tendency was not entirely unexpected because some members of the complex are known edaphic specialists, namely *Erigeron strigosus* var. *dolomiticola*, restricted to a single group of dolomite glades in central Alabama, and *E. strigosus* var. *calcicola*, restricted to calcareous glades in the Central Basin of Tennessee. In contrast, previous studies (Noyes et al. 2006; Noyes 2007) found that sexual diploid *E. strigosus* var. *strigosus* in the extreme Southeast in general, is not an edaphic specialist, rather preferring general sandy substrates from the Pineywoods of east Texas to the Southeastern Plains of Georgia and South Carolina. Alternative interpretations for the origin of edaphic specialization in Arkansas are possible. First, it may be that plants on these habitats are recent arrivals derived from ancestors adapted to sandy habitats from the south or southeast. That ancestral preference may then represent a predisposition for xeric and well drained soils that permitted encroachment onto more extreme glade and barren substrates of the Interior Highlands. This would be consistent with the hypothesis for the evolution of serpentine endemics (Krukeberg 1954; Pepper & Norwood 2001). Alternatively, edaphic specialization may represent the ancestral condition. In that case, plants occurring on non-specialized soils could represent recent expansion.

Phylogenetic results (Fig. 2) clearly show that sexual diploid *Erigeron strigosus* in Arkansas is not genetically distinct from populations further east. Arkansas sequences are virtually identical to sequences obtained from plants in Georgia and Alabama, and the Arkansas sequences closely align with other sequences in the rDNA type II and III clades of *E. strigosus* var. *strigosus*. Thus there is no support for an Arkansas or Interior Highlands clade. This is in contrast to the expectation, given the role of the Interior Highlands as a center of biodiversity and given their disjunct distribution, that sexual diploid *E. strigosus* found in this ecoregion would be genetically distinct.

The origin of the two rDNA haplotypes in Arkansas is unresolved as both (haplotypes II and III) are found throughout the Southeast (Noyes 2006). Instead we found two genetically distinct types both with genetic relatives in the eastern part of the range of the species. One possibility is that sexual diploid plants evolved in isolation in the Ozark and Ouachita interior highlands generating the type II and III rDNA haplotypes. Subsequent range expansion could have spread the haplotypes to the south and southeast to create the current distribution. Alternatively, the haplotypes could have arrived in Arkansas recently, having spread into the region from the south and southeast. Unfortunately with the present data, it is not possible to distinguish between these alternatives. Resolution would likely require the acquisition of population level genetic data and application of phylogeographic tools to properly model the history of the complex in the region. An additional caveat is that the current eight genetic samples from seven populations is small and may not reflect overall diversity in the region. Additional study will be required to determine the distribution, diversity, and evolutionary history of haplotypes in the region.

In contrast to sexual plants, polyploid apomictic *Erigeron strigosus* var. *strigosus* can occur in lowland regions characterized by alluvial clay soils, for instance of the Mississippi Alluvial Plain and the Interior River Lowland Ecoregions (Fig. 1). However, there is also evidence that polyploid apomicts can also occur in specialized habitats. For instance, collections by T. Witsell (ANHC) from natural areas in Arkansas that were mapped for this project show apomictic *E. strigosus* on shale glades and barrens in Saline (Witsell 07-142, 07-145, 07-153, 07-291, 07-394), Newton (07-402), and Garland (07-181, 07-184, 07-194) Cos., an igneous glade in Pulaski Co. (07-260), a dolomite glade in Marion Co. (07-411), and a chalk glade in Little River Co. (07-127). Thus it is evident that apomictic polyploid *E. strigosus* has the ability to utilize a broader spectrum of habitats than the sexual diploids. This breadth, probably in combination with asexual reproduction, likely accounts for their geographic predominance relative to sexual diploids. The polyploid condition has long been associated with expanded ecological repertoire (Ramsey & Schemske 1998; Soltis & Soltis 2000).

In contrast to their abundance in Arkansas, sexual diploid plants are apparently rare in Missouri, represented by only a single sexual diploid specimen from the southern part of the state about 35km N of the

Arkansas border. This limited distribution of sexual diploids in Missouri is somewhat surprising as suitable sites for sexual diploid plants that are comparable to those in Arkansas, for instance limestone glades, occur throughout the Ozark Highland Ecoregion of Missouri (Heikens 1999).

Finer, Level IV Ecoregion scale analysis of the distribution of sexual plants in the Ozarks shows that of the 13 sexual diploid specimens mapped, nine are located on the Springfield Plateau (including Springfield Plateau (1), Dissected Springfield-Elk River Hills (4), and the White River Hills (4; including the Missouri specimen) Ecoregions) that occupy the southwestern corner of the Ozark Highlands. The other four specimens, all in Arkansas, occur close to the Springfield Plateau but on the southern edge of the Central (or Salem) Plateau that forms the physiographic core of the Ozarks. Based on our admittedly limited sample, sexual diploids appear to be absent from the majority of the Central Plateau and also from the St. Francois Ecoregion of the Ozark Highlands. There are salient differences between the Springfield and Salem Plateaus, the former occurring at a somewhat higher elevation and overlying limestone and chert while the latter is at a somewhat lower elevation and overlies dolomite (Woods et al. 2004). It is unknown if these geologic differences or other climatic or historical events restrict sexual diploid populations to the southern part of the range surveyed. Survey of additional specimens from regional herbaria in Missouri or targeted surveying of *Erigeron strigosus* on glades and barrens in southern Missouri will be required to refine understanding of the distribution of sexual plants in the region.

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#### REFERENCES

- ADAMS, K.L. AND J.F. WENDEL. 2005. Polyploidy and genome evolution in plants. *Curr. Opin. Pl. Biol.* 8:135–141.
- ASKER S. AND L. JERLING. 1992. Apomixis in plants. CRC Press, Boca Raton, FL.
- BIERZYCHUDEK, P. 1985. Patterns in plant parthenogenesis. *Experientia* 41:1255–1264.
- BUGGS, R.J.A., P.S. SOLTIS, AND D.E. SOLTIS. 2011. Biosystematic relationships and the formation of polyploids. *Taxon* 60:324–332.
- COSENDAI A.-C., J. RODEWALD, AND E. HÖRANDL. 2011. Origin and distribution of autopolyploids via apomixis in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Taxon* 60:355–364.
- EPA. 2011. Level III Ecoregions of the Continental United States. National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency [http://www.epa.gov/wed/pages/ecoregions/level\\_iii\\_iv.htm](http://www.epa.gov/wed/pages/ecoregions/level_iii_iv.htm)
- FEHRER, J., K. KRAK, AND J. CHRTEK, JR. 2009. Intra-individual polymorphism in diploid and apomictic polyploid hawkweeds (*Hieracium*, Lactuceae, Asteraceae): disentangling phylogenetic signal, reticulation, and noise. *B.M.C. Evol. Biol.* 9:239. <http://www.biomedcentral.com/1471-2148/9/239>
- GRANT, V. 1981. Plant speciation. 2<sup>nd</sup> ed. Columbia Univ. Press, New York.
- GRUSZ, A.L., M.D. WINDHAM, AND K.M. PRYER. 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). *Amer. J. Bot.* 96:1636–1645.
- HEIKENS, A.L. 1999. Savanna, barrens, and glade communities of the Ozark Plateaus Province. In: Anderson, R.C., J.S. Fralish, J.M. Baskin, eds. Savannas, barrens, and rock outcrop communities of North America. Cambridge University Press, Cambridge, England. Pp. 220–230.
- KRUKBERG, A.R. 1954. The ecology of serpentine soils III. Plant species in relation to serpentine soils. *Ecology* 35:267–274.
- NE SOM, G. 2006. *Erigeron*. In: Flora of North America, vol. 20. Magnoliophyta: Asteridae (in part): Asteraceae, part 2. Flora of North America Editorial Committee, eds. 1993+. Flora of North America North of Mexico. 12+ vols. New York and Oxford. Pp. 256–348.
- NOYES, R.D. 2006. Intraspecific nuclear ribosomal DNA divergence and reticulation in sexual diploid *Erigeron strigosus* (Asteraceae). *Amer. J. Bot.* 93:470–479.
- NOYES, R.D. 2007. Reticulation and the evolution of apomixis in *Erigeron* sect. *Phalacrolooma* (Asteraceae) In: Hörandl, E.,

- U. Grossniklaus, P. Van Dijk, T. Sharbel, eds. Apomixis: evolution, mechanisms and perspectives. *Regnum Veg.* 147. Gantner Verlag, Ruggell, Liechtenstein. Pp. 337–358.
- NOYES, R.D. AND J.R. ALLISON. 2005. Cytology, ovule development and pollen quality in sexual *Erigeron strigosus* (Asteraceae). *Int. J. Plant Sci.* 166:49–59.
- NOYES, R.D., H. GERLING, AND C. VANDERVOORT. 2006. Sexual and apomictic prairie fleabane (*Erigeron strigosus* Muhl. ex Willd.) in Texas: Geographic analysis and a new combination (*Erigeron strigosus* var. *traversii* (Shinners) Noyes). *Sida* 22:265–276.
- NYLANDER, J.A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University. <http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html>
- OUACHITA ECOREGIONAL ASSESSMENT TEAM. 2003. Ouachita Mountains ecoregional assessment. The Nature Conservancy, Little Rock, AR and Tulsa, OK. [http://www.nature.org/ourinitiatives/regions/northamerica/unitedstates/oklahoma/explore/ouachita\\_mts-1.pdf](http://www.nature.org/ourinitiatives/regions/northamerica/unitedstates/oklahoma/explore/ouachita_mts-1.pdf)
- PEPPER, A.E. AND L.E. NORWOOD. 2001. Evolution of *Caulanthus amplexicaulis* var. *barbarae* (Brassicaceae), a rare serpentine endemic plant: a molecular phylogenetic perspective. *Amer. J. Bot.* 88:1479–1489.
- POSADA, D. AND K.A. CRANDELL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818. <http://darwin.uvigo.es/software/modeltest.html>
- RAMSEY J. AND D.W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Ann. Rev. Ecol. Syst.* 29:467–501.
- ROBISON, H., C. MCALLISTER, C. CARLTON, AND G. TUCKER. 2008. The Arkansas endemic biota: an update with additions and deletions. *J. Arkansas Acad. Sci.* 62:84–96.
- SCHRANZ, M.E., C. DOBEŠ, M.A. KOCH, AND T. MITCHELL-OLDS. 2005. Sexual reproduction, hybridization, apomixis and polyploidization in the genus *Boechea* (Brassicaceae). *Amer. J. Bot.* 92:1797–1810.
- SOLTIS, P.S. AND D.E. SOLTIS. 2000. The role of genetic and genomic attributes in the success of polyploids. *Proc. Natl. Acad. Sci. USA*, 97:7051–7057.
- STANLEY, R.G. AND H.F. LINSKENS. 1974. Pollen: biology, biochemistry, and management. Springer-Verlag, Berlin.
- STEBBINS, G.L. 1950. Variation and evolution in plants. Columbia Univ. Press, New York.
- SWOFFORD, D.L. 2002. PAUP\*: phylogenetic analysis using parsimony (\*and other methods), version 4.0. Sinauer, Sunderland, Massachusetts, USA.
- THORN, R.H. AND J.H. WILSON. 1980. The natural divisions of Missouri: an introduction to the natural history of the state. *Trans. Missouri Acad. Sci.* 14:9–23.
- USDA, 1999. Ozark-Ouachita Highlands Assessment: Terrestrial vegetation and Wildlife. General Technical Report SRS-35. <http://www.srs.fs.usda.gov/pubs/2039>
- WOODS A.J., T.L. FOTI, S.S. CHAPMAN, J.M. OMERNIK, J.A. WISE, E.O. MURRAY, W.L. PRIOR, J.B. PAGAN, Jr., J.A. COMSTOCK, AND M. RADFORD. 2004. Ecoregions of Arkansas (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,000,000). [http://www.epa.gov/wed/pages/ecoregions/ar\\_eco.htm](http://www.epa.gov/wed/pages/ecoregions/ar_eco.htm)