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CYTOLOGY, OVULE DEVELOPMENT, AND POLLEN QUALITY IN SEXUAL *ERIGERON STRIGOSUS* (ASTERACEAE)

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Erigeron strigosus is widespread in North America and has heretofore been considered to be uniformly apomictic. The recent description of new varieties of *E. strigosus* in Alabama, Georgia, and Tennessee on dolomitic and calcareous glades (*E. strigosus* var. *dolomiticola* and *E. strigosus* var. *calcicola*) and the discovery of a third, yet unnamed taxon on the coastal plain of the southeastern United States (*E. strigosus* var. nov.) prompted this investigation of their cytology and reproductive attributes. Here, we report that all three varieties are diploid (2n=18) and sexual, with tetrasporic female gametophyte development. In addition, B-chromosomes were observed in three instances in *E. strigosus* var. nov. On average, pollen of sexual *E. strigosus* is uniformly small (average 14.9 μm diameter) and of high quality (84.2% viability), although low pollen viability was observed in eight of 36 plants, and exceptionally large, putatively unreduced grains were observed in low frequency in two plants.

Keywords: apomictic complex, Asteraceae, B-chromosomes, chromosome numbers, *Erigeron*, glade, tetraspory.

Introduction

Apomixis, i.e., asexual reproduction through seed, is common in flowering plants, occurring in ca. 40 different plant families (Asker and Jerling 1992). Apomictic plants typically reside within apomictic complexes that include distinct, putatively ancestral sexual taxa (usually diploid) and a plethora of derived polyploid apomictic clones (Grant 1981). Morphologically distinct assemblages of the latter are often referred to as microspecies, apospecies, agamospecies, or formae apomictae. Evolutionary models proposed to explain the origin of apomixis usually invoke either (1) hybridization between sexual ancestors as the initial cause of the apomixis (Ernst 1918; Stebbins 1950; Carman 1997) or (2) genetic mutation within a single lineage that then spreads via hybridization among related species (Mogie 1992; Savidan 2000). In either case, subsequent crosses between facultative apomictic clones and backcrosses with sexual populations produce a myriad of distinct clonal genotypes. From a systematic viewpoint, apomictic complexes are problematic because (1) it is difficult to resolve biologically meaningful taxonomic entities among the apomictic clones (Dickinson 1998; Stace 1998), and (2) the apomictic clones within a complex may be so diverse and geographically widespread that they obscure the presence and distribution of sexual populations that typically have more restricted distributions (Grant 1981).

Biosystematic analysis of apomictic complexes invariably involves the discrimination of sexual and apomictic populations and the formal recognition of sexual taxa as a key first step toward understanding the evolution of the group (Grant 1981). Some of the best-studied apomictic complexes, for example *Crepis* L. (Babcock and Stebbins 1938), *Antennaria* Gaertn. (Bayer 1987), *Taraxacum* Wigg. (Kirschner and Štěpánek 1996), and *Rubus* L. (Gustafsson 1943; Weber 1996), were elucidated using this approach. In addition to potentially bringing to light new sexual taxa, knowledge of the geographic distributions of plants with different breeding systems provides insight into geographic and historical factors influencing sexual versus asexual reproduction in natural populations (Bierzychudek 1987).

Here we report the occurrence of sexual populations of *Erigeron strigosus* Muhl. ex Willd. (Asteraceae) in the southeastern United States. *Erigeron strigosus* and *Erigeron annuus* (L.) Pers. are traditionally classified as *Erigeron* L. sect. *Phalacroloma* (Cass.) Torr. and A. Gray. (Cronquist 1947; Nesom 1989). These taxa are readily distinguished from other *Erigeron* species because of their annual habit and because they have ray florets that lack conspicuous pappus bristles. Recent molecular phylogenetic data (Noyes 2000*a*) place *Erigeron tenuis* Torr. and A. Gray (which has pappose ray achenes) as the sister to *E. strigosus*, and morphological considerations suggest that *Erigeron geiseri* Shinners also has evolutionary affinities to the group. The upcoming *Flora of North America* taxonomic treatment of Asteraceae (G. Nesom, personal communication) will thus include four species (*E. annuus*, *E. geiseri*, *E. strigosus*, and *E. tenuis*) in *E.* sect. *Phalacroloma*.

Both *E. annuus* and *E. strigosus* are widespread in the United States, present in all states from the Great Plains to the Atlantic coast, but occurring only sporadically in the Rocky Mountains and the West (USDA plants database,

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http://plants.usda.gov, accessed June 16, 2004). *Erigeron annuus* is an old field and roadside colonizer, while *E. strigosus* tends to occur in drier habitats on forest margins. Examination of the serial *Index to Plant Chromosome Numbers* 1955–2000 (Cave 1958*a*, 1958*b*, 1959*a*, 1959*b*, 1960, 1961, 1962, 1963, 1964, 1965; Ornduff 1967, 1968; Moore 1973, 1974, 1977; Goldblatt 1981, 1984, 1985, 1988; Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000, 2003) and Federov (1969) shows that *E. annuus* is principally triploid (2n=27), although other counts (2n=26, 36, 54) have also been reported. *Erigeron strigosus* is also principally 2n=27 but includes 2n=18, 26, 28, 36, 54, and 72 cytotypes.

Apomixis has been described for *E. annuus* and *E. strigosus* (Tahara 1915; Holmgren 1919; Bergman 1944; Noyes 2000*b*), and both species have been considered to be uniformly apomictic in systematic (Cronquist 1947; Barkley 1986; Hickman 1993) and ecological (Hancock and Wilson 1976; Stratton 1991) works. Apomixis in *Erigeron* is diplosporous and is accomplished via a mitotic or mitotic-like division of the megaspore mother cell (MMC) that subsequently yields an unreduced female gametophyte (embryo sac). Embryos result from the parthenogenetic development of the unreduced egg cells.

Apomicts are nearly always polyploid (Savidan 2000); thus, when the first author began an investigation of the group in 1994, he was surprised to discover that nine diploid counts (2n=18) had been published for *E. strigosus* (table 1). Most of these occur in the southeastern United States, but one observation is reported from Nebraska. Note that Federov (1969) cites Land (1900) as reporting 2n=16 for *E. strigosus*. The Federov listing is erroneous, however, as Land (1900) clearly attributes 2n=16 to a species of *Silphium* L. and not *E. strigosus*. Following Nesom (1978), a plant was collected in disturbed forest in northern Alabama and used as a seed parent in a genetic mapping study of apomixis (Noyes 2000*b*; Noyes and Rieseberg 2000). Investigation showed that this plant was diploid (2n=18) and sexual, with tetrasporic gametophyte development and outcrossing. Tetraspory is a relatively rare developmental pattern in which all four nuclear products of meiosis divide mitotically to form a single genetically heterogeneous haploid female gametophyte. This is opposed to the common *Polygonum* pattern, in which the female gametophyte develops mitotically from only one meiotic product. Field observations by the first author indicate that plants similar in morphology to the sexual diploid Alabama plant occur on the coastal plain of Alabama, Florida, Georgia, and South Carolina. These plants differ from other *E. strigosus* in the production of spathulate rosette leaves that bear small, alternate, mucronate lobes along the petiole margin. In addition, the type specimen of *E. strigosus*, consequently typifying *E. strigosus* var. *strigosus*, was collected from Pennsylvania (Cronquist 1947). Although a thorough systematic revision of *E.* sect. *Phalacroloma* is needed, it would appear that because only polyploid apomictic plants are known north of Virginia, *E. strigosus* var. *strigosus* is likely an apomictic taxon. Consequently, the sexual plants discovered on the coastal plain of the southeastern United States are considered to be a new taxon and herein are referred to as *E. strigosus* var. nov.

Table 1

Published Diploid (2n=18) Chromosome Counts for *Erigeron strigosus*

State/county (U.S.)	Reference
Alabama:	
Marshall	Nesom 1978
Tuscaloosa	Nesom 1978
Winston	Nesom 1978; Noyes 2000*b*
Florida:	
Gadsden	Turner and Flyr 1966
Liberty	Nesom 1978
Mississippi:	
Harrison	Nesom 1978
Nebraska:	
Nehama	Semple and Chmielewski 1987
Tennessee:	
Morgan	Semple et al. 1989
Virginia:	
Smyth	Keil et al. 1988

Note. A count of 2n=16 for *Erigeron strigosus* attributed to Land (1900) in Federov (1969) is erroneous (see text).

In addition to *E. strigosus* var. nov., two new varieties of *E. strigosus* were recently discovered on distinctive habitats in the southeastern United States (Allison and Stevens 2001). These include *E. strigosus* var. *dolomiticola* J. Allison, which is restricted to a single dolomite glade complex in Bibb County, Alabama, and *E. strigosus* var. *calcicola* J. Allison, which occurs on calcareous glades in central Tennessee, northern Alabama, and northwestern Georgia. These two taxa are quite distinct from all other populations of *E. strigosus* in distribution, habit, and vegetative morphology. *Erigeron strigosus* var. *dolomiticola* has narrowly linear, very weakly dentate leaves in the rosette and on the flowering stem. *Erigeron strigosus* var. *calcicola*, on the other hand, has broader rosette and basal stem leaves with pronounced regular marginal lobes. Both glade taxa, in addition, are perennial, the former producing short rhizomes and the latter overwintering rosettes. Mode of reproduction for these glade taxa was unknown at the time of their publication.

The objective of this study was to evaluate mode of reproduction in the two glade taxa (*E. strigosus* var. *dolomiticola*, *E. strigosus* var. *calcicola*) and to further document mode of reproduction in *E. strigosus* var. nov. for different populations in the southeastern United States. Chromosome numbers, ovule development, and pollen quality were assessed for these three taxa and analyzed for patterns of variation. If apomictic, these taxa would represent mere extremes along a more or less continuous spectrum of morphological diversity in apomictic *E. strigosus*. However, if sexual, they would represent generally acceptable taxonomic units and would be valuable as distinct components of biological diversity. Elucidation of sexual populations also provides clues as to the origin and evolution of apomixis in *E.* sect. *Phalacroloma*.

Material and Methods

A total of 36 populations of *Erigeron strigosus* were studied (table 2; fig. 1). Two to seven live plants of *E. strigosus*

Table 2

Locality Data, Voucher Information, Chromosome Number, Mode of Development, Pollen Diameter, and Pollen Fertility for 36 Populations of *Erigeron strigosus*

Taxon/state/ county (U.S.)	Voucher number (R. D. Noyes no.)	Voucher distribution	Chromosome number (2n)	Pollen diameter (μm)[a]	Pollen fertility (%)
Erigeron strigosus var. *dolomiticola*:					
Alabama:					
Bibb	1546	COLO	18	15.1 (0.68)	88
	1545	COLO	18	14.1 (0.89)	81
Mean				14.6 (0.79)	84.5
Erigeron strigosus var. *calcicola*:					
Alabama:					
Franklin	1543	COLO	18	14.9 (0.68)	99
Tennessee:					
Bedford	1540	COLO, TENN, BRIT, US	18	15.0 (0.73)	76
Marshall	1557	COLO, TENN, BRIT	18	14.3 (0.76)	86
Mean				14.7 (0.72)	87.0
Erigeron strigosus var. nov.:					
Alabama:					
Lowndes[b]	1607	COLO, UNA	18	15.1 (0.47)	95
Marshall[b]	1608	COLO, UNA	18	15.1 (0.85)	81
Winston[b]	1609	COLO, UNA	18	14.7 (0.93)	58
	1225	COLO	18	15.6 (0.69)	65
Florida:					
Gadsden	1610	COLO, FLAS	18	15.2 (0.64)	68
Leon[b]	1611	COLO, FLAS	18	15.1 (0.68)	95
Georgia:					
Burke	1612	COLO, GA	18	15.1 (0.65)	60
Columbia	1613	COLO, GA	18	14.2 (0.52)	96
Columbia	1614	COLO, GA	18	15.8 (0.51)	97
Crawford	1615	COLO, GA	18	14.4 (0.61)	94
Greene	1616	COLO, GA	18	15.5 (0.72)	84
	1617	COLO, GA	18+4B	15.8 (0.68)	90
Jasper[b]	1560	COLO, GA	18	14.2 (0.56)	83
	1520	COLO, GA	18	15.2 (0.72)	89
Jenkins	1618	COLO	18	15.2 (0.81)	91
.	1519	COLO	18	14.7 (0.73)	94
Johnson	1619	COLO	18	14.2 (0.56)	98
Laurens	1620	COLO	18	14.7 (0.45)	93
Marion	1316	COLO, GA, BRIT	18	14.6 (0.73)	91
	1621	COLO	18	14.0 (0.60)	97
	1622	COLO, GA	18	14.7 (0.55)	94
	1623	COLO	18	14.5 (0.59)	95
McDuffie	1624	COLO, GA, BRIT, US	18+3B	15.6 (0.56)	94
Monroe[b]	1625	COLO, GA	18+7B	15.7 (0.81)	17
Morgan	1626	COLO, GA, BRIT	18	15.0 (0.63)	90
Putnam	1627	COLO, GA	18	16.3 (1.40)[c,d]	94
Schley	1323	COLO, GA, BRIT, US	18	14.2 (0.54)	88
Taliaferro	1628	COLO, GA	18	14.0 (0.51)	85
South Carolina:					
Edgefield	1629	COLO, USCH	18	15.3 (0.60)	66
	1630	COLO, USCH	18	15.9 (0.75)	76
McCormick	1631	COLO, USCH	18	14.9 (0.54)[d]	84
Mean				15.0 (0.66)	83.9
Grand mean				14.9 (0.68)	84.2

Note. Standard acronyms of herbaria are listed according to online Index Herbariorum, 8th ed., New York Botanical Garden, http://sciweb.nybg.org/science2/indexherbariorum.asp. Female gametogenesis is tetrasporic in all cases.

[a] Mean diameter, with standard deviation in parentheses.

[b] Occurs in population including both diploid sexual and apomictic polyploid plants.

[c] Pollen diameters not normally distributed.

[d] Plant produces large unreduced or partially unreduced pollen grains.

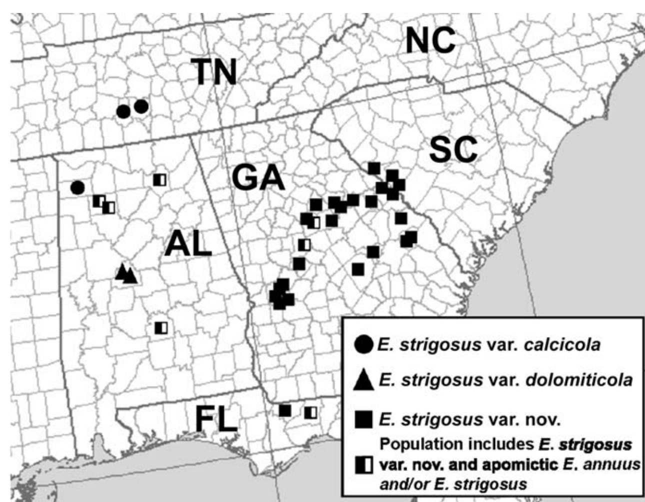

Fig. 1 Location of populations of *Erigeron strigosus* in the southeastern United States from which plants were sampled for analysis. Locality information, collection numbers, and voucher information are provided in table 2.

var. *dolomiticola* (from two populations) and *E. strigosus* var. *calcicola* (from three populations) were collected and maintained in greenhouse culture for study. Seeds from individuals representing 31 populations of *E. strigosus* var. nov. were collected from the southeastern United States (table 2) and stored at 4°C for a minimum of 2 mo. A small number of seeds (usually six) for each collection were germinated and grown to rosette stage. From these siblings, a single individual was arbitrarily selected for further analysis. All plants bolted after ca. 6 mo under standard greenhouse conditions. Seven of the populations from which plants of *E. strigosus* var. nov. were collected also included plants with pollen and morphology characteristic of polyploid apomictic *E. strigosus* or *Erigeron annuus* (table 2; fig. 1). Chromosome numbers and reproductive attributes of these plants will be presented elsewhere. Field survey of pollen and visual inspection of morphology indicated that the remaining 24 populations of *E. strigosus* var. nov. likely contained only sexual plants.

Root tips for each individual were collected, pretreated in 0.002 mol/L 8-hydroxyquinoline for 4 h at 14°C, fixed in 3 : 1 ethanol acetic acid, and stored in 70% ethanol at 4°C. Tips were digested in 15% HCl for ca. 25 min, stained in acetocarmine, and squashed under a coverslip, and five to eight well-separated spreads per sample were examined at ×1000 using an Olympus BX51 light microscope and standard bright-field optics. Optimal contrast of chromosomes against cellular debris was achieved when root tips were fixed for ca. 2 wk before being transferred to ethanol for storage.

To evaluate ovule development, young capitula were fixed in FAA, stored in 70% ethanol, and cleared in methyl salicylate following serial dehydration in ethanol, as previously described (Noyes 2000*b*). For each plant, cleared heads were shattered in a watch glass, and 50 to 75 ovaries were mounted in methyl salicylate on a slide under a 20 × 20 mm coverslip. Ovules were viewed at ×600 using differential interference contrast (DIC) optics. Ovules of meiotic and diplosporous origin are easily distinguished in the early stages of female gametogenesis. Tetrasporous ovules exhibit a linear tetrad of nuclei following meiosis but before vacuolization and subsequent mitotic divisions, while diplosporous ovules yield just two nuclei before further development. Subsequently, meiotic ovules produce a 2+2+2+2 nuclear arrangement, in contrast to diplosporous ovules, which yield a 2+2 nuclear stage. Using these early discriminatory stages, ovules for each sample were scored for mode of development. In addition, disk florets were also examined from representative individuals of the three varieties—*E. strigosus* var. *dolomiticola*, RDN 1546; *E. strigosus* var. *calcicola*, RDN 1543; and *E. strigosus* var. nov., RDN 1618—to evaluate the size and structure of mature female gametophytes. Capitula at anthesis were fixed for 48 h in 4% acrolein in 50 mmol/L PIPES buffer (pH 6.8 with 5 mmol/L EGTA and 1 mmol/L $MgSO_4$), stored in PIPES buffer, serially dehydrated in ethanol, and cleared in methyl salicylate as above. Forty to 60 cleared ovules from newly opened florets were inspected using DIC. Ovule images were obtained and manipulated using AnalySIS (version 3.1) image-capturing software (Soft Imaging System, GmbH 1989–2001) and Adobe Photoshop (version 6.0.1, Adobe Systems).

Fresh pollen from newly emerged anthers for all 36 plants was stained with cotton blue in lactophenol (Stanley and Linskens 1974) to evaluate pollen size and fertility. This stain was used because it permits simultaneous assessment of size and viability, and because it works equally well on herbarium specimens and fresh pollen, observations from an ongoing survey of herbarium specimens will be comparable to results reported here. To estimate percentage viability as an estimate of fertility, 300 grains were scored "viable" if they were uniformly and darkly stained blue or "nonviable" if they were clear or contained stained but shrunken cytoplasm within the grain wall. Care was taken to score regions of slides that by visual inspection showed no evidence of bias from differential migration of nonviable and viable grains. To evaluate variation in size among putatively viable pollen grains, images of 110–150 staining grains were captured at ×400 with bright-field optics, and grain diameter was measured using AnalySIS (version 3.1) image-capturing software. Although many grains were circular, some grains appeared slightly oval in outline. For these, diameter was measured at an intermediate point as an approximate average of extreme longitudinal and latitudinal distances. Diameter of the pollen grains was measured from the middle of the thin intine surrounding the pollen cytoplasm. This point was selected to avoid measurement bias from the irregular echinate pollen exterior. Tests (not shown) indicated that pollen size did not change over time in stain but that compression of grains as stain volume reduced under the coverslip could cause grains to appear up to 20% larger over time. Thus, to ensure equivalent and consistent estimates, abundant stain was added to samples 1 d before measurements. Normal distribution of pollen grain size was estimated using the Schafer-Wilk *W*-test (Royston 1992) with the Analyze-It plug-in program (version 1.60.0.1, Analyse-It Software, Leeds, U.K.) for Excel (version 10.0.4, Microsoft). Differences in grain size among the three varieties of *E. strigosus* were evaluated using ANOVA. To test if

drying (as would occur during the preparation of an herbarium specimen) had an appreciable effect on pollen size estimates, pollen was collected from samples of three specimens (RDN 1612, 1615, and 1629) that had been dried for ca. 2 mo at room temperature. Fifty grains were measured and compared with measurements made from fresh pollen for the same three plants. Differences between dried and fresh pollen samples were examined using a Student's *t*-test.

Results

Both plants of *Erigeron strigosus* var. *dolomiticola*, all three of *E. strigosus* var. *calcicola*, and 28 of 31 plants of *E. strigosus* var. nov. were diploid, with 2n=18 (table 2; fig. 2*A*–2*C*). At metaphase, chromosomes of all plants averaged between 2 and 3 μm and were metacentric to submetacentric. In addition, three plants, all *E. strigosus* var. nov., contained an A-complement identical in cytotype to other plants but also included small chromosomal fragments interpreted to be supernumerary (B-) chromosomes (RDN 1624, 2n=18+3B, fig. 2*D*; RDN 1617, 2n=18+4B, fig. 2*E*; RDN 1625, 2n=18+7B, fig. 2*F*). The B-chromosomes were ca. 1/5–1/3 the size of the A-chromosomes, measured ca. 0.5–1 μm in length, and clearly included a median heterochromatic centromere.

The ovaries of *E. strigosus* are small enough (ca. 0.3 mm in length) that an entire sample for a plant could be viewed

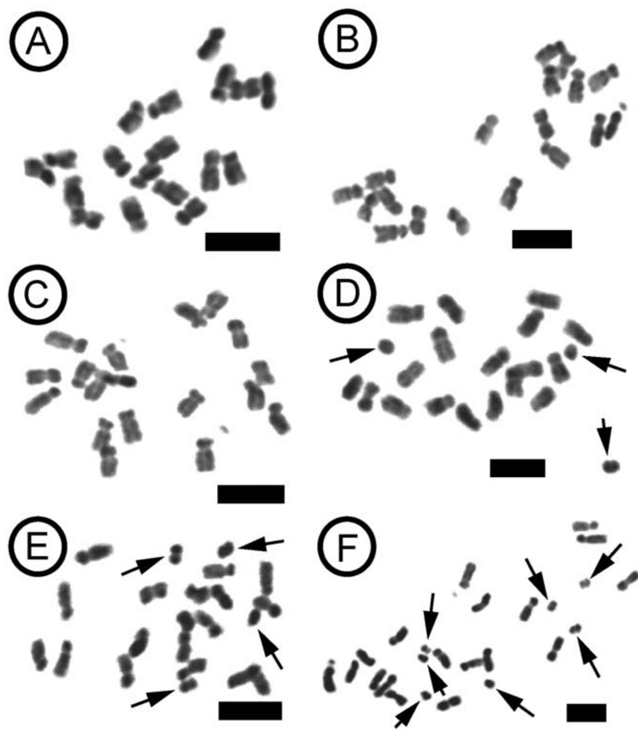

Fig. 2 Chromosomes of *Erigeron strigosus*. *A*, *E. strigosus* var. *dolomiticola*, RDN 1545, 2n=18. *B*, *E. strigosus* var. *calcicola*, RDN 1543, 2n=18. *C–F*, *E. strigosus* var. nov. *C*, RDN 1609, 2n=18. *D*, RDN 1624, 2n=18+3B. *E*, RDN 1617, 2n=18+4B. *F*, RDN 1625, 2n=18+7B. Arrows indicate B-chromosomes. Locality and voucher information provided in table 2. Bar = 5 μm.

on a single standard 75×25 mm microscope slide (fig. 3*A*–3*C*). All 36 plants examined exhibited exclusively (100%) tetrasporic female gametophyte development and no evidence of diplospory. Further, there were no conspicuous differences in development between the three varieties. Thus, the description that follows applies to all 36 plants examined for early (diagnostic) female gametogenesis and for the three plants selected to examine mature female gametophytes in the three varieties. Development is exemplified by *E. strigosus* var. *dolomiticola* (fig. 3*D*–3*I*). Ovaries contain a solitary anatropous ovule bearing a large central MMC (fig. 3*D*, 3*E*). Meiotic division of the MMC leads to four nuclear products arranged in a linear tetrad (fig. 3*F*), and as the female gametophyte expands, cytoplasm between the nuclei becomes vacuolated (fig. 3*G*). First mitotic division occurs more or less synchronously, yielding a 2+2+2+2 nuclear arrangement (fig. 3*H*). At this stage, the female gametophyte is weakly pandurate in outline, with one pair of nuclei occupying the broad micropylar end, a second pair of nuclei occurring at a weak constriction at the midpoint of the gametophyte, and two pairs of nuclei, noticeably smaller, within the chalazal terminus of the female gametophyte. Both micropylar nuclei undergo a second mitotic division to yield four nuclei. Three of the nuclei differentiate into an egg apparatus that consists of two wedge-shaped synergids at the micropylar terminus and a dome-shaped egg cell (fig. 3*I*) usually observed nestled between the synergids. The fourth micropylar nucleus (polar nucleus 1) occurs in the vicinity of the egg apparatus but within the nascent central cell. The second pair of nuclei (at the constriction of the young 2+2+2+2 gametophyte) also undergoes a second mitotic division to yield four nuclei, one of which (polar nucleus 2) becomes enveloped within the central cell, while the remaining three eventually differentiate into antipodals. Polar nucleus 2 appears to migrate toward polar nucleus 1 within the central cell as the flower approaches anthesis, and in a few instances the two polar nuclei were observed to be fusing. Mature female gametophytes (at anthesis) usually contained a single large fusion nucleus within the central cell proximal to the egg cell (fig. 3*I*). For *E. strigosus* var. *calcicola* (RDN 1543) and *E. strigosus* var. *dolomiticola* (RDN 1546), 45 of 45 and 53 of 53 ovules contained a fusion nucleus at anthesis, respectively. For *E. strigosus* var. nov. (RDN 1618), 47 of 48 female gametophytes contained a fusion nucleus, while one gametophyte included two distinct polar nuclei. The two chalazal pairs of nuclei in the young 2+2+2+2 female gametophyte appeared to undergo a subsequent mitotic division to yield additional antipodals. Division was inconsistent, however, and mature gametophytes contained seven to 10 antipodals within the tapered chalazal end of the mature gametophyte (fig. 3*I*). A ca. 14-fold expansion occurs during female gametogenesis, from an MMC 16 μm in length to a mature female gametophyte (including antipodals) of 225 μm. Young, intermediate, and mature stages for *E. strigosus* var. *calcicola* (fig. 3*J*–3*L*) and *E. strigosus* var. nov. (fig. 3*M*–3*P*) document the similarity between the three varieties in ovule development.

Mean pollen diameter across sexual plants ranged from 14.0 to 16.3 μm (average = 14.9 μm; table 2; fig. 4*A*, 4*B*). Grain diameters were similar across varieties, with average values of 14.6, 14.7, and 15.0 μm for *E. strigosus* var.

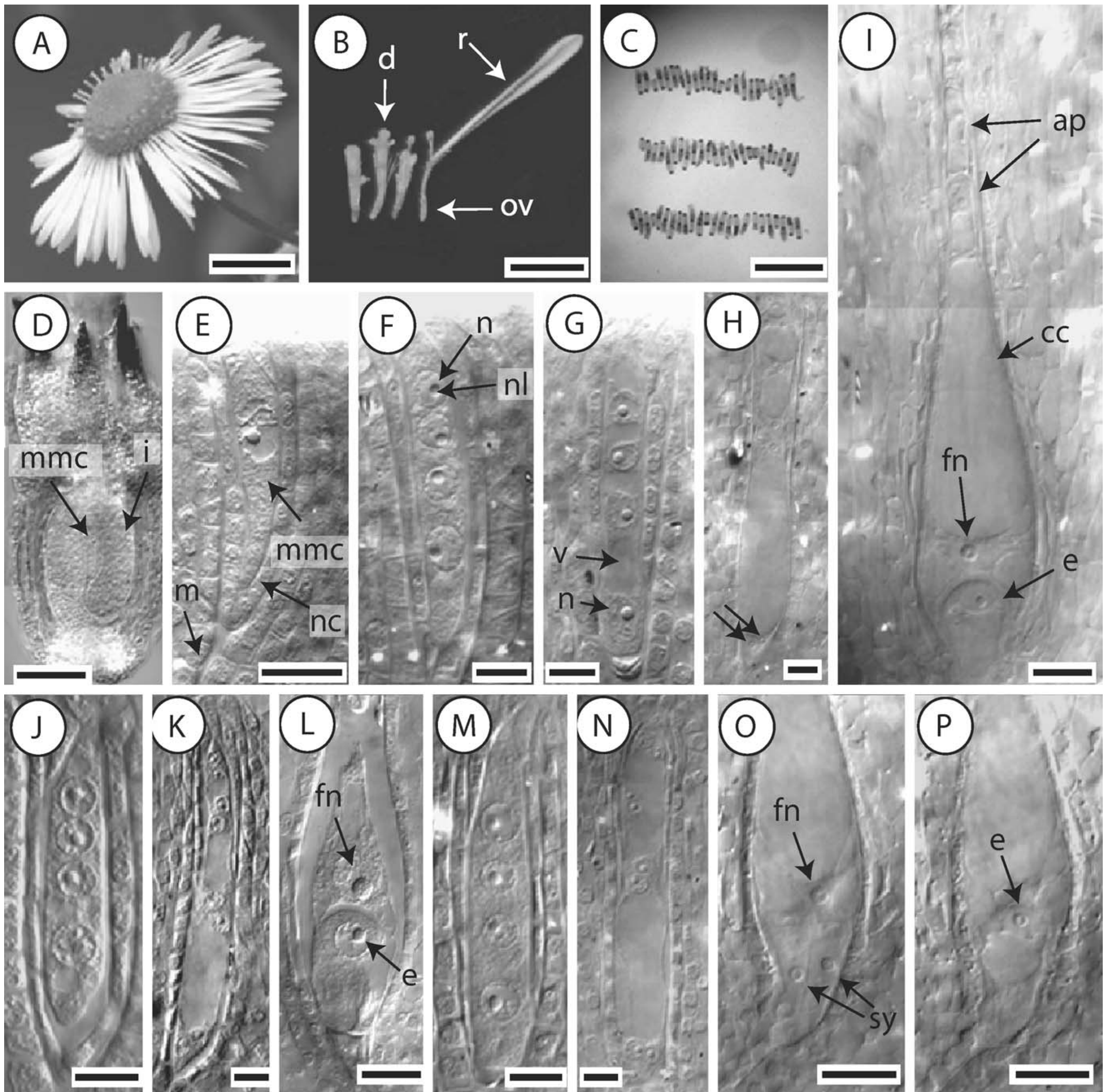

Fig. 3 Female gametogenesis for *Erigeron strigosus*. *A*, Mature capitulum of *E. strigosus* var. nov. Bar = 5 mm. *B*, Florets of *E. strigosus* var. nov. Bar = 2 mm. *C*, Cleared ovaries (71 count) mounted on standard 75 × 25 mm microscope slide. Bar = 2 mm. *D–I*, *E. strigosus* var. *dolomiticola*, RDN 1546. *D*, Whole ovary, showing single anatropous ovule. Bar = 100 μm. *E*, Details of young ovule. Bar = 20 μm. *F*, Linear tetrad resulting from meiosis of the megaspore mother cell (MMC). Bar = 10 μm. *G*, Elongation of tetrad. Bar = 10 μm. *H*, Eight nuclei in 2+2+2+2 arrangement resulting from first mitotic division of tetrad. Micropylar pair of nuclei indicated by arrows. Bar = 10 μm. *I*, Mature female gametophyte, synergids not in view. Bar = 20 μm. *J–L*, *E. strigosus* var. *calcicola*, RDN 1543. *J*, Linear tetrad. Bar = 10 μm. *K*, Eight-nucleate stage, showing 2+2+2+2 arrangement and vacuoles. Bar = 10 μm. *L*, Mature female gametophyte, synergids not in view. Bar = 20 μm. *M–P*, *E. strigosus* var. nov., RDN 1618. *M*, Linear tetrad. Bar = 10 μm. *N*, Eight-nucleate stage, showing 2+2+2+2 arrangement and vacuoles. Bar = 10 μm. *O*, Mature female gametophyte. Bar = 20 μm. *P*, Different view of the gametophyte in *O*. Bar = 20 μm. Locality and voucher information provided in table 2. *ap* = antipodals; *cc* = central cell; *d* = disk floret; *e* = egg cell; *fn* = fusion nucleus; *i* = integument; *m* = micropyle; *mmc* = megaspore mother cell; *n* = nucleus; *nc* = nucellus; *nl* = nucleolus; *ov* = ovary (inferior); *r* = ray floret; *sy* = synergids; *v* = vacuole.

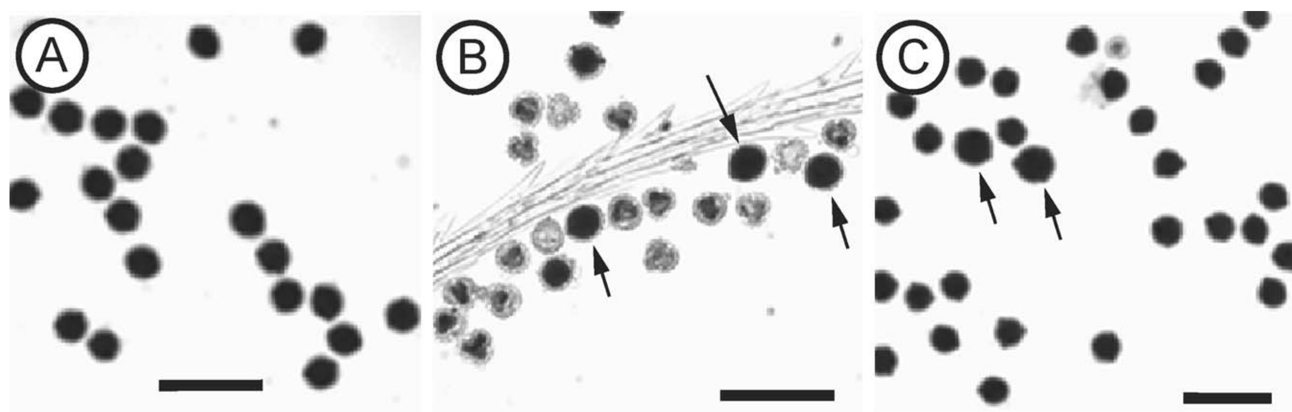

Fig. 4 Pollen samples of *Erigeron strigosus* var. nov. *A*, High-quality pollen (97% estimated viability) for RDN 1614, 2n=18. *B*, Low-quality pollen (17% estimated viability) for RDN 1625, 2n=18+7B. Arrows indicate three putatively viable grains among many nonviable grains. C, Large (presumably unreduced; indicated by arrows) and typical-sized (presumably reduced) pollen grains for RDN 1627, 2n=18. Grains stained in cotton blue in lactophenol. Darkly staining grains putatively viable; poorly staining grains putatively nonviable. Bar = 50 μm.

dolomiticola, *E. strigosus* var. *calcicola*, and *E. strigosus* var. nov., respectively. ANOVA of plant mean values indicated that the varieties are not significantly distinct ($P = 0.57$).

Pollen grains for 35 of 36 plants were normal in size distribution (Shafer-Wilk *W*-test, $P > 0.002$, Bonferroni-corrected $P_{crit} = 0.0014$; table 2). Standard deviations in grain diameter for these plants ranged from 0.47 to 0.93 μm. One exceptional plant, RDN 1627, exhibited nonnormal distribution for pollen diameter ($P < 0.0001$). Visual inspection of the pollen sample showed many conspicuously larger grains (fig. 4C). Of 140 grains, 10 (7.1%) yielded diameters of 18.5–23.3 μm, while the remaining 130 grains averaged only 15.9 μm. These larger grains are interpreted as unreduced or partially reduced. Atypically large grains were otherwise observed for only one other plant (RDN 1631), but these were quite infrequent (estimated 1 in 500–1000 grains). Because no larger grains were included in the measured sample, however, the distribution of pollen for this plant appeared normal. Pollen samples from fresh and dried capitula showed no significant difference for three different plants ($P > 0.05$ in all three comparisons; fig. 5).

Pollen fertility among plants ranged from 17% to 99%, with an overall average of 84% (table 2). Each of the varieties produced pollen of high fertility (84.5%, 87.0%, and 84.0% for *E. strigosus* var. *dolomiticola*, *E. strigosus* var. *calcicola*, and *E. strigosus* var. nov., respectively), indicating that there is no difference between geographically widespread and highly restricted taxa in ability to produce highly fertile pollen. However, across all plants, the distribution of fertility is approximately exponential in distribution (fig. 6). Most plants (28/36) produce pollen with >80% fertility (fig. 4*A*), while eight plants have <80% fertility. Three of these eight plants occur in populations that also include polyploid apomicts. The distribution of fertility values of plants from mixed populations, via comparison of the eight plants with low (<80%) fertility with the 28 plants with high (>80%) fertility, however, is not statistically different from that expected by chance (χ^2, $P > 0.10$). The plant with the lowest fertility (17%; RDN 1625; fig. 4*B*) has a cytotype that includes seven B-chromosomes and also occurs in a hybrid population.

Discussion

Sexual Erigeron strigosus

Diploidy and sexuality characterize *Erigeron strigosus* on the coastal plain of Georgia and in adjacent states (*E. strigosus* var. nov.) and also populations occupying glades in Alabama and Tennessee (*E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola*; table 2; fig. 1). Although diploid counts have been reported before for *E. strigosus* (table 1), sexuality has been unambiguously documented previously in only a single plant of *E. strigosus* var. nov. from Alabama (Noyes 2000*b*). Ironically, *E. strigosus* is cited (Raghavan 2003) as a member of the large group of angiosperms for which double fertilization was documented in 1900, just 2 yr after the discovery of the phenomenon by Nawaschin (1898). The observation for *E. strigosus* is attributed to Land (1900),

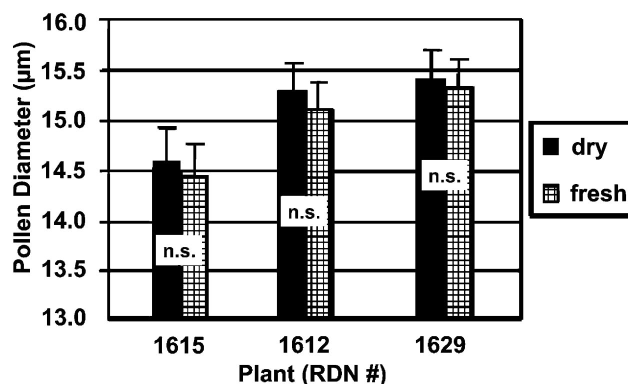

Fig. 5 Differences in pollen grain diameter between fresh and dried pollen samples for three specimens of *Erigeron strigosus* var. nov. Dried grains, $n = 50$; fresh grains, $n > 140$. Error bars = 1 SD from mean. Differences not significant (Student's *t*-test, $P > 0.05$).

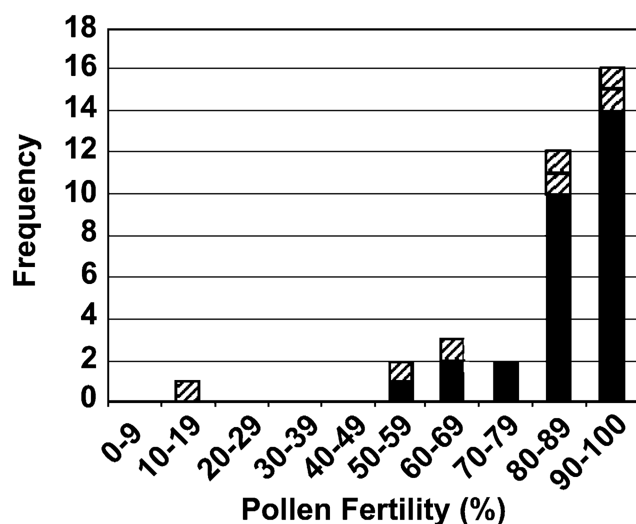

Fig. 6 Histogram of pollen fertility for 36 plants of sexual *Erigeron strigosus*. Hatched regions indicate plants from mixed sexual–apomictic populations. Fertility scores, locality data, and voucher information are provided in table 2.

who mentions collecting material for study of both *E. strigosus* and *Erigeron philadelphicus* L. from the Chicago vicinity. However, his figures illustrate double fertilization only for *E. philadelphicus*, and his concluding remarks note having documented double fertilization for *E. philadelphicus* and *Silphium laciniatum* but not *E. strigosus* (Land 1900). Given that apomictic but not sexual *E. strigosus* is known from the Chicago vicinity, it is probable that Land unknowingly collected apomictic plants and was not successful in documenting double fertilization for *E. strigosus*.

Initial screenings of pollen quality from herbarium specimens indicates that *E. strigosus* var. nov. ranges at least from Charleston, South Carolina, to eastern Texas. Given how geographically widespread these sexual plants are, it is initially surprising that the species has been considered to be exclusively asexual. However, observations by the first author indicate that mature sexual and apomictic *E. strigosus* in the field and even under greenhouse cultivation are often morphologically very similar. As a consequence, apomixis, widespread in North America except in the southeastern United States, had been assumed to characterize all populations of the species.

Evidence indicates that sexual populations are evolutionarily dynamic. Reduced pollen fertility (<80%), B-chromosomes, unreduced pollen production, and/or coexistence with apomictic plants characterizes 15 of the 31 populations of *E. strigosus* var. nov. (table 2). Ongoing research on mixed populations indicates that apomictic plants can fertilize sexual plants, with diverse fitness consequences for progeny (R. D. Noyes, unpublished data). For instance, seeds collected from sexual diploid plants in populations that also include triploid and tetraploid apomicts yield cohorts that include up to 20% polyploid apomicts. Hybrid dynamics thus likely closely parallel those recently documented for *Taraxacum* (Brock 2004). It is plausible, but warrants accumulation of additional data, that the observed cytological and fertility variation within *E. strigosus* var. nov. is the result of hybridization with apomictic plants.

Apomictic *E. strigosus* var. *strigosus* is distinguished from sexual plants in many characteristics (R. D. Noyes, unpublished data). First, apomicts are triploid (2n=27) or tetraploid (2n=36) and do not include B-chromosomes. Second, the pollen of apomictic *E. strigosus* is characterized by low viability (mean 21%, range 0%–83%, $n = 38$), high size variability, and the occurrence of nonviable micrograins. In addition, apomictic plants are usually exclusively diplosporous. Only two of 38 plants examined to date include both diplosporous and reduced (tetrasporic) female gametophytes.

Sexuality in *E. strigosus* var. *calcicola* and *E. strigosus* var. *dolomiticola* is particularly noteworthy. Because these taxa are now confirmed to be diploid and sexual rather than polyploid and apomictic they conform to more widely accepted units of biodiversity. As such, these taxa unquestionably can be added to the substantial list of plant taxa endemic to glade habitats in the southeastern United States (Estill and Cruzan 2001; Baskin and Baskin 2003). Additional investigation of *E. strigosus* on extreme edaphic habitats is warranted, and it would not be surprising if additional sexual populations worthy of taxonomic recognition are discovered.

B-Chromosomes

B-chromosomes, or accessory chromosomes, have been observed in >1360 flowering plant species and occur in both diploid and polyploid taxa (Jones 1995). They are defined as dispensable extra chromosomes that are found only in some individuals of a population and are not duplicates of any A-chromosome within the host genome (Jones 1995). Typically, three or fewer B-chromosomes are observed within an individual of a wild plant species, but up to 34 have been observed in maize (Jones and Rees 1982), and up to 20 are reported in *Allium schoenoprasum* L. (chives) (Bougourd et al. 1995).

B-chromosomes were observed in three plants in this study and have not been reported previously for *E. strigosus*. A survey of published counts (Cave 1958*a*, 1958*b*, 1959*a*, 1959*b*, 1960, 1961, 1962, 1963, 1964, 1965; Ornduff 1967, 1968; Federov 1969; Moore 1973, 1974, 1977; Goldblatt 1981, 1984, 1985, 1988; Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000, 2003) shows that only two of 920 reports for *Erigeron* (0.22%) reliably include B-chromosomes, indicating that accessory chromosomes are rare in the genus. One observation for *Erigeron inornatus* (Gray) Gray is 2n=18+0−5B (Semple 1985), while the other for *Erigeron glabellus* Nutt. subsp. *pubescens* (Hook.) Cronq. is 2n=36+1B (Chinnappa and Chmielewski 1987). In addition, *Erigeron annuus* is documented as 2n=18+0−8B (Rudyka 1988), but this report is considered unreliable because the species is typically 2n=27 and the count is provided only in a list without illustration or discussion. Nonetheless, the other two counts are reliable and demonstrate that B-chromosomes have been documented previously at both the diploid and tetraploid level in *Erigeron*.

Diminished fertility is often associated with an increase in B-chromosome number (Jones and Rees 1982). The pattern observed in this study is consistent with this trend because *E. strigosus* individuals with few Bs (three or four) have normal (high) pollen fertility, while the individual with seven Bs has

only 17% pollen fertility, the lowest observed among all sexual plants studied (table 1). The effect of B-chromosomes on female fertility is less clear, because the proportion of viable mature female gametophytes was not evaluated. However, the B-chromosomes do not appear to affect developmental mode, because all three plants with B-chromosomes were strictly tetrasporic, and no tendency toward other patterns of embryo sac development was observed. It is worth pointing out that although B-chromosomes have not been directly implicated as apomixis factors, many similarities in the genetic composition of B-chromosomes and the genomic regions linked to apomixis are noted by Roche et al. (2001). These authors hypothesize that it is possible that apomixis regions may have originated as accessory chromosomes with megasporogenesis-disrupting effects. The B-chromosomes in sexual *E. strigosus*, however, do not appear to be associated with transitions toward apomixis.

Interspecific hybridization is one possible mode of origin of B-chromosomes (Battaglia 1964), and their spontaneous origin has been observed in crosses between *Coix aquatica* Roxb. and *Coix gigantea* J. König ex Roxb. (Sapre and Deshpande 1987). In *E. strigosus*, it is enticing to speculate that B-chromosomes arose as a consequence of hybridization between sexual and apomictic plants. However, only plant RDN 1625, with seven B-chromosomes, occurs in a population with apomicts, and B-chromosomes were not observed in plants from six other populations containing sexual and apomictic plants. Further field and laboratory experiments are warranted to test the hypothesis of the origin of B-chromosomes via hybridization in sexual *E. strigosus*.

Tetraspory and Apomixis

The *Polygonum*-type, monosporic, eight-nucleate female gametophyte occurs in the majority of flowering plants and is considered ancestral (Palser 1975). Polyspory (bispory and tetraspory) is likely derived from monospory and has apparently evolved independently many times, because it occurs sporadically in 88 flowering plant families (Carman 1997). Tetraspory has been reported for 40 different flowering plant families, and several developmentally distinct forms of tetraspory have been described (Willemse and van Went 1984). In the Asteraceae, monospory is prevalent, but multiple types of polyspory have been observed (Pullaiah 1984). Tetraspory occurs in 15 different Asteraceae genera (observations pooled from Pullaiah 1984 and Carman 1997), but in tribe Astereae it is restricted to *Erigeron* and *Minuria* DC. *Erigeron*, which includes more than 400 species (Nesom 1994), includes species that exhibit monospory (*Polygonum* type), bispory (*Allium* type), and tetraspory (*Drusa*, *Peperomia* types). The *Drusa* type of tetraspory, reported here for *E. strigosus*, has previously been described for 12 other *Erigeron* species (Harling 1951; Pullaiah 1984).

To explain the origins of apomixis, Carman (1997) proposed the duplicate-gene asynchrony hypothesis, where apomixis arises in hybrids of sexual species or their derivatives as a result of conflict between divergent ovule development regimes. Accordingly, apomicts originate among cosmopolitan taxa, where onset of megasporogenesis, female gametogenesis, and embryony has diverged in timing because of ecological selection pressure. The apomixis-causing genetic interactions that occur in hybrids (or their derivatives) are stabilized by polyploidy and are thought to involve conflicts between competing asynchronously expressed ovule development programs such that apomictic seed forms.

Based on statistically significant correlations between (1) polysporic and apomictic genera (polyspory occurs in 31 of 33 apomictic genera) and (2) polyspory and high chromosome numbers, Carman (1997) further hypothesized that some polysporic species have evolved directly from apomicts. In such species, gene silencing, chromosome rearrangements, or base number modifications could eliminate developmental asynchronies required for apomixis but not affect other asynchronies that permit sexual reproduction through polyspory. The resultant neosexual plants are expected to occur either as polyploids, without change in chromosome base number, or in paleopolyploid groups, generally with high chromosome base numbers (transitional-phase hypothesis; Carman 1997). In this respect, sexual *E. strigosus* possesses the same base number ($x=9$) as other species in the genus (Nesom 1994). Hence, diploid polysporic *Erigeron* may have evolved from polyploid apomictic *Erigeron* through gene-silencing mechanisms, as suggested by Carman (1997), or directly from diploid monosporic *Erigeron*. Additional studies are required to differentiate among these possibilities.

While the transition from monospory to polyspory could have been selectively neutral, one could argue for various selective advantages of polyspory. Interestingly, formation of a *Drusa*-type tetrasporous female gametophyte involves only four rounds of nuclear division from the MMC, while the standard *Polygonum* female gametophyte requires five rounds. It is plausible, then, that the evolution of polysporic female gametophytes may have been favored in taxa (such as annuals or species of ephemeral habitats) under selection for rapid gametophyte development. Another possibility is that there may be a selective advantage for plants that possess genetically heterogeneous female gametophytes, i.e., containing nuclei derived from different meiotic products, as occurs in all polysporic taxa. Because *Erigeron* is developmentally diverse, it would be a highly suitable subject for testing hypotheses regarding female gametophyte evolution.

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