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INTRASPECIFIC NUCLEAR RIBOSOMAL DNA DIVERGENCE AND RETICULATION IN SEXUAL DIPLOID *ERIGERON STRIGOSUS* (ASTERACEAE)[1]

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Apomictic complexes in flowering plants often harbor multiple sexual taxa, which must be studied to interpret accurately the origin and diversity of apomictic derivatives. The daisy fleabane group in North America (*Erigeron* sect. *Phalacroloma*) includes the widespread polyploid apomictic taxa *E. annuus*, *E. strigosus*, and *E. tenuis*, as well as recently discovered sexual diploid populations in the southeastern United States (*E. strigosus* var. *calcicola*, *E. strigosus* var. *dolomiticola,* sexual *E. strigosus* var. *strigosus*). Phylogenetic analysis for 35 sexual populations was conducted using sequences from the internal and external transcribed spacers (ITS and ETS) of ribosomal DNA. Results indicate that the three groups of sexual plants form separate monophyletic clades and that edaphic specialization is ancestral in the group. Sequence analysis for sexual *E. strigosus* var. *strigosus* was confounded by the fact that 13 of 31 samples (42%) possessed nucleotide polymorphisms at 1.0–1.8% of sites. However, analysis of the genomic sequence for plants with low polymorphism levels in conjunction with analysis of cloned PCR products for plants with high levels of intra-individual polymorphism indicated that three combinations of haplotypes (N I + N II, two plants; N I + N III, five plants; N II + N III, six plants) accounted for the majority of sequence polymorphisms encountered. These data are consistent with historical diversification and subsequent reticulate evolution at the diploid level in sexual *E. strigosus* var. *strigosus*.

Key words: apomictic complex; Asteraceae; *Erigeron*; ETS; intraspecific; ITS; phylogenetics; rDNA polymorphisms; reticulation.

Apomixis in flowering plants, i.e., asexual reproduction by seed, is typically associated with the formation of a polyploid apomictic complex. The conventional evolutionary model for an apomictic complex includes a group of closely related sexual diploid ancestors and their polyploid apomictic derivatives (Grant, 1981). Polyploidy and apomixis are hypothesized to arise among hybrids between divergent sexual populations either as a form of reproductive assurance (Stebbins, 1950) or because of conflicting gene interactions (Carman, 1997). Multiple origins of polyploid apomicts, backcrossing between apomicts and sexual populations, plus occasional successful crosses between facultative apomicts, are thought to be the principal sources of the often bewildering array of diversity that is the hallmark of apomictic complexes (Asker and Jerling, 1992). Apomictic complexes are common components of temperate floras and in North America have evolved in a diversity of taxa including *Antennaria* (Bayer, 1987), *Boechera* (Schranz et al., 2005), *Bouteluoa* (Gould and Kapadia, 1964), *Crepis* (Babcock and Stebbins, 1938), *Poa* (Soreng, 1990), and *Townsendia* (Beaman, 1957).

The goals of systematic study of apomictic complexes are to circumscribe sexual taxa and characterize evolutionary relationships among them, to elucidate relationships between sexual progenitors and derivative polyploids, and to explore evolutionary processes involved in the diversification of apomicts (Grant, 1981). Sexual ancestors of apomictic complexes are often poorly known, no doubt because they are often restricted geographically and because they may be virtually indistinguishable from sympatric or parapatric apomicts with which they may hybridize. Nonetheless, study of sexual populations is key to understanding evolutionary processes in the complex. First, diversity among the progenitor diploids is likely to be a principal determinant of the ecological amplitude exhibited by the apomictic derivatives (Bayer et al., 1991). Second, characterization of phenological and developmental differences between sexual diploid populations could yield clues as to the origin of apomixis in hybrids (Carman, 1997). Third, analysis of diploid ancestors is essential for understanding the origin of genetic diversity in apomicts and permits, for instance, the ability to discriminate between diversity inherited from ancestors vs. novel variation acquired subsequent to polyploid formation. Lastly, study of sexual diploid ancestors is intrinsically important because of their contribution to biodiversity. Sexual ancestors are often restricted to specialized habitats and may therefore require special protection, compared to their widespread apomictic relatives, from anthropogenic habitat destruction. Also, because sexual and apomictic plants often occur in close proximity, gene flow via pollen from apomicts to sexual plants may lead to the spread of apomixis genes into the sexual population, compromising the integrity of the sexual population and placing the sexual population at risk of extinction (Brock, 2004).

This study involves the phylogenetic analysis of sexual diploid populations belonging to the *Erigeron* sect. *Phalacroloma* (Cass.) Torr. & A. Gray apomictic complex. This group has traditionally included only *Erigeron strigosus* Muhl. ex Willd. and *E. annuus* (L.) Pers. (Cronquist, 1947; Nesom, 1989), but recent molecular phylogenetic work (Noyes, 2000) indicates that *E. tenuis* Torr. & A. Gray is the sister taxon to *E. strigosus*, and consideration of morphology suggests that *E. geiseri* Shinners may also be closely related (G. Nesom, Botanical Research Institute of Texas, personal communica-

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tion). Both *E. annuus* and *E. strigosus*, the daisy and prairie fleabanes, respectively, are abundant in North America along roadsides, forest margins, and as old-field colonizers, occurring from the Atlantic coast to the Great Plains and sporadically in the West (USDA and NRCS, 2005). These taxa are tall, annual to weakly perennial herbs with conspicuous white to light violet ray florets and yellow disc florets. They are differentiated from other *Erigeron* taxa because their ray florets lack the conspicuous pappus bristles that are otherwise characteristic in the genus. Published chromosome counts for *E. annuus* are principally triploid ($2n = 27$), while diploid ($2n = 18$), triploid ($2n = 27$), and tetraploid ($2n = 36$) counts have been recorded for *E. strigosus*. Both taxa have been considered to be uniformly apomictic in monographs (Cronquist, 1947) and floristic treatments (e.g., Barkley, 1986; Hickman, 1993). *Erigeron tenuis* occurs principally in Texas, Louisiana, Arkansas, and Mississippi with a few additional collections from further north and east, while *E. geiseri* is known from a handful of collections from a broad north–south zone extending from southern Texas to Oklahoma. These latter two members of *E.* sect. *Phalacroloma* are poorly known, and their mode of reproduction has not been investigated.

Recent fieldwork in the southeastern United States led to the discovery of sexual diploid populations that are the likely progenitors of the polyploid apomicts in *E.* sect. *Phalacroloma* (Allison and Stevens, 2001; Noyes and Allison, 2005). The populations include *E. strigosus* var. *dolomiticola* J. Allison, restricted to a single dolomite glade complex in Bibb Co., Alabama, *E. strigosus* var. *calcicola* J. Allison, restricted to limestone glades in Tennessee, Alabama, and Georgia, and sexual populations of *E. strigosus* var. *strigosus*, which occur on the coastal plain of the southeastern United States. All sexual plants thus far investigated are diploid ($2n = 18$), have tetrasporic female gametophyte development (Noyes and Allison, 2005), and can usually be distinguished from apomictic relatives based on leaf characteristics.

The objectives of this study were to investigate the evolutionary relationship among sexual diploid populations of *Erigeron* sect. *Phalacroloma* to (1) discern the degree of divergence among the three sexual taxa and (2) explore the relationship of the two edaphic endemics to their nonspecialist relative. An additional, more long-term goal was to develop a phylogenetic framework that could be used for studying diversity and evolutionary origins of polyploid apomictic relatives. To these ends, the sequence was obtained for the internal and external transcribed spacers (ITS and ETS, respectively) of nuclear ribosomal DNA. High rates of nucleotide substitution and concerted evolution combine to make these markers highly valuable for constructing phylogenies at lower taxonomic levels (Hillis and Dixon, 1991; Baldwin, 1992; Baldwin et al., 1995; Baldwin and Markos, 1998). While the nrDNA sequence has been used to investigate intersectional relationships in *Erigeron* (Noyes, 2000), it has not been used previously in the genus to address intraspecific phylogenetic questions.

MATERIALS AND METHODS

Sampling and DNA extraction—A total of 37 ingroup and five outgroup plants were analyzed (Appendix), including three plants each of *Erigeron strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola*, and 31 plants of sexual *E. strigosus* var. *strigosus*. The outgroup taxa were selected to represent a range of closely and distantly related *Erigeron* species based on previous phylogenetic work (Noyes, 2000). Plants were grown from seed collected in the field, or, in a few cases, were collected in the field as nonflowering rosettes. All plants were maintained in the greenhouses at the University of Colorado. Total DNA was isolated for each plant using the DNeasy Plant Mini kit (Qiagen, Valencia, California, USA) from approximately 200 mg starting material and quantified using a Hoefer DyNA Quant 200 fluorometer (Amersham Biosciences, Piscataway, New Jersey, USA). All 37 *E. strigosus* plants included in this study were previously determined to be diploid ($2n = 18$) and sexual (Noyes and Allison, 2005).

rDNA amplification, sequencing, and cloning—Direct sequence was obtained for the ITS and ETS rDNA regions from genomic DNA. The ITS region (ITS1, 5.8S, and ITS2) was amplified via PCR using standard reagents, *Taq* polymerase (New England Biolabs, Beverly, Massachusetts, USA), primers ITSI (forward: GTC CAC TGA ACC TTA TCA TTT AG; Urbatsch et al., 2003) and ITS4 (reverse: TCC TCC GCT TAT TGA TAT GC; White et al., 1990), and cycling conditions of 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 1 min, with a final extension of 7 min. Forward and reverse ITS sequences from purified PCR products were obtained using primers ITS5 (forward: GGA AGG AGA AGT CGT AAC AAG G; White et al., 1990) and ITS4, and ca. one-fourth volume (12 μL) DYEnamic ET Terminator Cycle Sequencing reactions (Amersham Biosciences) consisting of 2 μL sequencing reagent premix, 0.28 μm/L primer, 45 ng template DNA, 25 mmoles/L Tris pH 9.0, and 0.63 mmoles/L $MgCl_2$. All sequencing products were resolved on a BaseStation DNA fragment analyzer (MJ Research, Waltham, Massachusetts, USA).

The ETS region was amplified from genomic DNA using primers (AST-8, forward: TTC TCT TCG TAT CGT GCG GT; 18S, reverse: ACT TAC ACA TGC ATG GCT TAA TCT) and cycling conditions (94°C denaturing, 55°C annealing, and 72°C extension for 1 min, for 39 cycles, final extension of 7 min) recommended by Markos and Baldwin (2001), and using *Taq* polymerase. ETS sequences from purified PCR products were obtained using the amplification primers. Sequence chromatograms for both ITS and ETS were analyzed using Sequencher (version 4.1.4, Gene Codes, Ann Arbor, Michigan, USA). Sequences were subsequently exported to BioEdit (version 5.0.9, http://www.mbio.ncsu.edu/BioEdit) and alignment was conducted visually. BioEdit alignments were exported as NEXUS files for phylogenetic analysis.

PCR products were cloned for all plants exhibiting a high frequency of nucleotide polymorphisms in direct sequences of genomic DNA. To minimize likelihood of error due to methodology, amplifications of ITS and ETS were re-performed, as above, except that a proofreading polymerase ($Vent_R$ DNA polymerase; New England Biolabs) was used to amplify the regions from genomic DNA. This necessitated lowering the annealing temperature for both ITS and ETS cycling programs to 47°C, reducing the concentration of polymerase to 0.5 U/50 μL PCR reaction, adding 0.6 mg/mL BSA to the reaction mixture, and increasing the amount of template DNA to 300 ng/reaction. To facilitate cloning, Vent PCR products were incubated at 72°C for 10 min with *Taq* polymerase to provide 5′ terminal thymidine overhangs. Incorporation of PCR product into plasmid vector and transformation were performed using the TOPO TA cloning kit (Invitrogen Life Technologies, Carlsbad, California, USA). Positive colonies were grown in overnight cultures and plasmids were isolated using the QIAprep Spin Miniprep kit (Qiagen). Cycle sequencing of plasmid inserts was accomplished using M13 forward and/or M13 reverse primers (as warranted) using the conditions described and ~350 ng plasmid template. A minimum of 12 clones was sequenced per individual. To facilitate analysis, BioEdit files for individual plants were generated that included the complete set of cloned sequences plus the original genomic sequence. For all analyses, determination of boundaries for the ITS and ETS regions followed Noyes and Rieseberg (1999) and Markos and Baldwin (2001), respectively.

Analyses—Preliminary parsimony analyses using PAUP* (version 4.0b.10; Swofford, 2002) of ITS and ETS data sets, separately, yielded topologies consistent with the absence of phylogenetic conflict between the two gene regions. This conclusion was supported by the results of the incongruence length difference test (ILD; Farris et al., 1994) performed in PAUP* with 1000 replicates, simple sequence addition, and tree-bisection-reconnection (TBR) branch swapping using the CHARPARTITION and HOMPART commands ($p = 0.15$). All analyses were subsequently performed on the combined ITS and ETS data. Phylogenetic analyses were conducted on data sets consisting of (1) genomic sequences exhibiting low levels of intra-individual polymorphism and (2) the former plus cloned sequences for individuals with high levels of intra-

individual polymorphism. Weighted parsimony analysis was conducted in PAUP* using empirically determined transition/transversion (ti/tv) ratios, with and without the GAPMODE = NEWSTATE option. When gaps were treated as informative, they were assigned a weight equivalent to the highest value in the nucleotide matrix. Heuristic searches included 100 random order additions, ACCTRAN, MULPARS, TBR, and STEEPEST DESCENT options. Bootstrap analyses (Felsenstein, 1985) with 100 random addition replicates were conducted for the genomic sequence data set. For the larger data set that included cloned sequences, number of trees saved per bootstrap replicate was limited to 1000.

Maximum likelihood analyses were conducted in PAUP* using evolutionary model parameters proscribed by Modeltest 3.6 (Posada and Crandall, 1998; http://www.darwin.uvigo.es). To evaluate support for trees, Bayesian analysis using MrBayes (version 3; Ronquist and Huelsenbeck, 2003; http://morphbank.ebc.uu.se/mrbayes) was conducted using Modeltest likelihood parameters. Bayesian analyses included four parallel chains run for 1 000 000 generations, with sampling every 1000 generations, and BURNIN = 1000 trees. Final consensus trees with 50% clade credibility values were produced from the remaining 9000 trees.

To interpret the origin of intra-individual polymorphism in the group, diagnostic rDNA haplotype sequences (ITS plus ETS) were determined for major clades resulting from analyses of plants lacking substantial intra-individual polymorphism. Haplotype sequences and sequence polymorphism patterns were compared to identify instances of haplotype additivity. In addition, individual cloned ITS and ETS sequences for polymorphic individuals were classified as to haplotype. Two composite (ITS plus ETS) cloned sequences (corresponding to the same haplotype) were selected to represent polymorphic plants in phylogenetic analyses.

Split decomposition was performed to evaluate potential conflict in the sequence data sets using SplitsTree (Huson, 1998; http://www-ab.informatik.uni-tuebingen.de/software/splits). Also, a global tree-based relative rates test of molecular clock (Felsenstein, 1988) was implemented in PAUP* by evaluating the difference in likelihood values between optimal trees produced with proscribed Modeltest parameters vs. those constrained by the assumption of a molecular clock (LSET CLOCK=YES). The difference between these two trees (2 ×[likelihood constrained − likelihood unconstrained]) was then evaluated against a chi-square distribution with df equal to the number of sequences. Finally, to estimate divergence times, average HKY85 genetic distances between clades (÷ 2.0) were compared to ITS nucleotide substitution rates (reported here as percentage change per million years) for *Robinsonia* (0.78%; Sang et al., 995b), *Lepidium* (0.44 and 0.88%; Mummenhoff et al., 2004), Cucurbitaceae (0.36%; Jobst et al., 1998), *Eupatorium* (1.17%; Schmidt and Schilling, 2000), and *Soldanella* (0.834%; Zhang et al., 2001). The exceptionally slow rate reported for Madiinae (0.23%; Asteraceae; B. Baldwin, University of California, Berkeley, personal communication); and the exceptionally fast rate reported for Primulaceae (3.73%, Zhang et al., 2001) were not considered in calculating divergence dates for sexual *Erigeron*.

RESULTS

Genomic sequences—The ITS region was 626 bp in length for all ingroup plants except for no. 1545, which was 625 bp long. Alignment with outgroup taxa required the insertion of one 6-bp and one 4-bp gap into ingroup sequences (final aligned ITS of 636 bp). In the final alignment, ITS1 was 254 bp, the 5.8S region was 164 bp, and ITS2 was 218 bp in length. For the ingroup, ETS was either 597 bp (for *Erigeron strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola)* or 596 bp (sexual *E. strigosus* var. *strigosus*) due to a single deletion. Alignment with outgroup taxa required the insertion of two 2-bp and one 1-bp gaps (final aligned ETS of 602 bp). The final data matrix consisted of ITS followed by ETS in a single block 1238 bp in length (available by request from the author). Reference to specific nucleotides hereafter refers to positions in this merged data matrix.

There were a total of 52 potentially informative sites (4.2%) in the data matrix, including one gap in ETS (Table 1). The ITS region (ITS1, 5.8S, ITS2) contained 18 potentially phylogenetically informative sites (2.8%); nine sites in ITS 1, one site in 5.8S, and eight sites in ITS2. Excluding the 5.8S region, there were 17 phylogenetically informative sites (3.5%). ETS contained 34 potentially informative sites (5.6%).

Plants varied in the number of intra-individual nucleotide polymorphisms observed in the direct genomic sequence of ITS and ETS (i.e., the number of multiple peaks observed at single nucleotide positions on sequence chromatograms; Table 2). Polymorphic positions were infrequent (0 to 2) in *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola*. However, for sexual *E. strigosus* var. *strigosus*, although 18 of 31 plants had few polymorphisms (0 to 7; mean = 0.2% polymorphisms/plant), 13 plants had 12 to 22 polymorphisms (mean = 1.3% polymorphisms/plant). Furthermore, of the total 44 polymorphisms observed in the 18 plants with low polymorphism levels, 21 (48%) were uninformative in that they occurred at positions that otherwise did not vary across the entire data set. In contrast, among the 13 plants with high polymorphism levels, only 19 of 204 polymorphisms were uninformative (9.3%). In addition, an intra-individual length polymorphism in ITS resulted from a 2-bp indel for no. 1323 (Table 2). This indel produced equivocal genomic sequence that was resolved upon cloning.

rDNA diversification—Phylogenetic analysis for the 24 ingroup plants with low levels of intra-individual polymorphism (three samples each for *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola* plus 18 samples for sexual *E. strigosus* var. *strigosus*; Table 2) yielded maximum parsimony and maximum likelihood phylogenies with considerable resolution (Fig. 1). Weighted parsimony analyses (A-C = A-T = C-G = G-T = 2.24, A-G = C-T = 1.0), with and without gaps treated as informative, each yielded 156 shortest trees and identical consensus trees (Fig. 1A), with consistency index values (excluding uninformative characters) of 0.82 and 0.83, respectively. Including gaps as informative in the analyses had little effect except that it increased support for the sexual *E. strigosus* var. *strigosus* clade from 76 to 92%. The Akaike information criterion implemented in ModelTest selected a general time reversible evolutionary model (Tavaré, 1986) with gamma distributed rate variation (GTR + G) with R[A-C] = 1.23, R[A-G] = 3.47, R[A-T] = 2.72, R[C-G] = 0.66, R[C-T] = 9.07, R[G-T] = 1.0. Maximum likelihood analysis in PAUP* employing GTR + G parameters yielded six trees (Fig. 1B). The Bayesian 50% compatibility tree (with NST = 6 and RATES = gamma) and the 50% majority rule consensus bootstrap tree from weighted parsimony analysis were identical in topology. Clade credibility values from Bayesian analysis were similar to or higher than corresponding bootstrap values for the same nodes (Fig. 1A).

The topology supported by phylogenetic analyses (Fig. 1) shows that *Erigeron strigosus* var. *calcicola* (CA) occurs basal to *E. strigosus* var. *dolomiticola* (DO) and sexual E. *strigosus* var. *strigosus*. In addition, sexual *E. strigosus* var. *strigosus* includes three well-supported, major clades (N I, N II, and N III). The results of split decomposition yielded a figure with strict dichotomous branching, thus providing no evidence of conflict within the data set. The phylogenetic pattern is mirrored in the HKY85 genetic divergences for ITS and ETS among clades (Table 3). There is relatively low genetic divergence within each of the five ingroup clades (CA, DO, N I, N II, N III; 0.04 to 0.33%, mean = 0.15%). In contrast, between-group divergences ranged from 0.88% (N II vs. N III) to 1.68% (DO to N II).

TABLE 1. Informative rDNA (ITS and ETS) nucleotide positions for diploid *Erigeron strigosus*. CA = *E. strigosus* var. *calcicola*; DO = *E. strigosus* var. *dolomiticola*; N I, N II, N III = rDNA haplotype groups for sexual *E. strigosus* var. *strigosus*; H I·II, H I·III, and H II·III = hybrid haplotypes for sexual *E. strigosus* var. *strigosus*. Shaded nucleotides for CA, DO, and N I, N II, and N III are clade informative. Eighteen positions shaded for N I, N II, and N III define haplotypes that show additivity in hybrid individuals.

		ITS1									5.8S	ITS2								ETS																																	
Taxon	Plant No.	28	68	78	128	138	203	208	226	229	391	433	451	458	493	522	562	566	603	656	666	668	680	683	684	736	758	832	838	839	858	863	876	938	960	973	975	995	996	1049	1071	1076	1078	1094	1096	1101	1105	1142	1144	1149	1178	1203	1223
CA	1540	C	C	G	T	T	C	A	G	C	T	C	T	C	T	C	C	G	C	T	T	T	C	C	C	G	G	C	C	A	A	T	C	A	T	T	C	T	T	A	T	C	G	C	C	G	T	C	G	G	C	C	C
	1543	C	C	G	T	T	C	A	G	C	T	C	T	C	T	C	T	G	C	T	T	T	C	C	T	G	G	C	C	A	A	T	C	A	T	T	C	T	T	A	T	C	G	C	C	G	T	C	G	T	C	C	C
	1557	C	C	G	T	T	C	A	G	C	T	C	T	C	T	T	C	G	C	T	T	T	C	C	C	G	G	C	C	A	A	T	C	A	T	T	C	T	T	A	T	C	G	C	C	G	T	C	G	G	C	C	C
DO	1545	C	C	G	T	T	C	A	G	C	T	C	T	T	C	T	T	G	C	C	T	T	C	T	C	G	G	C	C	G	A	T	C	A	C	T	C	T	C	A	T	G	G	C	T	G	C	C	G	G	C	C	C
	1546	C	C	G	T	T	C	A	G	C	T	C	T	T	C	T	T	G	C	C	T	T	C	T	C	G	G	C	C	G	A	T	C	A	C	T	C	T	C	A	T	G	G	C	Y	G	C	C	G	G	C	C	C
	1632	C	C	G	T	T	C	A	G	C	T	C	T	T	C	T	T	G	C	C	T	T	C	T	C	G	G	C	C	G	A	T	C	A	C	T	C	T	C	A	T	G	G	C	T	G	C	C	G	G	C	C	C
N I	1225	C	C	G	T	T	C	A	G	T	T	C	T	C	C	C	C	T	C	C	A	—	C	C	T	A	A	C	T	A	A	T	C	A	C	T	C	A	T	A	T	G	A	C	C	G	T	T	A	G	C	C	C
	1316	C	C	G	T	T	C	A	G	T	T	C	T	C	C	C	C	T	C	C	A	—	C	C	T	A	A	C	T	A	A	T	C	A	C	T	C	A	T	A	T	G	A	C	C	G	T	T	A	G	C	C	C
	1628	C	C	G	T	T	C	A	G	T	T	C	T	C	C	C	C	T	C	C	A	—	C	C	T	A	A	C	T	A	A	T	C	A	C	T	C	A	T	A	T	G	A	C	C	G	T	T	A	G	C	C	C
	1631	C	C	G	T	T	C	A	G	T	T	C	T	C	C	C	C	T	T	C	A	—	C	C	T	A	A	C	T	A	A	T	C	A	C	T	C	A	T	A	T	G	A	C	C	G	T	T	A	G	C	C	C
N II	1608	T	T	A	G	T	Y	W	G	C	T	C	T	C	C	C	Y	G	C	C	A	—	T	C	C	G	A	C	C	A	T	T	C	A	C	T	C	T	T	G	A	G	G	A	C	G	T	C	G	G	C	Y	T
	1609	T	T	A	K	T	Y	A	G	C	T	C	T	Y	C	C	C	G	Y	C	A	—	T	C	C	G	A	C	C	A	T	T	C	A	C	T	C	T	T	G	A	G	G	A	C	G	T	C	G	G	Y	Y	T
	1616	T	T	A	T	C	C	T	G	C	C	C	T	C	C	C	T	G	C	C	A	—	T	C	C	G	A	C	C	A	T	T	C	A	C	T	C	T	T	G	A	G	G	A	C	G	T	T	G	G	C	C	T
	1617	T	T	A	T	C	C	T	G	C	C	C	T	C	C	C	T	G	C	C	A	—	T	C	C	G	A	C	C	A	T	T	C	A	C	T	C	T	T	G	A	G	G	A	C	G	T	T	G	G	C	C	T
	1621	T	T	A	T	T	C	W	R	C	T	C	T	C	C	C	Y	G	C	C	A	—	T	C	C	G	A	C	C	A	T	T	Y	A	C	T	C	T	T	G	A	G	G	A	C	G	T	C	G	G	C	C	T
N III	1519	T	C	G	T	T	C	A	G	C	T	C	T	C	C	Y	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	G	T	C	G	G	C	C	T
	1520	T	C	G	T	T	C	A	G	C	T	Y	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	R	T	C	G	G	C	C	T
	1612	T	C	G	T	T	C	A	G	C	T	C	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	G	T	C	G	G	C	C	T
	1613	T	C	G	T	T	C	A	G	C	T	C	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	G	T	C	G	G	C	C	T
	1614	T	C	G	T	T	C	A	G	C	T	C	Y	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	R	T	G	G	C	C	G	T	C	G	G	C	C	T
	1618	T	C	G	T	T	C	A	G	C	T	T	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	R	T	C	G	G	C	C	T
	1620	T	C	G	T	T	C	A	G	C	T	C	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	T	T	T	A	T	G	G	C	C	G	T	C	G	G	C	C	T
	1626	T	C	G	T	T	C	A	G	C	T	Y	T	C	C	C	T	G	C	C	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	R	T	C	G	G	C	C	T
	1630	T	C	G	T	T	C	A	G	C	T	C	T	C	C	C	T	G	C	T	A	—	C	C	C	G	A	C	C	A	A	T	C	A	C	A	C	T	T	A	T	G	G	C	C	G	T	C	G	G	C	C	T
H I·II	1607	Y	Y	R	T	T	C	A	G	Y	T	C	T	C	C	C	C	K	C	C	A	—	Y	C	Y	R	A	Y	Y	A	W	T	C	A	C	T	C	W	T	R	W	G	R	M	C	G	T	T	R	G	C	C	Y
	1611	Y	Y	R	T	T	C	W	G	Y	T	C	T	C	C	C	Y	K	C	C	A	—	Y	C	Y	R	A	C	Y	A	W	T	C	A	C	T	C	W	T	R	W	G	R	M	C	G	T	Y	R	G	C	C	Y
H I·III	1323	Y	C	G	T	T	C	A	G	Y	T	C	T	C	C	C	Y	K	C	Y	A	—	C	C	Y	R	A	C	Y	A	A	T	C	A	C	W	C	W	T	A	T	G	R	C	C	G	T	Y	R	G	C	C	Y
	1560	Y	C	G	T	T	C	A	G	Y	T	C	T	C	C	C	Y	K	C	C	A	—	C	C	Y	R	A	C	Y	A	A	T	C	A	C	W	C	W	T	A	T	G	R	C	C	G	T	Y	R	G	C	C	Y
	1615	Y	C	G	T	T	C	A	G	Y	T	Y	T	C	C	C	Y	K	C	C	A	—	C	C	Y	R	A	C	Y	A	A	T	C	W	C	W	C	W	T	A	T	G	R	C	C	R	T	Y	R	G	C	C	Y
	1623	Y	C	G	T	T	C	A	G	Y	T	C	T	C	C	C	Y	K	C	C	A	—	C	C	Y	R	A	C	Y	A	A	Y	C	W	C	W	C	W	T	A	T	G	R	C	C	G	T	Y	R	K	C	C	Y
	1629	Y	C	G	T	T	C	A	G	Y	T	C	Y	C	C	C	Y	K	C	C	A	—	C	C	Y	R	A	C	Y	A	A	T	C	A	C	W	C	W	T	A	T	G	R	C	C	G	T	Y	R	G	C	C	Y
H II·III	1610	T	Y	R	T	T	C	W	G	C	T	C	T	C	C	C	T	G	C	C	A	—	Y	C	C	G	A	C	C	A	W	Y	C	A	C	W	Y	T	T	R	W	G	G	M	C	G	T	Y	G	G	C	C	T
	1619	T	Y	R	T	T	C	A	G	C	T	C	T	Y	C	C	Y	G	Y	C	A	—	Y	C	C	G	A	C	C	A	W	T	C	A	C	W	C	T	T	R	W	G	G	M	C	G	T	C	G	G	Y	C	T
	1622	T	Y	R	T	T	C	W	G	C	T	C	T	C	C	C	T	G	C	C	A	—	Y	C	C	G	A	C	C	A	W	Y	Y	A	C	W	C	T	T	R	W	G	G	M	C	G	T	C	G	G	C	C	T
	1624	T	Y	R	T	T	C	W	G	C	T	C	T	C	C	C	T	G	C	C	A	—	Y	C	C	G	A	C	C	A	W	T	C	A	C	W	Y	T	T	R	W	G	G	M	C	G	T	Y	G	G	C	C	T
	1625	T	Y	R	T	T	C	A	G	C	T	C	T	Y	C	C	Y	G	Y	C	A	—	Y	C	C	G	A	Y	C	A	W	T	C	A	C	W	C	T	T	R	W	G	G	M	C	G	T	C	G	G	Y	C	T
	1627	T	Y	R	T	T	C	A	R	C	T	C	T	Y	C	Y	Y	G	Y	C	A	—	Y	C	C	G	A	C	C	A	W	T	C	A	C	W	C	T	T	R	W	G	G	M	C	G	T	C	G	G	Y	C	T

Average clade-based HKY85 genetic distances for ITS1 and ITS2 (472 bp) were calculated as 0.96% for node 1 (CA: [DO, N I, N II, N III]), 1.05% for node 2 (DO: [N I, N II, N III]), 1.17% for node 3: (N I: [N II, N III]), and 0.81% for node 4 (N II: N III). Using published substitution rates for ITS, these corresponded to divergence times ($\pm$95% confidence interval) of 0.760 mya ($\pm$0.290) for node 1, 0.833 mya ($\pm$0.318) for node 2, 0.925 mya ($\pm$0.353) for node 3, and 0.646 mya ($\pm$0.247) for node 4. All divergence dates fell within the Pleistocene (1.8–1.6 to 0.01 mya). Global relative rate tests indicated clock-like molecular evolution for the 24 sexual *E. strigosus* sequences (ITS1 + ITS2, $\chi^2 = 17.0$, df = 24, $P = 0.85$; ITS + ETS, $\chi^2 = 23.8$, df = 24, $P = 0.47$).

Visual inspection of informative sites (Table 1) indicated 18 nucleotide positions (28, 68, 78, 229, 566, 680, 684, 736, 838, 858, 973, 995, 1049, 1071, 1078, 1094, 1144, and 1223) that yielded unique sequences for the three clades of sexual *Erigeron strigosus* var. *strigosus*. These sequences, which were diagnostic for three principal rDNA haplotypes, were CCG TTC TAT ATA ATA CAC for clade N I, TTA CGT CGC TTT GAG AGT for N II, and TCG CGC CGC AAT ATG CGT for N III. Note that bp 1049 was included in defining haplotypes even though one N III individual (no. 1614) was polymorphic (R instead of A) for that position. Five and 13 of the diagnostic haplotype nucleotides occurred within ITS and ETS, respectively.

Without exception, the observed polymorphism patterns at the 18 haplotype nucleotide positions in the 13 plants with high levels of intra-individual polymorphism were explained by the combinations of haplotypes N I and N II (= H I·II [two plants]), N I and N III (= H I·III [five plants]), or N II and N III (= H II·III [six plants]) (Table 1). For instance, the 17 polymorphisms in genomic sequence for plant no. 1607 was accounted for by the combination of haplotypes N I and N II. Haplotype additivity accounted for 17, 11, and 9 polymorphic positions for H I·II; H I·III; and H II·III hybrids, respectively, and 76% (143 of 188) of all potentially informative polymorphisms in the data set.

Diversity of cloned sequences—Twelve to 17 clones of ITS and ETS were sequenced for each of the 13 plants with high levels of intra-individual polymorphism (Appendices S1 and S2, see Supplemental Data with the online version of this article). A total of 170 ITS and 161 ETS clones were analyzed. Two classes of cloned sequences were recognized: (1) those that corresponded in sequence to haplotypes N I, N II, or N III (84% of all sequenced clones) or (2) putative single and double recombinants between haplotypes (16% of all cloned sequences). For all plants with high polymorphism levels except one, the two parental haplotypes postulated from the genomic sequence were observed among the cloned sequences. The exception was plant no. 1611 for which haplotype N I and putative recombinants were observed, but no cloned sequence corresponded in entirety to haplotype N II.

TABLE 2. Nucleotide polymorphisms in genomic ITS and ETS sequence for diploid *Erigeron strigosus*. CA = *Erigeron strigosus* var. *calcicola*; DO = *E. strigosus* var. *dolomiticola*; N I, N II, and N III = rDNA haplotypes for sexual *E. strigosus* var. *strigosus*; H I·II, H I·III, and H II·III = hybrid haplotypes for sexual *E. strigosus* var. *strigosus*.

Taxon	Plant no.	ITS	ITS-I[a]	ETS	ETS-I[a]	Total	Total-I[a]
CA	1540	1	0	0	0	1	0
	1543	0	0	0	0	0	0
	1557	1	0	0	0	1	0
DO	1545	0	0	1	0	1	0
	1546	0	0	1	1	1	1
	1632	1	0	0	0	1	0
N I	1225	0	0	2	0	2	0
	1316	1	0	0	0	1	0
	1628	1	0	0	0	1	0
	1631	2	0	0	0	2	0
N II	1608	3	3	1	1	4	4
	1609	4	4	3	2	7	6
	1616	2	0	0	0	2	0
	1617	1	0	0	0	1	0
	1621	5	3	2	1	7	4
N III	1519	1	1	0	0	1	1
	1520	1	1	1	1	2	2
	1612	0	0	0	0	0	0
	1613	0	0	2	0	2	0
	1614	1	1	1	1	2	2
	1618	0	0	1	1	1	1
	1620	1	0	0	0	1	0
	1626	3	1	1	1	4	2
	1630	0	0	0	0	0	0
H I·II	1607[b]	7	6	13	13	20	19
	1611[b]	8	7	14	13	22	20
H I·III	1323[b]	8[c]	4	10	10	18	14
	1560[b]	5	4	10	9	15	13
	1615[b]	6	5	13	11	19	16
	1623[b]	4	4	12	12	16	16
	1629[b]	6	5	9	9	15	14
H II·III	1610[b]	3	3	10	9	13	12
	1619[b]	6	5	7	7	13	12
	1622[b]	3	3	9	8	12	11
	1624[b]	3	3	9	8	12	11
	1625[b]	5	5	9	8	14	13
	1627[b]	8	7	7	7	15	14

[a] No. of polymorphisms that are potentially informative.
[b] Cloned. See Appendices S1 and S2 in Supplemental Data with online version of this article.
[c] Includes a 2-bp indel polymorphism.

Phylogenetic analyses including cloned sequences—The 13 plants with high levels of intra-individual polymorphism were represented in phylogenetic analysis by two sequences, each a composite of ITS plus ETS sequences corresponding to the same haplotype (Appendices S1, S2, see Supplemental Data with the online version of this article). For no. 1611, for which a clone representing only one haplotype for ITS was recovered, the second sequence representing a complete, hypothetical, second haplotype was determined from genomic sequence. For each plant, selected clones were complementary at all polymorphic positions and thus account for all the variation observed in the genomic sequence.

Phylogenetic analysis included 55 sequences—five outgroup, 24 for plants with low intra-individual polymorphism, and 26 cloned sequences representing the 13 plants with high intra-individual polymorphism. Weighted parsimony analysis (A-C = A-T = C-G = G-T = 2.34, A-G = C-T = 1.0), with and without gaps treated as informative, resulted in 34.5 k shortest trees in both cases and strict consensus trees with identical topologies (Fig. 2A) and consistency index values (excluding uninformative characters) of 0.78 and 0.79, respectively. Bootstrap scores for the two analyses were similar, although support for the node supporting the monophyly of sexual *Erigeron strigosus* var. *strigosus* increased from 77 to 94% with gaps treated as informative. The Akaike information criterion in ModelTest selected a GTR + G evolutionary model with R[A-C] = 1.21, R[A-G] = 3.12, R[A-T] = 2.30, R[C-G] = 0.61, R[C-T] = 8.74, R[G-T] = 1.0. Maximum likelihood analysis in PAUP* employing these evolutionary parameters yielded 25 trees (Fig. 2B). The Bayesian 50% compatibility tree (with NST = 6, RATES = gamma) was identical in topology to the maximum parsimony strict consensus tree except for the presence of one additional weakly supported grouping in clade N II consisting of four plants, with clade credibility value of 69%, and one additional association in clade N III consisting of two plants, with clade credibility value of 79% (cladogram not shown). Clade credibility values were similar to bootstrap values for all branches (Fig. 2A). Comparison of bootstrap and credibility values between the reduced and more inclusive analyses (Fig. 1A vs. Fig. 2A) indicated little change in support for the CA, DO, N I, N II, and N III clades. In other words, the addition of the cloned sequences did not substantially reduce phylogenetic resolution.

Global relative rate test supported the hypothesis of molecular clock-like divergence for the data set that included cloned sequences ($\chi^2 = 37.4$, df = 50, $P = 0.91$). In addition, split decomposition analysis yielded a tree with strict branching, thus providing no evidence of data conflict. To test the sensitivity of split decomposition, an analysis was conducted that included one intentionally chimeric molecule comprising ITS for haplotype N I and ETS for haplotype N II

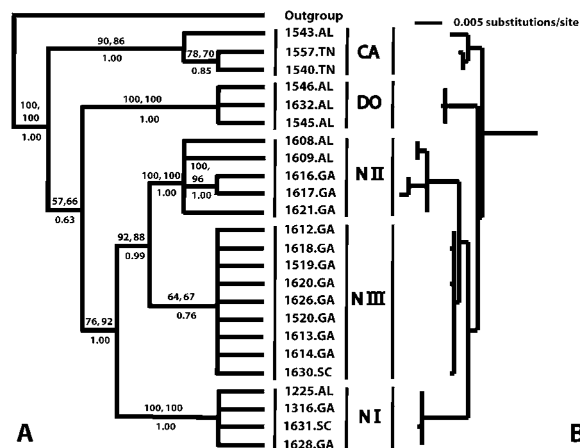

Fig. 1. rDNA phylogeny based on ITS plus ETS sequence for 24 sexual diploid *Erigeron strigosus* with low levels of intra-individual polymorphism. (A) Strict consensus tree for 156 weighted parsimony trees (ti/tv = 2.24; GAPS = MISSING or NEWSTATE). Maximum likelihood consensus is identical in topology except for one weakly supported branch uniting nos. 1608 and 1609. Numbers above branches are bootstrap support from weighted parsimony analyses, with gaps missing or informative, respectively; numbers below branches are clade credibility values from Bayesian analysis. (B) One of five trees resulting from maximum likelihood analysis under a GTR + G model of evolution. CA = *E. strigosus* var. *calcicola*; DO = *E. strigosus* var. *dolomiticola*; N I, N II, N III = separate clades of sexual *E. strigosus* var. *strigosus*.

TABLE 3. Genetic distance (HKY85) based on ITS and ETS sequences within and between clades (Fig. 1) of sexual diploid *Erigeron strigosus*. CA = *E. strigosus* var. *calcicola*, DO = *E. strigosus* var. *dolomiticola*, N I, N II, N III = haplotype groups for sexual *E. strigosus* var. *strigosus*.

	Genetic distance				
Clade	CA	DO	N I	N II	N III
CA	0.33%	—	—	—	—
DO	1.05%	0.05%	—	—	—
N I	1.37%	1.60%	0.04%	—	—
N II	1.60%	1.68%	1.66%	0.27%	—
N III	0.94%	1.01%	1.12%	0.88%	0.05%

haplotype for plant no. 1611. The resultant reticulating network indicated that split decomposition analysis would be sensitive to even single recombinant molecules within the data set.

To represent both diversification of rDNA haplotypes (N I, N II, and N III) and recombination of haplotypes in putative hybrids (H I·II, H I·III, and H II·III), a phylogeny reflecting reticulation was drafted (Fig. 3). There is little apparent geographic pattern in the distribution of rDNA haplotypes of sexual *Erigeron strigosus* var. *strigosus* (Fig. 4). Although there may be a weak concentration of N III (in non-hybrids and hybrids) in southeast Georgia, in most instances throughout the region, divergent rDNA haplotypes occur in close geographic proximity.

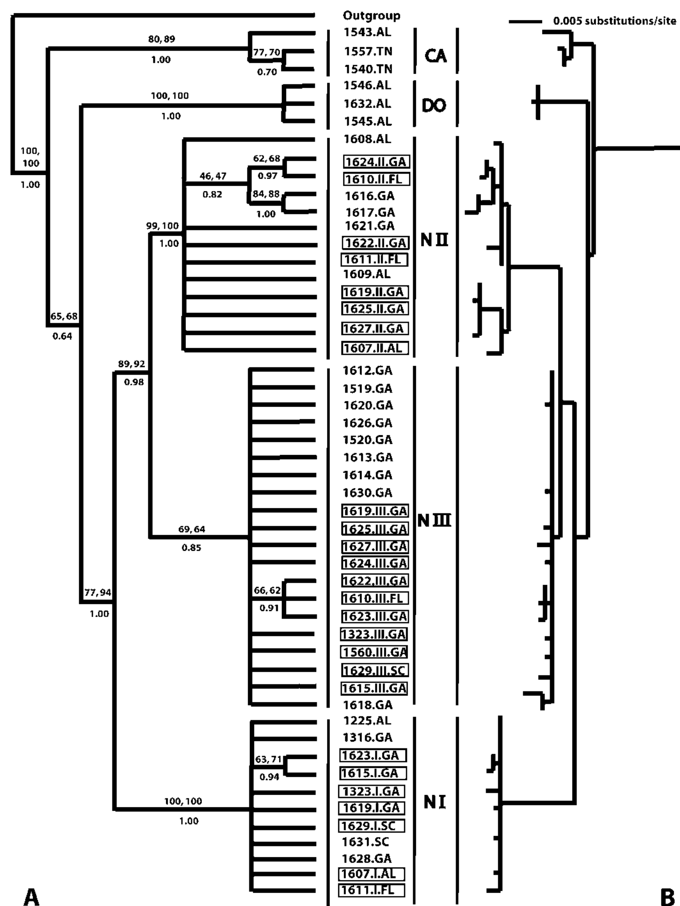

Fig. 2. rDNA phylogeny based on ITS plus ETS sequence for sexual diploid *Erigeron strigosus* that includes 24 plants with low intra-individual polymorphism (as in Fig. 1) plus two cloned sequences for each of 13 plants (boxed) with high levels of intra-individual polymorphism. (A) Strict consensus of 34.5 k shortest weighted parsimony trees (ti/tv = 2.34; GAPS = MISSING or NEWSTATE). Numbers above branches are bootstrap support for analyses with gaps missing or informative, respectively; numbers below branches are clade credibility values from Bayesian analysis. (B) One of 25 trees resulting from maximum likelihood analysis under a GTR + G model of evolution. CA = *E. strigosus* var. *calcicola*; DO = *E. strigosus* var. *dolomiticola*; N I, N II, N III = separate clades of sexual *E. strigosus* var. *strigosus*.

DISCUSSION

rDNA evidence and hybridization—Although rDNA has been widely used to construct phylogenies among closely related taxa, its reliability for documenting hybridization has been questioned (Rauscher et al., 2002; Álvarez and Wendel, 2003; Small et al., 2004). This is because in an individual that combines divergent rDNA sequences through hybridization, concerted evolution or unequal crossing-over may act quickly to diminish or eliminate copies of one of the parental repeat types (Wendel et al., 1995; Fuertes Aguilar et al., 1999; Volkov et al., 1999; Rauscher et al., 2002). Because of these phenomena, hybrid origins may be obscured. A related problem is that while intra-individual polymorphic rDNA genomic sequences are often obtained in broad phylogenetic studies (e.g., Baker et al., 2000; Denduangboripant and Cronk, 2000; Wichman et al., 2002; Andreasan and Baldwin, 2003; Lindqvist et al., 2003), hybrid origins cannot be assumed. This is because repeat diversity within an individual may also arise due to the evolution of nonfunctional nrDNA repeats (pseudogenes) or to the intragenomic evolution of functional, but divergent, paralogous gene copies (Buckler et al., 1997; Bailey et al., 2003).

In this study, intra-individual rDNA polymorphisms were frequent. However, the inference that the origin of the polymorphisms was primarily due to hybrid recombination of divergent rDNA types rather than alternative molecular mechanisms is robust, because in each case the polymorphisms could be traced to isolated rDNA haplotypes found in other individuals. This level of resolution was only possible, however, with extensive intraspecific sampling in sexual *Erigeron strigosus* var. *strigosus* in conjunction with analysis of multiple cloned PCR products for each polymorphic individual. In addition, while the majority of intra-individual rDNA diversity was explained by the additivity of haplotypes through recombination, additional mechanisms and hypotheses must be invoked to explain the diversity of rDNA sequences among clones, including recombinants, obtained for single individuals (Appendices S1, S2).

Other examples in which nrDNA additivity has successfully been used to document hybridization at the diploid level in vascular plants include *Armeria* (Fuertes Aguilar and Nieto Feliner, 2003; Nieto Feliner et al., 2004), *Bursera* (Weeks and Simpson, 2004), *Clausia* (Franzke et al., 2004), *Coprosma* (Wichman et al., 2002), *Helianthus* (Rieseberg, 1991), *Hippophae* (Sun et al., 2003), *Paeonia* (Sang et al., 1995a), and *Pinus* (Quijada et al., 1997). As in sexual *Erigeron strigosus*, the recovery of parental sequences in hybrids in these examples was possible either because the hybridization event occurred recently (i.e., not enough time had passed for concerted

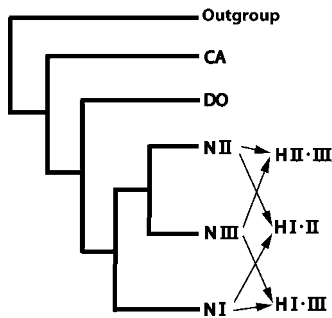

Fig. 3. Inferred phylogeny for sexual diploid *Erigeron strigosus* showing diversification of rDNA haplotypes NI, NII, and NIII and their subsequent recombination in hybrids H I·II, H I·III, and H II·III.

evolution to act), hybridization was recurrent, or perhaps the two parental rDNA loci occupied distinct chromosomal positions, thus preventing loss through segregation alone and/or reducing the action of concerted evolution.

Evolutionary inference for sexual Erigeron strigosus—The rDNA phylogeny for sexual diploid *Erigeron strigosus* (Figs. 2, 3) provides insight into the evolution of sexual populations of *Erigeron* sect. *Phalacroloma*. First, *E. strigosus* var. *calcicola* occurs basal to *E. strigosus* var. *dolomiticola* and sexual *E. strigosus* var. *strigosus*. This is consistent with the hypothesis that the glade endemics, which are perennial through the production of stolons or overwintering rosettes, are ancestral to the typical annual or weakly perennial forms of *E. strigosus*, including sexual *E. strigosus* var. *strigosus* (Allison and Stevens, 2001). Glades in the southeastern United States are harsh and variable environments, prone particularly to extreme heat and desiccation (Baskin and Baskin, 1999). The perennial habit may foster survival and reproduction during prolonged water stress. This phylogenetic pattern indicates that the nonspecialized habit of sexual *E. strigosus* var. *strigosus* is likely derived from an ancestor that was an edaphic specialist. This is counter to the conventional model that posits that edaphic plant specialists usually evolve from nonspecialist progenitors (MacNair and Gardner, 1998; Rajakaruna, 2004). The sister group of *E.* sect *Phalacroloma* is a diverse group of *Erigeron* species that occurs in the southwestern United States and northern Mexico (Noyes, 2000). This group is poorly known, however, and thus it is not presently possible to evaluate whether the perennial habit and edaphic specialization of sexual *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola* was inherited from distant ancestors or evolved more recently in the group.

Quaternary origins of intraspecific rDNA diversity—The impact of Quaternary climatic oscillations on plants with temperate, continental distributions has long been debated (Stebbins, 1950; Willis and Niklas, 2004). However, a number of recent phylogeographic studies have yielded data that are consistent with the hypothesis that many plant species may have fractured into isolated, independently evolving populations that then came back into contact during interglacial periods (Comes and Kadereit, 1998). The principal effects of global climate change may thus have been to increase genetic variance within plant species. This view is supported by studies based on cpDNA diversity (Soltis et al., 1997; Ferris et al., 1998) and a recent set

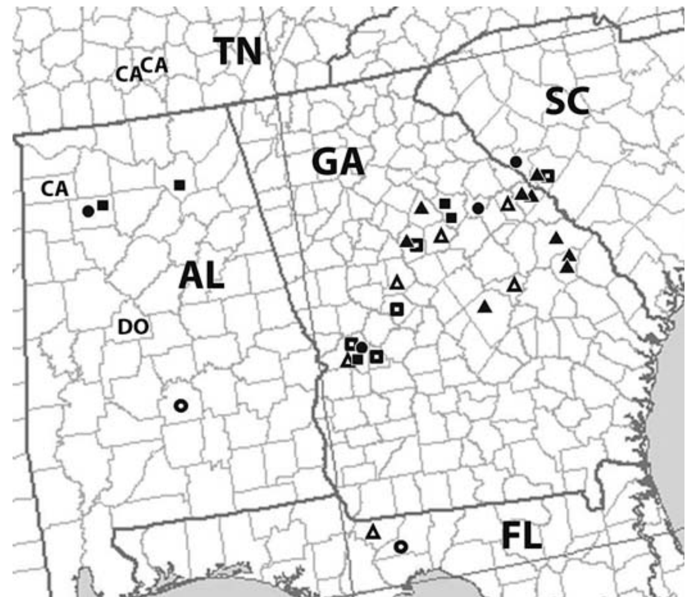

Fig. 4. Geographic distribution of rDNA haplotypes in diploid sexual plants of *Erigeron strigosus*. CA = *E. strigosus* var. *calcicola*; DO = *E. strigosus* var. *dolomiticola*; symbols represent sexual *E. strigosus* var. *strigosus* haplotypes: ● = N I; ■ = N II; ▲ = N III; ○ = H I·II; □ = H I·III; △ = H II·III. DO includes three plants sampled in close proximity.

of studies using rDNA. For instance, in *Clausia* (Brassicaceae) a diploid perennial herb of the Eurasian steppe (Franzke et al., 2004), divergence in rDNA (ITS) sequence between eastern and western populations is consistent with contraction of populations ca. 1 mya, followed by range expansion and subsequent recontact of populations. In this and other studies, it thus appears that population subdivision during recent climactic events contributed to genetic diversity, but not speciation.

The genetic distances among and within the five clades of sexual *E. strigosus* (Table 3) are also consistent with divergences in the middle Pleistocene. These dates are highly speculative due to the high standard errors of the estimates and the absence of a fossil record for *Erigeron* that would provide more local calibration. Nonetheless, the entire range of dates for each node lies well within the Pleistocene, and thus the divergences appear to correlate with Quaternary climactic fluctuations. In theory, periodic isolation of populations fostered divergence of rDNA haplotypes in geographic isolation, followed by their admixture, at least among populations of sexual *E. strigosus* var. *strigosus*, subsequent to range expansion.

The lack of geographic pattern in the distribution of haplotypes N I, N II, and N III (Fig. 4) is consistent with extensive gene flow among populations and Mendelian segregation of haplotypes. While reticulate evolutionary patterns have been documented for apomictic polyploids in apomictic complexes (e.g., Campbell et al., 1997), the data for *Erigeron strigosus* indicate that reticulate evolutionary processes may also be prominent at the diploid level among sexual populations. Curiously, the data indicate that *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola* have not participated in reticulation (Fig. 3). The lack of evidence for reticulation involving these localized taxa might be explained by (1) pre- or postzygotic reproductive isolation, (2) limited

sampling of sexual plants in the vicinity of glades, (3) the geographic isolation of edaphic specialists, or (4) selection against hybrids between edaphic specialists and nonspecialists. An additional variable that is conspicuously absent in this evolutionary model is the possible influence of apomictic polyploid *E. annuus* and *E. strigosus* in facilitating gene flow among populations.

Recent study indicates that sexual diploid populations of *Erigeron strigosus* var. *strigosus*, in addition to the region sampled for this study, also occur in the Pineywoods of East Texas and adjacent Louisiana, and in the Ozarks of Arkansas and adjacent Oklahoma (Noyes, 2006). Study of these populations may well reveal additional rDNA diversity or genetic structure among sexual diploid populations. Apomicts in *E.* sect. *Phalacroloma*, particularly *E. annuus* and *E. strigosus*, are widespread and morphologically diverse. Their origin in relation to sexual diploid populations is yet unknown.

LITERATURE CITED

ALLISON, J. R., AND T. E. STEVENS. 2001. Vascular flora of Ketona Dolomite outcrops in Bibb County, Alabama. *Castanea* 66: 154–205.

ÁLVAREZ, I., AND J. F. WENDEL. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

ANDREASEN, K., AND B. G. BALDWIN. 2003. Nuclear ribosomal DNA sequence polymorphism and hybridization in checker mallows (*Sidalcea*, Malvaceae). *Molecular Phylogenetics and Evolution* 29: 563–581.

ASKER, S. E., AND L. JERLING. 1992. Apomixis in plants. CRC Press, Boca Raton, Florida, USA.

BABCOCK, E. B., AND G. L. STEBBINS. 1938. The American species of *Crepis*. Their interrelationships and distribution as affected by polyploidy and apomixis. Carnegie Institution of Washington Publication No. 504, Washington, D.C., USA.

BAILEY, C. D., T. G. CARR, S. A. HARRIS, AND C. E. HUGHES. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435–455.

BAKER, W. J., T. A. HEDDERSON, AND J. DRANSFIELD. 2000. Molecular phylogenetics of the subfamily *Calamoideae* (Palmae) based on nrDNA ITS and cpDNA *rps*16 intron sequence data. *Molecular Phylogenetics and Evolution* 14: 195–217.

BALDWIN, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.

BALDWIN, B. G., AND S. MARKOS. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S-26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* 10: 449–463.

BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.

BARKLEY, T. M. [ed.]. 1986. Flora of the Great Plains. University Press of Kansas, Lawrence, Kansas, USA.

BASKIN, J. M., AND C. C. BASKIN. 1999. Cedar glades of the southeastern United States. *In* R. C. Anderson, J. S. Fralish, and J. M. Baskin [eds.], Savannas, barrens, and rock outcrop plant communities of North America, 206–219. Cambridge University Press, Cambridge, UK.

BAYER, R. 1987. Evolution and phylogenetic relationships of the *Antennaria* (Asteraceae: Inuleae) polyploid agamic complexes. *Biologisches Zentralblatt, Leipzig* 106: 683–698.

BAYER, R. J., B. G. PURDY, AND D. G. LEBEDYK. 1991. Niche differentiation among eight sexual species of *Antennaria* Gaertner (Asteraceae: Inuleae) and *A. rosea*, their allopolyploid derivative. *Evolutionary Trends in Plants* 5: 109–123.

BEAMAN, J. 1957. The systematics and evolution of *Townsendia* (Compositae). *Contributions of the Gray Herbarium* 183: 1–151.

BROCK, M. T. 2004. The potential for genetic assimilation of a native dandelion species, *Taraxacum ceratophorum* (Asteraceae), by the exotic congener *T. officinale*. *American Journal of Botany* 91: 656–663.

BUCKLER, E. S. I., A. IPPOLITO, AND T. P. HOLTSFORD. 1997. The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

CAMPBELL, C. S., M. F. WOJCIECHOWSKI, B. G. BALDWIN, L. A. ALICE, AND M. J. DONOGHUE. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81–90.

CARMAN, J. 1997. Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony (review). *Biological Journal of the Linnean Society* 61: 51–94.

COMES, H. P., AND J. W. KADEREIT. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3: 432–438.

CRONQUIST, A. 1947. Revision of the North American species of *Erigeron*, north of Mexico. *Brittonia* 6: 121–302.

DENDUANGBORIPANT, J., AND Q. C. B. CRONK. 2000. High intraindividual variation in internal transcribed spacer sequences in *Aeschynanthus* (Gesneriaceae): implications for phylogenetics. *Proceedings of the Royal Society of London, B, Biological Sciences* 267: 1407–1415.

FARRIS, J. S., M. KÄLLERSJÖ, A. G. KLUGE, AND C. BULT. 1994. Testing significance of congruence. *Cladistics* 10: 315–319.

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

FELSENSTEIN, J. 1988. Phylogenies from molecular sequences: inference and reliability. *Annual Review of Genetics* 22: 521–565.

FERRIS, C., R. A. KING, R. VAINOLA, AND G. M. HEWITT. 1998. Chloroplast DNA recognizes three refugial sources of European oaks and suggests independent eastern and western immigrations to Finland. *Heredity* 5: 584–593.

FRANZKE, A., H. HURKA, D. JANSSEN, B. NEUFFER, N. FRIESEN, M. MARKOV, AND K. MUMMENHOFF. 2004. Molecular signals for Late Tertiary/Early Quaternary range splits of an Eurasian steppe plant *Clausia aprica* (Brassicaceae). *Molecular Ecology* 13: 2789–2795.

FUERTES AGUILAR, J., AND G. NIETO FELINER. 2003. Additive polymorphisms and reticulation in an ITS phylogeny of thrifts (*Armeria*, Plumbaginaceae). *Molecular Phylogenetics and Evolution* 28: 430–447.

FUERTES AGUILAR, J., J. A. ROSSELLO, AND G. NIETO FELINER. 1999. Nuclear ribosomal DNA (nrDNA) concerted evolution in natural and artificial hybrids of *Armeria* (Plumbaginaceae). *Molecular Ecology* 8: 1341–1346.

GOULD, F. W., AND Z. J. KAPADIA. 1964. Biosystematic studies in the *Bouteloua curtipendula* complex. II. Taxonomy. *Brittonia* 16: 182–207.

GRANT, V. 1981. Plant speciation, 2nd ed. Columbia University Press, New York, New York, USA.

HICKMAN, J. C. [ed.]. 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley, California, USA.

HILLIS, D. M., AND M. T. DIXON. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology* 66: 411–453.

HUSON, D. H. 1998. SplitsTree: a program for analyzing and visualizing evolutionary data. Bioinformatics 14, 68–73.

JOBST, J., K. KING, AND V. HEMBLEN. 1998. Molecular evolution of the internal transcribed spacers (ITS1 and ITS2) and phylogenetic relationships among species of the family Cucurbitaceae. *Molecular Phylogenetics and Evolution* 9: 204–219.

LINDQVIST, C., T. J. MOTLEY, J. J. JEFFREY, AND V. A. ALBERT. 2003. Cladogenesis and reticulation in the Hawaiian endemic mints (Lamiaceae). *Cladistics* 19: 480–495.

MACNAIR, M. R., AND M. GARDNER. 1998. The evolution of edaphic endemics. *In* D. J. Howard and S. H. Berlocher [eds.], Endless forms:

species and speciation, 157–171. Oxford University Press, New York, New York, USA.

MARKOS, S., AND B. G. BALDWIN. 2001. Higher-level relationships and major lineages of *Lessingia* (Compositae, Astereae) based on nuclear rDNA internal and external transcribed spacer (ITS and ETS) sequences. *Systematic Botany* 26: 168–183.

MUMMENHOFF, K., P. LINDER, N. FRIESEN, J. L. BOWMAN, J.-Y. LEE, AND A. FRANZKE. 2004. Molecular evidence for bicontinental hybridogenous genomic constitution in *Lepidium* sensu stricto (Brassicaceae) species from Australia and New Zealand. *American Journal of Botany* 91: 254–261.

NESOM, G. L. 1989. Infrageneric taxonomy of New World *Erigeron* (Compositae: Astereae). *Phytologia* 67: 67–93.

NIETO FELINER, G., B. GUTIERREZ LARENA, AND J. FUERTES AGUILAR. 2004. Fine-scale geographical structure, intra-individual polymorphism and recombination in nuclear ribosomal internal transcribed spacers in *Armeria* (Plumbaginaceae). *Annals of Botany* 93: 189–200.

NOYES, R. D. 2000. Biogeographical and evolutionary insights on *Erigeron* and allies (Asteraceae) from ITS sequence data. *Plant Systematics and Evolution* 220: 93–114.

NOYES, R. D., 2006. Reticulation and the evolution of apomixis in *Erigeron* sect. *Phalacroloma (Asteraceae). In* E. Hörandl, U. Grossniklaus, P. Van Dijk, and T. Sharbel [eds.], Apomixis: evolution, mechanisms and perspectives. *Regnum Vegetabile* 147: in press.

NOYES, R. D., AND L. H. RIESEBERG. 1999. ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* s.l. *American Journal of Botany* 86: 398–412.

NOYES, R. D., AND J. R. ALLISON. 2005. Cytology, ovule development and pollen quality in sexual *Erigeron strigosus* (Asteraceae). *International Journal of Plant Sciences* 166: 49–59.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

QUIJADA, A., A. LISTON, W. ROBINSON, AND E. ALVAREZ-BUYLLA. 1997. The ribosomal ITS region as a marker to detect hybridization in pines. *Molecular Ecology* 6: 995–996.

RAJAKARUNA, N. 2004. The edaphic factor in the origin of plant species. *International Geology Review* 46: 471–478.

RAUSCHER, J. T., J. J. DOYLE, AND A. H. D. BROWN. 2002. Internal transcribed spacer repeat-specific primers and the analysis of hybridization in the *Glycine tomentella* (Leguminosae) polyploid complex. *Molecular Ecology* 11: 2691–2702.

RIESEBERG, L. H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218–1237.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SANG, T., D. J. CRAWFORD, AND T. F. STUESSY. 1995a. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: implications for biogeography and evolution. *Proceedings of the National Academy of Sciences, USA* 92: 6813–6817.

SANG, T., D. J. CRAWFORD, T. F. STUESSY, AND O. SILVA M. 1995b. ITS sequences and phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55–64.

SCHMIDT, G. J., AND E. E. SHILLING. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. *American Journal of Botany* 87: 716–726.

SCHRANZ, M. E., C. DOBEŠ, M. A. KOCH, AND T. MITCHELL-OLDS. 2005. Sexual reproduction, hybridization, apomixis, and polyploidization in the genus *Boechera* (Brassicaceae). *American Journal of Botany* 92: 1797–1810.

SMALL, R. L., R. C. CRONN, AND J. F. WENDEL. 2004. Use of nuclear genes for phylogeny reconstruction in plants. *Australian Systematic Botany* 17: 145–170.

SOLTIS, D. E., M. A. GITZENDANNER, D. D. STRENGE, AND P. S. SOLTIS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.

SORENG, R. 1990. Chloroplast DNA phylogenetics and biogeography in a reticulating group: study in *Poa* (Poaceae). *American Journal of Botany* 77: 1383–1400.

STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.

SUN, K., R. J. MA, X. L. CHEN, C. B. LI, AND S. GE. 2003. Hybrid origin of the diploid species *Hippophae goniocarpa* evidenced by the internal transcribed spacers (ITS) of nuclear rDNA. *Belgian Journal of Botany* 136: 91–96.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer, Sunderland, Massachusetts, USA.

TAVARÉ, S. 1986. Some probabilistic and statistical problems in the analysis of DNA sequences. *In* R. M. Miura [ed.], Some mathematical questions in biology: DNA sequence analysis, 57–86. American Mathematical Society, Providence, Rhode Island, USA.

URBATSCH, L., R. P. ROBERTS, AND V. KARAMAN. 2003. Phylogenetic evaluation of *Xylothamia*, *Gundlachia*, and related genera (Asteraceae: Astereae) based on ETS and ITS nrDNA sequence data. *American Journal of Botany* 90: 634–649.

USDA AND NRCS. 2005. The PLANTS database, version 3.5. Data compiled from various sources by Mark W. Skinner. National Plant Data Center, Baton Rouge, Louisiana, USA. Available at website, http://plants.usda.gov [accessed 15 April 2005].

VOLKOV, R., N. BORISJUK, I. PANCHUK, D. SCHWEIZER, AND V. HEMLEBEN. 1999. Elimination and rearrangement of parental rDNA in the allotetraploid *Nicotiana tabacum*. *Molecular Biology and Evolution* 16: 311–320.

WEEKS, A., AND B. B. SIMPSON. 2004. Molecular genetic evidence for interspecific hybridization among endemic Hispaniolan *Bursera* (Burseraceae). *American Journal of Botany* 91: 976–984.

WENDEL, J. F., A. SCHNABEL, AND T. SEELANAN. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences, USA* 92: 280–284.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: a guide to methods and application, 315–322. Academic Press, New York, New York, USA.

WICHMAN, S. R., S. D. WRIGHT, E. K. CAMERON, D. J. KEELING, AND R. C. GARDNER. 2002. Elevated genetic heterogeneity and Pleistocene climatic instability: inferences from nrDNA in New Zealand *Coprosma* (Rubiaceae). *Journal of Biogeography* 29: 943–954.

WILLIS, K. J., AND K. J. NIKLAS. 2004. The role of Quaternary environmental change in plant macroevolution: the exception or the rule? *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 359: 159–172.

ZHANG, L.-B., H. P. COMES, AND J. W. KADEREIT. 2001. Phylogeny and quaternary history of the European montane/alpine endemic *Soldanella* (Primulaceae) based on ITS and AFLP variation. *American Journal of Botany* 88: 2331–2345.

APPENDIX. Voucher information for taxa in this study, including EMBL database accession numbers for ITS and ETS genomic sequence for outgroup taxa and sexual diploid *Erigeron strigosus*.

Taxon—Locality[a]; Voucher (*R.D. Noyes* no.); EMBL no. (ITS, ETS).

Outgroup

Erigeron acris L.; France: Meurthe et Moselle[b]; *1191*; AF118496, AY871356.

E. divergens Torr. and A. Gray—NM: Eddy Co.; *1253*; AY871317, AY871359.

E. hyssopifolius Michx.—VT: Orange Co.; *1473*; AY871316, AY871355.

E. pinnatisectus (A. Gray) A. Nels.; WY: Albany Co.; *1146*; AF118501, AY871357.

E. speciosus (Lindl.) DC.—NM[c]; *1188*; AF118479, AY871358.

Ingroup

E. strigosus* var. *calcicola J. Allison—**1.** AL: Franklin Co.; *1543*; AY871318, AY871360. **2.** TN: Bedford Co.; *1540*; AY871320, AY871362. **3.** TN: Marshall Co.; *1557*; AY871319, AY871361.

E. strigosus* var. *dolomiticola J. Allison—1. AL: Bibb Co.; *1545*; AY871323, AY871365. **2.** AL: Bibb Co.; *1546*; AY871321, AY871363. **3.** AL: Bibb Co.; *1632*[d]; AY871322, AY871364.

E. strigosus* var. *strigosus—**1.** AL: Lowndes Co.; *1607*; AY871342, AY871384. **2.** AL: Marshall Co.; *1608*; AY871328, AY871370. **3.** AL: Winston Co.; *1609*; AY871331, AY871373. **4.** AL: Winston Co.; *1225*; AY871324, AY871366. **5.** FL: Gadsen Co.; *1610*; AY871354, AY871396. **6.** FL: Leon Co.; *1611*; AY871343, AY871385. **7.** GA: Burke Co.; *1612*; AY871333, AY871375. **8.** GA: Columbia Co.; *1613*; AY871339, AY871381. **9.** GA: Columbia Co.; *1614*; AY871340, AY871382. **10.** GA: Crawford Co.; *1615*; AY871346, AY871388. **11.** GA: Greene Co.; *1616*; AY871329, AY871371. **12.** GA: Greene Co.; *1617*; AY871330, AY871372. **13.** GA: Jasper Co.; *1560*; AY871347, AY871389. **14.** GA: Jasper Co.; *1520*; AY871338, AY871380. **15.** GA: Jenkins Co.; *1618*; AY871334, AY871376. **16.** GA: Jenkins Co.; *1519*; AY871335, AY871377. **17.** GA: Johnson Co.; *1619*; AY871348, AY871391. **18.** GA: Laurens Co.; *1620*; AY871336, AY871378. **19.** GA: Marion Co.; *1316*; AY871325, AY871367. **20.** GA: Marion Co.; *1621*; AY871332, AY871374. **21.** GA: Marion Co.; *1622*; AY871352, AY871393. **22.** GA: Marion Co.; *1623*; AY871344, AY871386. **23.** GA: McDuffie Co.; *1624*; AY871353, AY871395. **24.** GA: Monroe Co.; *1625*; AY871350, AY871392. **25.** GA: Morgan Co.; *1626*; AY871337, AY871379. **26.** GA: Putnam Co.; *1627*; AY871351, AY871394. **27.** GA: Schley Co.; *1323*; AY871345, AY871387. **28.** GA: Taliaferro Co.; *1628*; AY871327, AY871369. **29.** SC: Edgefield Co.; *1629*; AY871348, AY871390. **30.** SC: Edgefield Co.; *1630*; AY871341, AY871383. **31.** SC: McCormick Co.; *1631*; AY871326, AY871368.

[a] USA, two-letter state abbreviation: county, unless noted otherwise. AL = Alabama, FL = Florida, GA = Georgia, NM = New Mexico, SC = South Caroline, TN = Tennessee, WY = Wyoming.

[b] Seed ex Conservatoire et Jardins Botaniques de Nancy, France; #97.

[c] Seed ex Plants of the Southwest, Sante Fe, NM, USA.

[d] Same site as RN 1545; not reported in Noyes and Allison (2005).