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BIAS IN INHERITANCE OF CHLOROPLAST DNA AND MECHANISMS OF HYBRIDIZATION BETWEEN WIND- AND INSECT-POLLINATED EUPATORIUM (ASTERACEAE)

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Hybrids of the wind-pollinated *E. capillifolium* and the insect-pollinated *E. serotinum* were found to occur in the Coastal Plain of Louisiana, Mississippi, and southern Arkansas and on the Piedmont in three locations. Interspecific cross-pollination occurs when wind causes the flexible flowering branches of *E. capillifolium* to whisk over the stiff upright branches of *E. serotinum* plants. Interspecific pollen was found to be captured by *E. serotinum* more effectively than by *E. capillifolium.* However, analysis of cpDNA of natural field hybrids indicated that most were maternally descended from *E. capillifolium.* This latter bias can be explained by the far greater number of flowers, and thus greater seed producing capacity, of *E. capillifolium* relative to *E. serotinum.* Morphological variability observed among field hybrids suggests that backcrossing has occurred; however, field hybrids and progeny grown from field hybrid achenes generally had low pollen stainability, irregular meiosis, and reduced achene set. Furthermore, field hybrids were found to have a chromosome number of $2n = 20$, like the parental species, whereas progeny grown from field hybrids had deficiencies and duplications of chromosome numbers. Because these chromosome number deviations were not seen in field hybrids it is logical to suggest that either they are F_1s, or that strong selective forces in the field eliminated the progeny of hybrids with deficiencies and duplications.

Eupatorium capillifolium (Lam.) Small (Asteraceae) and *E. serotinum* Michx. are weedy perennial plants of wide range in eastern North America, occurring in old-fields, cleared pinelands, and other disturbed habitats. *Eupatorium serotinum* occurs further north than *E. capillifolium,* while *E. capillifolium* occurs outside the range of *E. serotinum* in the Bahamas and Cuba (Fig. 1). However, the distributions of the two species overlap throughout much of their respective ranges. In early seral stages in cleared forests, pastures, and fallow fields at certain geographic locations, plants of the two parental species form dense intermixed populations where hybrids can be found.

King and Robinson (1987) used the discarded generic names, *Uncasia* Greene and *Traganthes* Raf., to refer to groups of species that include *E. serotinum* and *E. capillifolium,* respectively. Although these subgeneric categories lack legitimate taxonomic status, they will be used herein for convenience.

Plants of *E. serotinum* and *E. capillifolium* have a chromosome number of $n = 10$ and reproduce sexually (Maurushat, 1969; Sullivan, 1976), in contrast to many species of the *Uncasia* group, which include agamospermous polyploids (Montgomery and Fairbrothers, 1970; Sullivan, 1976). Sullivan (1975) found that *E. capillifolium* possesses a suite of characters correlated with anemophilous pollinating mechanisms (Whitehead, 1969): smaller pollen grains than members of the *Uncasia* group, pollen with reduced spines, and capitulescences with flexible branches. In a study of allergens, Smith (1984) captured airborne pollen of *E. capillifolium* during its flowering period using a Rotorod Sampler, thus corroborating the morphological evidence that the species is anemophilous. The species of the *Uncasia* group have entomophilous features, including stiff upright capitulescence branches and larger, long-spined pollen grains.

In addition, *E. capillifolium* and other species of the *Traganthes* group differ from those of the *Uncasia,* to which *E. serotinum* belongs, in having highly dissected leaves with filiform to slightly broader lobes, diffuse paniculate capitulescences, and long collecting papillae

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Fig. 1. Distributions of parental species (below C line = inclusive range of *E. capillifolium* and below S line = inclusive range of *E. serotinum*), known locations of hybrids (solid circles), and mixed parental populations with no hybrids (*X*s).

above the distal end of the stigmatic region; all members of the *Uncasia* have undissected leaves, cymose capitulescences, and only short papillae on the style branches.

Recent studies of wind pollination have illustrated the specialized nature of female organs for entrapment of intraspecific pollen (Niklas and Paw U, 1982, 1983; Niklas, 1985; Niklas, Buchmann, and Kerchner, 1986; Paw U and Hotton, 1989). The evidence would suggest that natural hybridization between wind- and insect-pollinated species is highly unlikely. Wind-pollinated *E. capillifolium* is reported to hybridize naturally with three other entomophilous species in the southeastern United States: *E. perfoliatum* L. (Fryar, 1964) of the *Uncasia* group and *E. compositifolium* Walt. and *E. leptophyllum* DC. (Maurushat, 1969) of the *Traganthes* group. The mechanisms by which these hybridizations occur has not been examined.

The goals of this work were to discover how interspecific pollination between *E. capillifolium* and *E. serotinum* occurs, to analyze the origin of field hybrids, and to assess the possibility of introgression. To determine whether preferential backcrossing occurs, cpDNA of parental and hybrid plants was analyzed and pollen flow between parental plants was directly observed. Principal component analysis of morphological variation was used to predict origins of hybrids; reciprocal interspecific crosses were made and fertility of hybrids was examined.

MATERIALS AND METHODS

In order to discover how the hybridizations occur, pollinators were observed during peak flowering periods in isolated and mixed populations of *E. capillifolium* and *E. serotinum.* A total of 8 hours of observations of pollinators of both species in the field were conducted throughout the flowering periods.

The distinctly different pollen morphology of the parent species of the hybrids made possible identification of grains on style branches. Pollen on stigmas of spatially isolated and contacting parental plants was identified in order to determine whether interspecific pollination occurred in intermixed populations and if so, to ascertain whether physical contact was necessary for transfer to occur. Capitulescence branches of interspecifically isolated and interspecifically contacting parental plants were collected and placed individually in separate Ziploc bags labeled "isolated" or "contacting." Interspecifically isolated plants were those in intermixed populations separated by sufficient distances so that they could not physically touch plants of the other species; interspecifically contacting plants could physically contact plants of the other species. In the laboratory, styles were pulled from flowers, placed on glass slides in a drop of aceto-orcein stain, and all pollen grains on stigmatic surfaces were counted and the parental source identified. Dissecting tools were rinsed in ethanol and wiped clean between samples to prevent contamination.

Counts of conspecific and interspecific pollen were made on the stigmatic surfaces of a total of 430 styles collected from 44 *E. capillifolium* and 44 *E. serotinum* individual plants. Because pollen grain numbers on styles from the same plant were observed to be positively correlated, statistical comparisons were based on means of the counts from each plant rather than counts from individual styles. *t*-tests were used to compare mean pollen counts between different interspecifically isolated and contacting plants of each species. Although not all the data met the normality assumption of the *t*-test, comparisons of means were included because the mean number of pollen grains per style is expected to reflect rates of seed production. In addition, the nonparametric Wilcoxon's rank-sums test (Hollander and Wolfe, 1973) was used to compare rank-sums of pollen counts between different interspecifically isolated and contacting plants of each species.

Interspecific hybrids of *E. capillifolium* and *E. serotinum* are found over only a small part of the range of overlap of the parental plants. One reason hypothesized for this narrow hybrid range was that the flowering periods of the species do not coincide throughout their entire overlapping ranges. Therefore, flowering dates of over 500 parental plant specimens from throughout their distributional ranges were recorded.

One hundred seventy-four plants of *E. capillifolium, E. serotinum,* and their hybrids were scored for 25 characters that appeared to be good discriminators of parental species (Table 1). Using these characters, principal component analysis (PROC PRINCOMP) was performed with SAS version 5 (1985) run on the University of Southwestern Louisiana IBM 3090 mainframe computer. Scattergrams of principal components were generated. Single digit numbers in parentheses (1–6) refer to classification labels in the scattergrams. To examine their full range of variation and to identify possible variation that could have resulted from hybridization, 47 plants of *E. capillifolium* (1) and 38 plants of *E. serotinum* (2) from population samples and individual herbarium specimens collected from throughout their ranges in North America were analyzed. Hybrid plant sources were as follows: 23 plants from a single population in Sabine Parish, Louisiana (3345, 5); 47 individual herbarium specimens from Louisiana and Arkansas (3); nine progeny grown from achenes of a field hybrid (4); and 13 artificial F_1 hybrids (6).

Self-incompatibility of hybrids and parental species was tested in the field in two ways: 1) immature capitula were enclosed in nylon hose and later examined for fruit set. Hand self-pollinations were not made, however, as in other species of the Asteraceae, during anthesis the style pushes through the anther ring and becomes covered with self-pollen. 2) Immature capitula were unbagged at anthesis, self-pollinated using fine brushes, rebagged, left for a period of 3 days, and fixed in FAA (five parts formalin : five parts glacial acetic acid : 90 parts 70% ethanol) overnight. Styles were pulled from the fixed flowers with cleaned forceps, softened in 10% KOH for an hour, rinsed in water for an hour, then stained with aniline blue. The styles were wet-mounted on glass slides; pollen tubes, styles and stigmas, and adhering pollen grains were observed for callose fluorescence using fluorescence microscopy. Because our results show that inhibition was not at the ovules but in the pollen and stigmatic papillae, examination of control plants for seed set was not necessary.

Meiosis in pollen mother cells of five field hybrids (Lafayette Parish site, 1941) was examined in order to assess the likelihood that they could produce gametes with full haploid

TABLE 1. *Characters used in morphometric analyses of* E. capillifolium, E. serotinum, *and hybrids*

Acronym	Character	Scale
LOBNO	No. leaf lobes/teeth along one side	mm
LOBLEN	Length (Lg.) of lowest leaf lobe/tooth	mm
HDLEN	Lg. of capitulum, excl. style	mm
CORLEN	Lg. of corolla	mm
FLWNO	No. of flowers in head	5 to 13
CLOBLEN	Lg. of corolla lobes	mm
CLOBWID	Width of corolla lobes	mm
STYAP	Lg. of style appendage, excl. STIGLEN	mm
STIGLEN	Lg. of stigmatic surface	mm
STYBAS	Lg. of style base, excl. STYAP and STIGLEN	mm
PAPLEN	Lg. of pappus	mm
TIPWID	Width of leaf tip 1/3 from tip	mm
MIDWID	Width of leaf at midpoint excl. lobes/teeth	mm
LOBWID	Width of lowest lobe/tooth	mm
STYTOT	STYAP + STIGLEN + STYBAS	mm
STAPRAT	STYAP/STYTOT	
STLENRAT	STIGLEN/STYTOT	
STBASRAT	STYBAS/STYTOT	
CORCLRAT	CLOBLEN/CORLEN	
CORLRAT	CLOBLEN/CLOBWID	
STYAPRAT	STIGLEN/(STYAP + STIGLEN)	
LOBRAT	LOBWID/LOBLEN	
TPRAT	TIPWID/MIDWID	
TLRAT	TIPWID/LOBWID	
NOLENRAT	LOBNO/LOBLEN	

sets of chromosomes. Flower buds were fixed overnight in acetic-alcohol (one part glacial acetic acid : three parts 95% ethanol) then stored in 70% ethanol at 10 C. Somatic chromosome numbers of 33 field hybrids (Sabine Parish, LA, 3342 and 3345) and 13 progeny grown from field hybrid achenes were determined from root tips. Root tips were treated according to the oxyquinoline-acetic-orcein squash method of Radford et al. (1974). As a measure of viability, the percentage of pollen grains stained uniformly dark with cotton blue in lactophenol out of a total of 200 to 400 grains was calculated.

Chloroplast DNA (cpDNA) was extracted from 27 field hybrid plants (Sabine Parish site, 3345), eight plants of an isolated population of *E. capillifolium* (Lafayette Parish site, 3335), and five plants of an isolated population of *E. serotinum* (Lafayette Parish site, 3334) cultivated in a greenhouse. Chloroplast DNA was extracted and purified by the method of Palmer (1982). Chloroplasts were isolated on sucrose step gradients, and cpDNA from lysed chloroplasts was purified by CsCl/ethidium bromide gradient centrifugation.

Chloroplast DNA samples were prepared for Southern blot analysis (Southern, 1975) as follows. One-μg samples of genomic DNA were digested by restriction endonucleases that recognize either four or six base sites according to the supplier's directions. The digests were concentrated by ethanol precipitation, and resuspended in 15 μl TE (10 mM Tris, 1 mM EDTA, pH 8.0) with 2 μl loading buffer. Samples were electrophoresed along with fragment length standards (phage lambda DNA digested with Hind III) in 20 × 25 × 0.4 cm 0.7% agarose gels with TBE (0.089 M Tris-borate, 0.089 M boric acid, 0.01 M EDTA, pH 8.0) at 50 V for 5 hr, stained with ethidium bromide, and photographed under ultraviolet illumination.

Gels were prepared for Southern transfers following the procedures of Maniatis, Fritsch, and Sambrook (1982). DNA was blotted onto Magnagraph nylon (Micron Separations, Inc., Westboro, MA) overnight and the filters baked at 80 C for 2 hr. Gels were placed on a stack of Whatman 3MM paper soaked in 10× SSC (1.5 M NaCl, 0.15 M sodium citrate).

Chloroplast DNA clones of lettuce (*Lactuca sativa*) in the plasmid vector pUC 12 were a generous gift of Robert Jansen. Clone numbers 5 and 7 (approximately 28 to 46 kb, and 91 to 98 kb on the restriction map in Fig. 2 of Jansen and Palmer, 1987) were used in our analysis.

Probe DNA was labeled with the hapten digoxigenin by random primer extension with the Klenow subunit of DNA polymerase (Ausubel et al., 1988). Genomic hybridizations were performed overnight at 65 C in 5× SSC and 10–50 ng probe per ml hybridization solution. The probe was visualized by incubation

TABLE 2. *Mean stigmatic pollen counts for* E. capillifolium *and* E. serotinum *in isolation and in contact with members of the other species. Each observation consisted of an average of counts from up to six separate styles from each plants*

		Conspecific pollen			Interspecific pollen		
	N	[a]Proportion	Mean	Variance	[a]Proportion	Mean	Variance
Isolated							
capillifolium	19	1.00	40.8	461	0.11	0.0211	0.00398
serotinum	22	1.00	34.8	469	0.55	0.462	0.479
In contact							
capillifolium	25	1.00	34.6	554	0.40	1.89	20.38
serotinum	22	1.00	30.6	414	0.95	5.75	55.7

[a] Proportion of total number of plants in sample.

with polyclonal sheep anti-digoxigenin Fab fragments conjugated to alkaline phosphatase (Boehringer Mannheim), which produces a localized color reaction when incubated with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate toluidinium.

In order to determine whether relative seed setting capacity influenced the numbers and the maternal source of field hybrids, densities of parental plants in a mixed population were determined. The best choice for study was the population in Sabine Parish, Louisiana where hybrids for cpDNA analysis were collected. However, by the time such studies were planned, 20 months later, all *E. serotinum* plants and hybrids had disappeared from that site. An adjacent site was chosen with very similar characteristics and from which a hardwood forest had been cut the previous winter. At this site, 20 4-m^2 circular plots at randomly selected intervals along a line transect were surveyed for numbers of plants of each species. Stems of the tallest plant of each species were counted at randomly selected intervals along a line transect. The number of flowers per stem were counted for several plants of each of the two species.

RESULTS

Natural hybridization—Pollinators were not observed visiting flowers of *E. capillifolium*; however, flowers of *E. serotinum* were visited by honeybees and a diversity of moths and skippers (not specifically identified). Thus, interspecific cross-pollination by insects seemed an unlikely means of hybridization.

It was observed that when plants of the two species were in close contact the lax capitulescence branches of *E. capillifolium* were whisked by wind over the stiff capitulescences of *E. serotinum.* Pollen count data from interspecifically isolated and contacting plants are summarized in Table 2. Conspecific pollen was found on at least some styles of all plants examined, and the number of conspecific pollen grains per style averaged between 30.6 and 40.8 for both species, whether interspecifically isolated or in contact. In contrast, interspecific pollen was not found on the styles of all plants examined, and counts of interspecific pollen were relatively low, averaging between 0.0211 and 5.75 grains per style.

Statistical comparisons of pollen counts from interspecifically isolated plants vs. those from interspecifically contacting plants indicate that capture of interspecific pollen is facilitated by contact (Table 3). The proportion of plants with interspecific pollen was higher for interspecifically contacting plants, and average pollen counts were 12 times higher for *E. capillifolium* and 90 times higher for *E. serotinum.* Two-sided *t*-tests of these differences in mean pollen counts were significant (alpha = 0.05) for *E. serotinum* but not *E. capillifolium.* However, corresponding Wilcoxon's rank-sums tests were significant for both species.

TABLE 3. *Statistical tests of differences in interspecific pollen counts. Significance levels are based on two-sided alternatives*

	Probability	
Comparison	Wilcoxon	*t*-test
Isolated vs. In contact		
capillifolium	0.0106	0.0790
serotinum	<0.0001	0.0019
capillifolium vs. *serotinum*		
Isolated	0.0023	0.0087
In contact	0.0001	0.0349

Eupatorium serotinum appears to be more effective at capture of *E. capillifolium* pollen than the reverse. The proportion of plants with interspecific pollen was higher for *E. serotinum,* whether interspecifically contacting or not. This bias was also reflected in average pollen counts, which were 21.9 times higher for interspecifically isolated plants and three times higher for interspecifically contacting

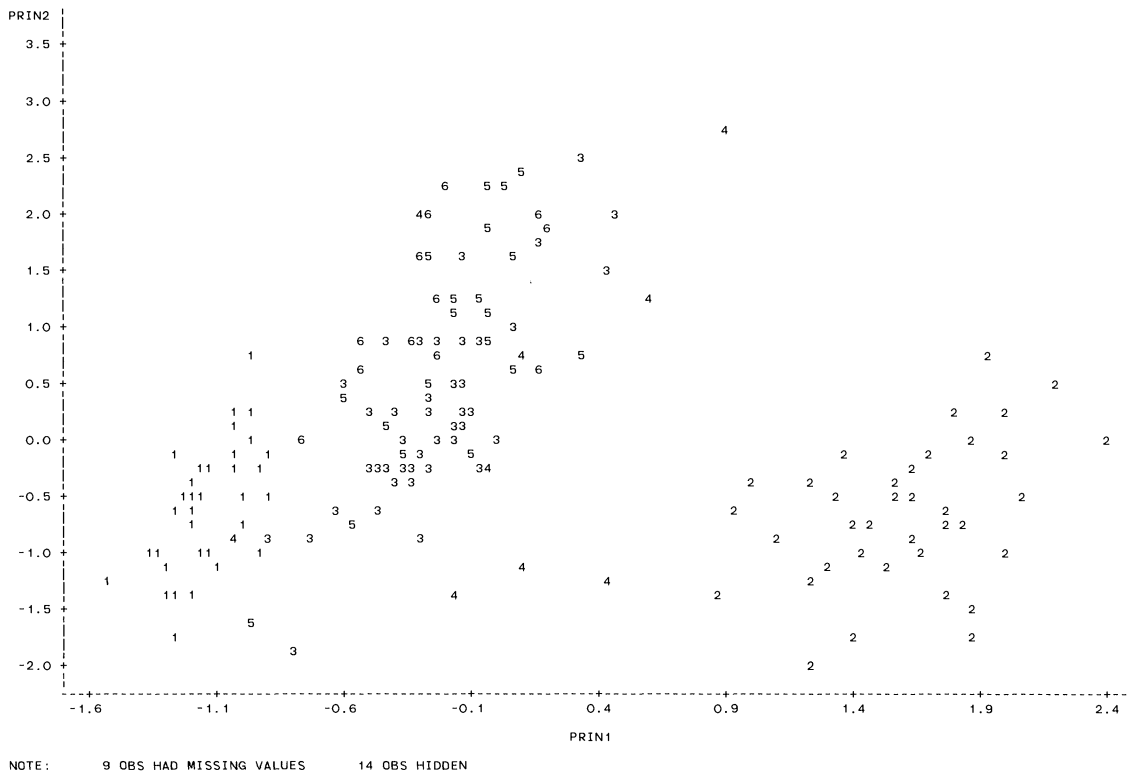

Fig. 2. Scattergram of principal components 1 vs. 2 based on 25 characters of parental plants (1 = *E. capillifolium*, 2 = *E. serotinum*) and hybrids (3 = herbarium, 4 = progeny from field hybrid achenes, 5 = population sample, 6 = F_1s).

plants. These differences in pollen counts were significant both in two-sided *t*-tests and Wilcoxon rank-sums tests.

Hybrid distribution—The hybrids predominate in the western portion of the range of overlap of the parental species. Field observations and examination of herbarium specimens from throughout the overlapping distributional ranges of the parental species revealed that hybrids occur commonly in Louisiana and southern Arkansas and infrequently in southern Mississippi and the Piedmont of Alabama and North Carolina. Intermixed parental populations were discovered in several sites in Mississippi, but hybrids were not present (Fig. 1).

The greater abundance of hybrids along the western edge of the range of parental species was hypothesized to result from lack of overlapping flowering periods of parental species elsewhere. However, it was found that throughout most of their ranges flowering periods do coincide. The overlapping of flowering periods of the two parental species is during the first 2 weeks of October, which is near the end of the flowering period of *E. serotinum* (ca. end of August to second week of October). Only certain populations of *E. serotinum* in parts of Florida were found to flower earlier (ca. end of June to first of August) than *E. capillifolium* (ca. October until November). Plants of the two flowering races of *E. serotinum* transplanted to a greenhouse retained their distinct flowering periods.

Morphometric analyses—Morphometric analyses of parental and hybrid plants were based on 25 characters (Table 1). Data and simple statistics can be obtained from V. Sullivan. Principal component analyses were performed using a full set of 25 characters and a reduced set of 16 characters that excluded the nine leaf characters. Leaf morphologies of the species are markedly different, and homologies were difficult to ascertain. In comparing leaf structure, leaf lobes of *E. capillifolium* were considered to be homologous with teeth of *E. serotinum* leaves because the number of lobes of hybrids seemed to be influenced by number of teeth of *E. serotinum* leaves. For principal component analyses using 25 characters, 70.9% of variation is accounted for in three principal component axes. Using 16 characters, 76.9%

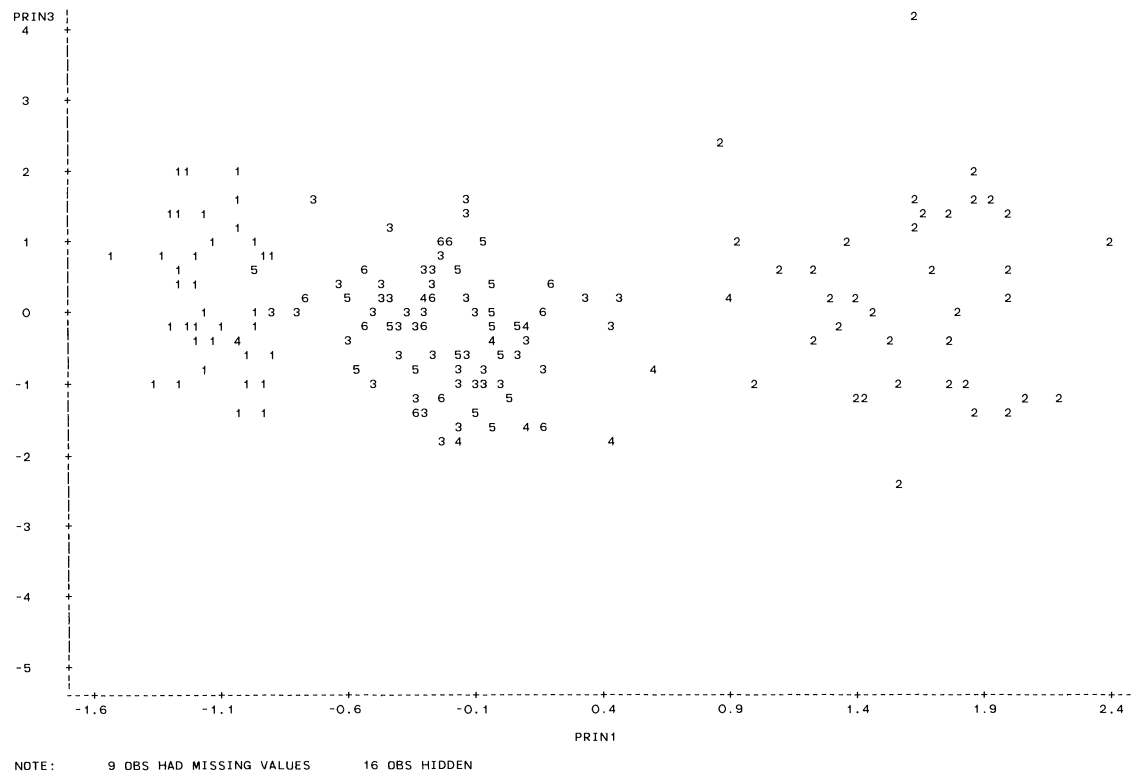

Fig. 3. Scattergram of principal components 1 vs. 3 based on 25 characters of parental plants (1 = *E. capillifolium*, 2 = *E. serotinum*) and hybrids (3 = herbarium specimens, 4 = progeny from field hybrid achenes, 5 = population samples, 6 = F_1s).

of variation is accounted for in three principal component axes.

Results using the full and reduced data sets differed as can be seen in scattergrams of principal components 1 vs. 2 and 1 vs. 3 for each analysis (Figs. 2–5). When leaf characters were included in the analysis, most hybrids resembled *E. capillifolium* more than they did *E. serotinum.* Hybrid plants all had lobed leaves like the *E. capillifolium* parent, and these similarities could have skewed the results in that direction. However, when leaf characters were excluded, most hybrids appeared intermediate and formed a group distinct from *E. capillifolium.*

Interfertility—Reciprocal hand interspecific pollinations in a greenhouse yielded a small number of viable achenes. The percentage yield was not determined. The fertility of the resulting F_1 hybrids was low as measured by an average pollen stainability of 17%. Five reciprocal backcrosses to each parent produced a total of six achenes.

Ripe achenes were formed by 0 to 5.6% of ovaries from bagged capitulescence branches of parental plants suggesting that they were self-incompatible. The small percentage seed set probably resulted from fertilization by contaminating pollen on capitulescence branches before bagging. Bagged capitulescence branches of F_1 hybrids and natural hybrid plants produced no achenes, suggesting that they are also self-incompatible; however, because pollen stainability was very low, lack of achene set could have been due to infertility. Fluorescent callose plugs were found in the germination pores of self-pollen grains and in tips of stigmatic papillae of hand self-pollinated styles of parental plants; these are also features characteristic of self-incompatibility.

The ability of hybrids to reproduce would appear to be reduced by infertility; however, reproduction also may be reduced by deficiency of the pollinating mechanism. Only two field hybrid plants were observed to attract pollinators in the field and these resembled the *E. serotinum* parent in having showier flowers. However, artificial F_1 hybrids were visited by foraging honeybees and various lepidopterans in an open greenhouse. The potential for aerial dispersal of hybrid pollen is unknown. Distinguishing parental and hybrid pollen on stigmas of hybrids was abandoned because of the con-

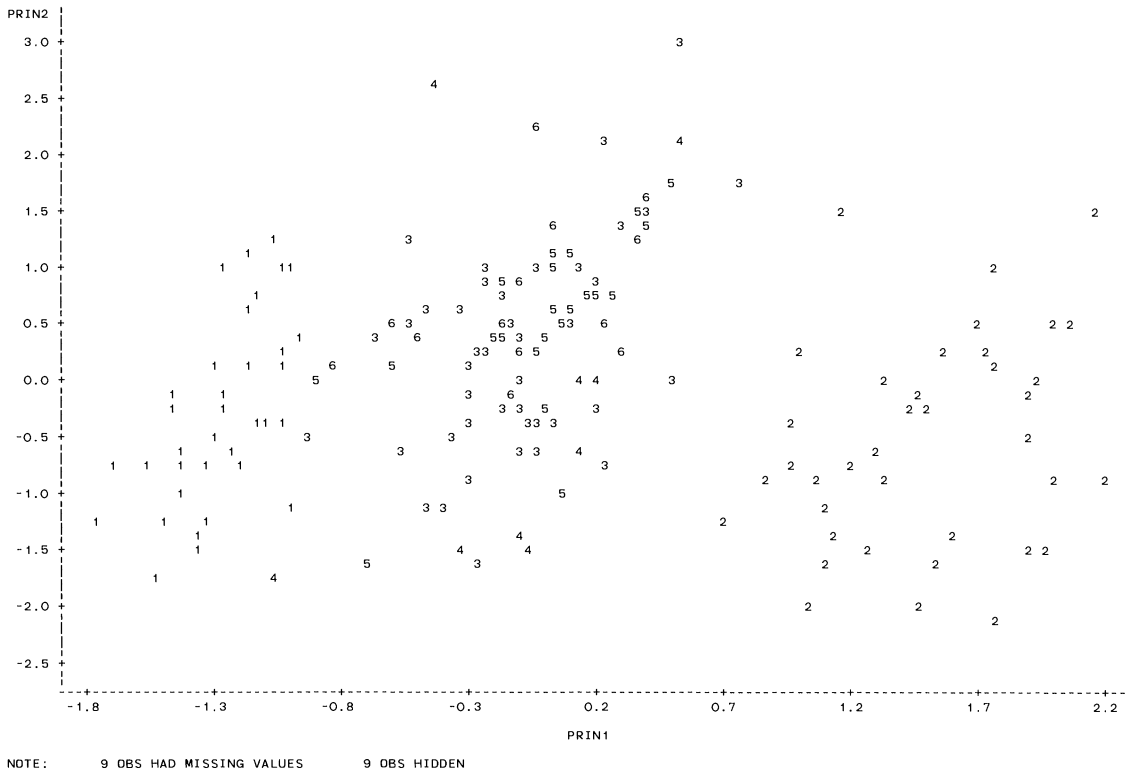

Fig. 4. Scattergram of principal components 1 vs. 2 based on 16 characters (excluding leaf features) of parental plants (1 = *E. capillifolium* and 2 = *E. serotinum*) and hybrids (3 = herbarium specimens, 4 = progeny from field hybrid achenes, 5 = population samples, 6 = F_1s).

fusing array of recombinant pollen morphologies.

Fruit set of natural hybrids in the field was found to be low in all those examined except one. Percentage achene set based on numbers of black achenes in 150 capitula of each of 24 plants (3345) was between 0.38% and 18.7%, excluding the 100% fruit set of a hybrid with $2n = 30$ chromosomes ($\bar{X} = 14.06\%$, SD 26.1%). The remaining 23 plants had $2n = 20$ as do parental plants.

Of 38 field hybrids examined (3345, 1941), 37 had $2n = 20$ and one plant had $2n = 30$ chromosomes. However, 13 progeny grown in a greenhouse from field hybrid achenes had various somatic chromosome numbers: $2n = 20$ in four plants, $2n = 21$ in one, $2n = 22$ in one, $2n = 26$ in two, $2n = 28$ in one, $2n = 29$ in three, and $2n = 31$ in one. Six of the hybrid progeny showed sublethal growth, and their chromosome numbers could not be determined.

Among a total of 178 pollen mother cells of five field hybrids (1941) 66% to 94% had two to seven univalents, 0 to 20% had one to two trivalents, and 0 to 19% had one to two quadrivalents during diakinesis of meiotic prophase. Only 6% to 40% of pollen mother cells had ten bivalents. At anaphase I and II, lagging univalents and bivalents were frequent. The consequences of meiotic abnormalities were apparent in low percentage pollen stainability in cotton blue in lactophenol. Percentage stainable pollen in 71 field hybrids was from 0 to 60% ($\bar{X} = 13.2\%$, SD 11.87%) in counts of 200 to 400 grains. Only four plants had greater than 30% stainable pollen.

Chloroplast DNA analyses —Chloroplast DNA was isolated from parental and hybrid plants, and two cloned cpDNA probes in combination with the following eight restriction endonucleases were surveyed: Ava I, Ban I, Bgl II, Cla I, EcoR V, Hae III, Kpn I, and Xba I. Three of the enzymes, Cla I, Hae III, and Xba I, produced fragment patterns that distinguished the two species (Fig. 6). For the enzymes that recognize six-base sequences (all except Hae III, which recognizes four-base sequences), 19 hybridizing cpDNA fragments were shared out of a total of 27 fragments. For Hae III, seven out of eight fragments were shared. A comparison of the fragment sizes indicated that the observed differences are con-

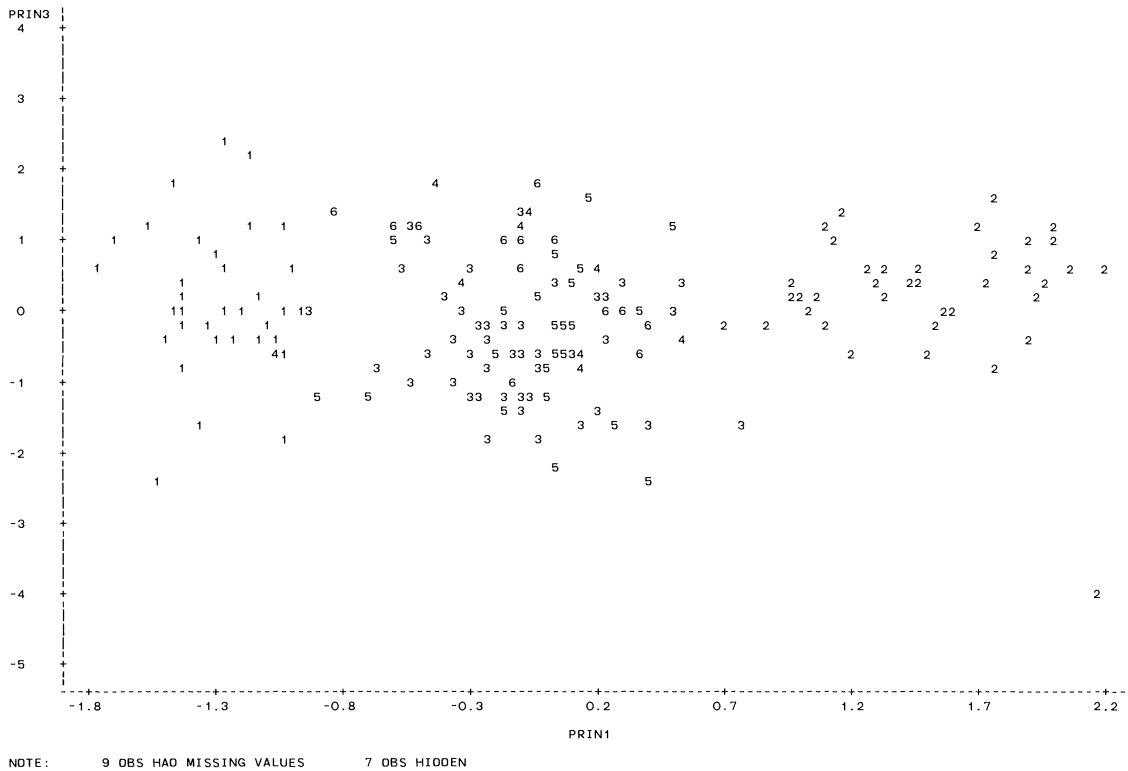

Fig. 5. Scattergram of principal components 1 vs. 3 based on 16 characters (excluding leaf features) of parental plants (1 = *E. capillifolium* and 2 = *E. serotinum*) and hybrids (3 = herbarium specimens, 4 = progeny from field hybrid achenes, 5 = population samples, 6 = F_1s).

sistent with independent restriction site mutations, rather than multiple effects of a single insertion/deletion. An Xba I fragment of approximately 15 kb was unique to the *E. serotinum* pattern while two fragments, about 7.8 kb and 5.6 kb, were unique to the *E. capillifolium* pattern. A Cla I fragment of approximately 3.6 kb in *E. serotinum* was replaced by fragments of approximately 1.9 and 1.0 kb in *E. capillifolium.* An Hae III fragment of approximately 695 bp was found only in *E. serotinum* while a fragment of 455 bp was found in *E. capillifolium.* All of the 27 morphologically designated hybrids had either the *E. serotinum* or the *E. capillifolium* cpDNA types as characterized by the species-specific fragment patterns described above.

Population analysis—In May 1990 *E. serotinum* and hybrids were no longer present at the Sabine Parish, Louisiana site where in October 1988 hybrids were collected. Field observations in several sites indicate that parental species and hybrids become established in open sites and are eliminated as succession proceeds. In order to determine the approximate time of clearing and establishment of the parental species in the Sabine Parish site, small trees of *Fraxinus* sp., *Quercus marylandica* Muench., *Rhus copallina* L., and *Pinus elliottii* Engelm. were cut and annual rings were counted. All the trees were beginning their fourth spring, indicating that they established in the spring of 1986, likely following clearcutting. That year also probably marked the date of establishment of parental plants, and possibly hybrids, recruited through achene dispersal from other sites. By fall 1986, these newly established parental plants could have flowered and crossed interspecifically, yielding hybrid achenes that could establish and flower by fall 1987. Also hybrids recruited in 1986 would have had an opportunity to cross with parental plants and with other hybrid plants. By October 1988, when collections were made for this study, progeny from interspecific and interhybrid crosses and backcrosses could have established at this site. By spring 1990, only scattered *E. capillifolium* plants remained in site 3345.

In an adjacent field to 3345, cleared the previous winter of 1989–1990, a total of 356 plants was counted in a survey of 78.8 m^2. Plants of *E. capillifolium* outnumbered those of *E. serotinum* in a mixed population in 80% of plots

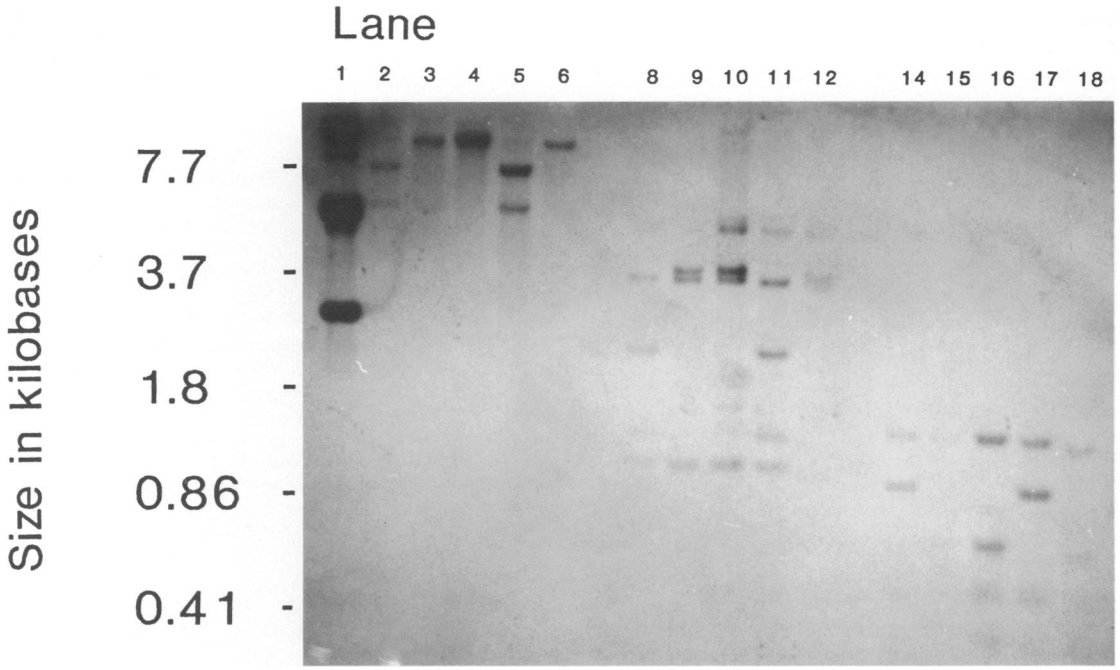

Fig. 6. Southern hybridization of cpDNA digests of *Eupatorium* probed with lettuce probe 7. Lane 1 is a positive control of undigested probe. Lanes 2–6 are Xba 1 digests, lanes 8–12 are Cla I digests, and lanes 14–18 are Hae III digests. Lanes 2, 8, and 14 are *E. serotinum,* and lanes 3, 9, and 15 are *E. capillifolium.* Three hybrids between *E. serotinum* and *E. capillifolium* are shown in lanes 4, 10, and 16; lanes 5, 11, and 17 and lanes 6, 12, and 18, respectively. Two of the hybrids show the *E. capillifolium* pattern, while the other shows the *E. serotinum* pattern.

and by a 1.5:1 ratio in all plots. In number of flowering stems from the 198 plants counted, *E. capillifolium* outnumbered *E. serotinum* by a 4.5:1 ratio. Based on calculations from counts of the average number of ovaries per stem of parental plants, the potential seed set per plant of *E. capillifolium* is higher (ca. 330,000) than that of *E. serotinum* (ca. 40,000) by an 8:1 ratio. In this population hybrid plants were present indicating they hybrid achenes had dispersed from other areas.

Taxonomy—The type specimen of *E. eugenei* Small illustrated by Mohr (1901) from the Piedmont of eastern Alabama is identical to certain hybrids of *E. capillifolium* × *serotinum.* Mohr states that the plant is rare and local. Several features common to hybrids examined in this study and the type specimen are: number of flowers per head, petiolate leaf base, pubescence, and dome-shaped capitulescence branches.

DISCUSSION

Evidence from cpDNA that *E. capillifolium* is more often the maternal parent of the population of hybrids examined here is consistent with the eightfold greater seed setting potential of *E. capillifolium* than *E. serotinum* in mixed populations. The slightly greater ability of *E. serotinum* styles to capture pollen of *E. capillifolium* when plants of the two species are in contact does not appear to be sufficient to compensate for the greater seed setting capacity of *E. capillifolium.* The narrow overlap of flowering toward the end of the flowering period of *E. serotinum* is also likely to reduce the probability of its maternal role in crosses.

Only 66% of progeny grown from field hybrids flowered and only 22% had $2n = 20$ chromosomes. The remaining progeny had duplications and deficiencies of chromosomes resulting from irregular meiosis of their maternal parent and possibly from a hybrid paternal parent. In addition, all hybrids were highly infertile as measured both by pollen viability and achene set. Based on this evidence, it appears most likely that the field hybrids are F_1s.

The barriers to hybridization between *E. capillifolium* and *E. serotinum* apparently have broken down in certain areas of their overlapping distributions and remain effective in others. Edaphic factors could play a part in separating the species elsewhere in their overlapping ranges. In contrast to *E. capillifolium, E. serotinum* appears to be edaphically

restricted to lower, wetter sites of fine loamy soils in the eastern Coastal Plain and does not occupy the more common porous sandy soils of that area.

The general pattern of hybridization between *E. capillifolium* and *E. perfoliatum* of the *Uncasia* group is probably similar in many respects to the hybridization considered here. *Eupatorium perfoliatum* is insect pollinated, so that crosses likely occur by physical contact, and meiotic irregularities and low fertility are characteristic of the hybrids (Sullivan, unpublished data). However, hybrids between *E. capillifolium* and other members of the *Traganthes, E. compositifolium* and *E. leptophyllum,* are highly fertile (Maurushat, 1969). *Eupatorium compositifolium* and *E. leptophyllum* have conspicuously echinate pollen associated with insect pollination (Sullivan, 1975), and Sullivan (unpublished data) has observed that *E. compositifolium* attracts pollinators. The hybridizations of *E. capillifolium* with these other species are now being compared to that of *E. capillifolium* × *serotinum.* It is expected that differences in fertility of hybrid combinations, in lengths of flowering period overlap, and in seed setting potential among the various hybrid combinations with *E. capillifolium* could be valuable in examining the evolutionary significance of hybridization and selection of pollinating mechanisms.

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