GENETIC DIVERSITY AND ENDEMISM IN NORTH AMERICAN *CAREX* SECTION *CERATOCYSTIS* (CYPERACEAE): A PHYLOGEOGRAPHIC CONTEXT

by

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Genetic diversity and endemism in North American *Carex* section *Ceratocystis* (Cyperaceae): a phylogeographic context

Thesis directed by Associate Professor Leo P. Bruederle

ABSTRACT

Two described species of *Carex* section *Ceratocystis* (Cyperaceae) are endemic to North America: *C. lutea* Le Blond is restricted to a single watershed along the Atlantic Coastal Plain of North Carolina; *C. cryptolepis* Mack. is distributed across formerly glaciated Eastern North America. Allozyme diversity within and among populations of the respective taxa is used to assess the impact of restricted distribution on genetic diversity in species of *Carex* section *Ceratocystis*. *Carex lutea* maintains high levels of genetic diversity relative to *C. cryptolepis* and an endemic species new to science, of intermediate distribution. Mean proportion of loci polymorphic (P = 20.0%), number of alleles per polymorphic locus (A_p = 2.15), and observed and expected heterozygosities (H_o = 0.028, H_e = 0.049) were all greater in *C. lutea* than *C. cryptolepis*; the new species maintained no allozyme variation. *Carex lutea* and *C. cryptolepis* had similar heterozygote deficiencies (f = 0.431 and f = 0.485, respectively), and large positive inbreeding coefficients in both taxa were generally correlated with significant deviations from Hardy-

Weinberg equilibrium. A large degree of population differentiation was observed in both *C. lutea* (F_{ST} = 0.412) and *C. cryptolepis* (F_{ST} = 0.796). Aspects of the evolutionary history of these taxa, particularly relating to Pleistocene climate change and consequential shifts in species' distributions, might account for the observed patterns of genetic diversity.

This abstract accurately represents the content of the candidate's thesis. I recommend its publication.

Signed [redacted]

Leo P. Bruederle

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1.1 Genetic diversity expectations

Endemic species are geographically restricted in distribution; however, the degree of restriction can vary significantly. Endemics may be broadly distributed, occurring across relatively large areas, or more narrowly distributed. For any given species, the causes of endemism may differ, as well. Recently diverged species may be localized to the region of origin (e.g., Pleasants and Wendel, 1989). Events such as climate change may initiate a range contraction and restrict the species to a reduced portion of its original distribution (e.g., Maki and Asada, 1998). Adaptation to a specific successional stage, community, or habitat type of limited distribution will restrict the species to only those areas that are ecologically appropriate (e.g., Purdy et al., 1994; Neel and Ellstrand, 2003).

All endemic species are found in a limited geographic region, one criterion for rareness, and many are habitat specialists, a second criterion (Rabinowitz, 1981). Rare plants are a conservation concern, as rarity is expected to increase susceptibility to extinction. Measures of genetic diversity have been used by conservation biologists as a means of assessing the threat of extinction for a particular species and setting management

practices. Low genetic diversity may predict an inability to adapt to changing conditions, and an increased risk of extinction (Huenneke, 1991). Consequently, characterization of the relationship between endemism and levels of genetic diversity maintained within populations, and the degree to which populations are differentiated, has been a major area of investigation.

The general consensus has been that widespread species can be expected to maintain high levels of genetic diversity relative to species with restricted distribution (e.g., Hamrick et al., 1979; Hamrick and Godt, 1989): species of recent origin will harbor a subset of the progenitor's allelic variation; small populations will exhibit inbreeding and increased fixation indices; alleles will be lost due to genetic drift following range reduction; natural selection will purge non-adaptive alleles; and patchy habitats will reduce gene flow.

The expected pattern of relatively low levels of genetic diversity in species with limited distribution has been reported frequently (e.g., Pleasants and Wendel, 1989; Purdy et al., 1994; Coates et al., 2003). In the most recent review, Gitzendanner and Soltis (2000) compared genetic diversity in widespread and rare species pairs from the same genus and found a significant correlation between rarity and lower levels of genetic diversity at the population level (proportion polymorphic loci, mean alleles per locus, and observed heterozygosity) and at the species level (proportion polymorphic loci

and alleles per locus). However, relative levels of genetic diversity in some congeneric widespread and rare species pairs have been observed to deviate from expectations (e.g., Linhart and Premoli, 1993; Maki and Asada, 1998; Neel and Ellstrand, 2001; Dodd and Helenurm, 2002; Coates et al., 2003; Neel and Ellstrand, 2003; Broadhurst and Coates, 2004). Additionally, disparate levels of genetic diversity have been observed in comparisons of unrelated species with congruent natural history and distribution size (Edwards et al., 2004; Helenurm and Hall, 2005). Considering these previous studies, researchers are well advised to choose comparisons between related taxa, thereby controlling for confounding factors such as life history traits, rate of evolution, and relative ages of species being compared (Karron, 1991). Sister species, when available, would provide the most appropriate comparison in assessing the influence of endemism on levels of genetic diversity.

1.2 *Carex* section *Ceratocystis*

1.2.1 Taxa occurring in North America

Crins and Ball (1988) used morphological, micromorphological, and ecological characters to support the monophyly of *Carex* section *Ceratocystis*, sister to section *Spirostachyae* Drejer (Bailey); ITS sequence data provides additional support for this relationship (Hiendrichs et al., 2004). *Carex lutea* Le Blond (Cyperaceae) is a recently described species in section *Ceratocystis* Dumort. from the Atlantic Coastal Plain of North Carolina (Le Blond et al.,

1994). In addition to *C. lutea*, the other members of the section occurring in North America are *C. cryptolepis* Mack., *C. flava* L., *C. hostiana* DC., and *C. viridula* Michaux (Crins, 2001). *Carex cryptolepis* and *C. lutea* are the only North American endemic species in the section, with the other species also occurring in Europe (*C. flava, C. hostiana*, and *C. viridula*), Asia (*C. flava* and *C. viridula*) and northwestern Africa (*C. viridula*) (Crins and Ball, 1987, 1989a, 1989b). Robertson (1980) described *C. saxilittoralis* as a narrow endemic from Labrador, Newfoundland and Nova Scotia, although Crins and Ball (1989b) subsequently relegated this taxon to varietal status, *C. viridula* subsp. *brachyrryncha* (Celakovský) B. Schmid var. *saxilittoralis* (Robertson) Crins (Crins, 2001).

1.2.2 Previous genetic diversity studies

A number of previous studies have examined genetic (allozyme) diversity within and among taxa comprising section *Ceratocystis*, specifically: North American populations of *C. viridula* subsp. *viridula* var. *viridula* (Kuchel and Bruederle, 2000); European populations of *C. flava* and *C. viridula* (Bruederle and Jensen, 1991); and *C. viridula* subsp. *brachyrryncha* in Sweden (Hedren and Prentice, 1996). In Europe, species of section *Ceratocystis* exhibit levels of genetic diversity and population differentiation typical of caespitose sedges (Bruederle and Jensen, 1991; Bruederle et al., in press; Hedren and Prentice, 1996). However, Kuchel and Bruederle (2000)

found no allozyme variation in the North American populations of *C. viridula* subsp. *viridula* var. *viridula*, presumably due to bottlenecks during dispersal to the continent. Describing patterns of genetic diversity in the North American endemics *C. cryptolepis* and *C. lutea* could increase our understanding of evolution in the section, as well as provide an excellent comparison between the impact of broad versus narrow endemism on levels and apportionment of genetic diversity. This investigation is particularly critical for *C. lutea*, an endangered species of conservation concern.

1.2.3 The North American endemics

Carex lutea comprises eight populations localized to Pender and Onslow counties in North Carolina. All known populations of *C. lutea* are restricted to a portion of the Cape Fear River watershed, within a several kilometer radius (Fig. 1). This distribution is highly disjunct from that of other members of the section: *Carex viridula* subsp. *viridula* var. *viridula* extends as far south as New Jersey, about 750 km north (Le Blond et al., 1994), and an isolated occurrence of *C. flava* in Virginia (itself disjunct) is approximately 400 km north (Wieboldt et al., 1998). The habitat of *C. lutea* is highly specific, occurring as "phytogeographic islands:" soils are sandy and wet, overlying coquina limestone deposits; microsites for which data are available have a pH of 5.6; and frequent fires are necessary to suppress shrub dominance (Le Blond et al., 1994). In contrast, *C. cryptolepis* is broadly

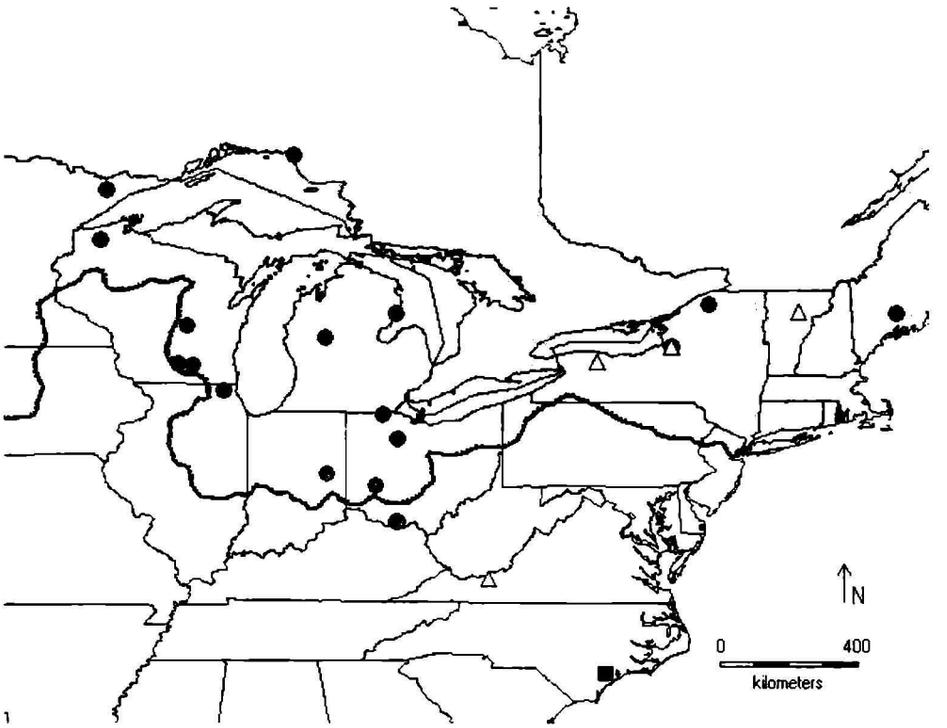


FIGURE 1. SAMPLED POPULATIONS. Approximate locations of five populations of *Carex lutea* Le Blond (black square), 21 populations of *C. cryptolepis* Mack. (black and grey filled circles), and four populations of *C. flava* (open triangles) sampled for allozyme analysis. The furthest extent of glacial ice during the Wisconsin Glaciation is illustrated by a heavy black line; the grey filled circles represent populations that are possibly refugial, or near-refugial, for *C. cryptolepis*.

distributed across northeastern Eastern North America (Fig. 1). This species occupies wet, sandy, frequently acidic soils; competition is not tolerated; and the required open sites are typically disturbance maintained (Crins and Ball, 1989b; Derieg, pers. obs.). As with other species in section *Ceratocystis, C. lutea* and *C. cryptolepis* are self-compatible caespitose perennials. Primarily

selfing plants are expected to maintain lower levels of genetic diversity relative to outcrossing species (Hamrick et al., 1979), and this pattern emerges when caespitose carices, which appear to have high selfing rates, are compared with rhizomatous carices, which exhibit the effects of outcrossing (Bruederle et al., in press).

1.2.4 Systematics of *Carex* section *Ceratocystis*

The systematics of section *Ceratocystis* has been addressed by a number of authors over the last three decades; however, a clear understanding of relationships within the section has been elusive. Crins and Ball (1988) proposed an evolutionary progression of increasing chromosome number, elaboration of silica bodies, and decreasing longevity (i.e., more r-selected); additionally, diversification of the section in Europe, with subsequent dispersal to North America is suggested. In this phylogenetic model, *C. cryptolepis* roughly intermediate between an ancestral group including *C. flava*, *C. hostiana* and *C. durieui*, and the more recently derived subspecific taxa of *C. viridula*. *Carex cryptolepis* was hypothesized to have diverged in eastern North America from *C. flava* (Crins and Ball, 1989b) and Le Blond et al. (1994) suggested a close relationship between *C. lutea* and *C. cryptolepis*, but were careful to highlight the tentative nature of their conclusion. In a broad analysis of sectional relationships in *Carex* using ITS sequence data, Hiendrichs et al. (2004) found strong support for a monophyletic section

Ceratocystis (North American endemics were not included), with *C. sylvatica* as the sister taxon. They resolved some aspects of the within-section relationships, generally matching the hypothesized phylogeny of Crins and Ball (1988), but mention that some taxa were excluded from the analyses because of poor resolution.

2. Hypotheses

2.1 Genetic diversity: levels and apportionment

Given the putative common evolutionary history, similar ecology (habitat specificity, habitat patchiness, and disturbance regime), and similar breeding systems it is hypothesized that, due to narrow endemism, *C. lutea* will exhibit low levels of genetic diversity relative to the broad endemic *C. cryptolepis*.

2.2 Evolutionary relationships

An understanding of systematic relationships is critical to interpreting patterns of genetic diversity within the North American endemic species. In particular, the hypothesis that these species are a monophyletic group that diversified in North America from *C. flava* is addressed using analysis of allozyme allele frequency data, as well as nuclear ribosomal DNA sequence data sets.

3. Materials and Methods

3.1 Field collections

Samples of leaf tissue were collected for allozyme analysis from five of the eight known populations of *Carex lutea*, as well as 19 populations of *C. cryptolepis* from across its distribution (Table 1, Fig. 1). Individuals representing relevant taxa were sampled for nrDNA analyses (Table 2). Sampling of Pleistocene refugial populations of *C. cryptolepis* was considered a priority, since such populations were expected to maintain levels of genetic diversity higher than populations founded following Wisconsin glaciation. I obtained tissue from the only reported populations of *C. cryptolepis* south of the Last Glacial Maximum, occurring in the Edge of Appalachia Preserve, as well as from a number of populations along the southern extent of the species' distribution (Fig. 1). In several populations of mixed species composition (*C. cryptolepis* plus *C. flava* and/or *C. viridula* subsp. *viridula* var. *viridula*), limited sampling of non-target taxa was undertaken in order to ascertain whether gene flow occurs among taxa. The disjunct occurrence of *C. flava* in Virginia consists of three known populations in close proximity (although topography suggests limited gene flow); two of these populations were located and sampled. As species are caespitose, a discrete clump was treated as an

TABLE 1. Populations sampled for allozyme analyses. Five populations of *Carex lutea* Le Blond, 19 populations purported to be *C. cryptolepis*, and four populations of *C. flava* L. sampled for allozyme analysis in this study (collections by NJD and LPB during 2005 and 2006, as well as others previously).

	Site	Country	Latitude	Longitude	Abbreviation
Carex lutea	Haws Run Savanna	USA	NA	NA	HR
	Neck Savanna	USA	NA	NA	NS
	Sandy Run Savanna	USA	NA	NA	SRS
	Shaken Creek Savanna	USA	NA	NA	SC
	Watkins Savanna	USA	NA	NA	WS
C. cryptolepis	Cambridge	USA	39.7404	-84.0084	CM
	Irwin Prairie	USA	43.0050	-89.0194	IP
	Marl Flat	USA	38.7569	-83.4037	MF
	MN02	USA	40.0650	-85.3618	MNO2
	Muck Lake	USA	41.6580	-83.7799	ML
	Pic River	Canada	38.7646	-83.4129	PR
	Pickerel Creek	USA	42.3339	-88.1526	PC
	Sayles Road	USA	47.7430	-91.4186	SR
	Sloperville Bog	USA	46.3922	-91.5533	SB
	Spring Lake	USA	48.7005	-86.2441	SP
	Springville Marsh	USA	43.7417	-85.3917	SM
	Tuttle Marsh	USA	44.6371	-74.9382	TM
	Tyler Pond	USA	43.5035	-75.9553	TP
	UW Arboretum	USA	44.0860	-89.1907	UW
	Ankeney Fen	USA	41.0067	-83.4011	AF
	Cline Road	USA	44.3958	-83.4214	CR
	IMI Fen	USA	44.3954	-69.8226	IMI
	Lynx Prairie	USA	43.0535	-89.4185	LP
	Wilderness Road	USA	38.7785	-83.4006	WR
C. flava	Bergen Swamp	USA	43.0984	-77.9590	BS
	Sloperville Bog	USA	43.5035	-75.9553	SBF
	Walnut Flat	USA	37.1981	-80.8867	WF
	Woodbury	USA	44.4408	-72.4169	WB

individual. Populations were sampled exhaustively or systematically (e.g., at approximately 5 m intervals) depending on size. Field collections were maintained in cool, moist conditions until protein extraction. Tissue was also silica gel dried for DNA extraction. When sufficient tissue was available, a voucher for each specimen was retained; in the case of sensitive populations, a single individual served as voucher. This method allowed the cross-referencing of allozyme data, nuclear ribosomal DNA sequence data, and morphological characters for specific individuals comprising each population.

TABLE 2. Individuals sampled for DNA analyses. Taxa were sequenced at all or some of the nuclear ribosomal regions ETS-1f, ITS-1, 5.8S and ITS-2; sequence data for additional *Carex* section *Ceratocystis* taxa, as well as several outgroup taxa, were obtained from GenBank (only sect. *Ceratocystis* taxa and the three most relevant outgroup taxa are listed here).

	Site	Country		GenBank Accession Number	
				ITS	ETS
Carex lutea	Haws Run Savanna	USA	luteaHR07, luteaHR26	NA, NA	NA, NA
	Neck Savanna	USA	luteaNS19	NA	NA
	Watkins Savanna	USA	luteaOB36	NA	NA
	Sandy Run Savanna	USA	luteaPL08	---	NA
	Shaken Creek Savanna	USA	luteaSC18	---	NA
C. cryptolepis	Muck Lake	USA	cryptML08	NA	NA
	French River	Canada	cryptFR29	NA	NA
	6260502	USA	crypt0604	NA	NA
	Irwin Prairie	USA	cryptIP37	NA	NA
	Taylor Pond	USA	cryptTP03	---	NA
	Ankeney Fen	USA	lgcryptAF0	NA	NA
	Wilderness Road	USA	lgcryptLQ32	NA	NA
	IMI Fen	USA	lgcryptIMI38	---	NA
C. flava	Sloperville Bog	USA	flavaSB06	NA	NA
	Walnut Flat	USA	flavaWFB16	NA	NA
	BBFa	Canada	flavaBBFa02	NA	NA
	BBFb	Canada	flavaBBFb04	NA	NA
	Rosentorp	Sweden	flavaRT03	NA	NA
	Trunnahute	Austria	alpinaTH16	---	NA
	(GenBank)	Canada	GBflava03	AY757596	AY757657
	(GenBank)	Russia	GBflava04	AF285007	---
	(GenBank)	NA	GBflava02	DQ384144.1	---
	(GenBank)	Germany	GBflavaEU	AY278310	---
C. flaviformis	(GenBank)	New Zealand	GBflavi	AY699610.1	---
C. viridula	Green Lake	Canada	viridGL39	NA	NA
subsp. *viridula*	Asa	Sweden	viridAsa10	---	NA
var. *viridula*	(GenBank)	Switzerland	GBviridEU	AY278290	---
	(GenBank)	Canada	GBviridNA	AY278308	AY757658.1
	(GenBank)	Canada	GBvirid02	AY757597	---
subsp. *brachyrrhyncha*	Grunsee	Austria	elatiorGS10		
var. elatior	(GenBank)	NA	GBelatior1	DQ384164.1	---
	(GenBank)	Germany	GBelatior2	AY278293.1	---
subsp. *nevadensis*	(GenBank)	Spain	GBnevadens	DQ384172.1	---
subsp. *oedocarpa*	(GenBank)	Germany	GBdemissa1	AY278307.1	---
	(GenBank)	NA	GBdemissa2	DQ384119.1	---
C. hostiana	(GenBank)	France	GBhostiana	AY278309.1	---
C. sylvatica	(GenBank)	Switzerland	GBsylvatica	AY757660	AY757599
C. pendula	(GenBank)	UK	GBpendula	AY757661	AY757600
C. punctata	(GenBank)	UK	GBpunctata	AY757659	AY757598

3.2 Collection and analysis of allozyme data

Protein extractions followed standard methods effective for *Carex* section *Ceratocystis* (e.g., Bruederle and Jensen 1991). Approximately 1–2 cm^2 of leaf tissue were ground with washed sea sand and an extraction buffer consisting of 0.1 M tris-HCl buffer (pH 7.5), 20% polyvinylpyrrolidone (PVP-40), and 0.1% beta mercaptoethanol. Following absorption of the protein extract onto #17 chromatography paper (Whatman, Maidstone, England), wicks (2 X 12 mm) were stored at -70°C.

Electrophoresis was conducted using 11% starch gels (Starch Art, Austin, Texas, USA) with three gel-buffer systems (Bruederle and Fairbrothers, 1986; Bruederle and Jensen, 1991). A histidine-HCl system (constant current, 130 ma) consisted of a 0.02 M L-histidine-HCl gel buffer (pH 7.0) and a 0.4 M solution of citric acid trisodium hydrate as electrode buffer (pH 8.0) (Gottlieb, 1981). A lithium-borate system (constant voltage, 270 V) consisted of a 0.02 M tris, 0.007 M citric acid monohydrate, 0.004 M lithium hydroxide, and 0.025 M boric acid gel buffer and a 0.263 M boric acid and 0.039 M lithium hydroxide electrode buffer (Soltis et al., 1983). A tris-citrate system (constant current, 60 ma) consisted of a 0.223 M tris and 0.086 M citric acid monohydrate electrode buffer (pH 7.5); a 3.5% dilution of the electrode buffer was used as the gel buffer (Solits et al., 1983).

Enzyme systems coded by 19 putative loci were visualized using 14 substrate specific stains: isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and shikimic acid dehydrogenase (SDH) were stained on the histidine-HCl system; alcohol dehydrogenase (ADH), diaphorase (DIA), malic enzyme (ME), menadione reductase (MNR), superoxide dismutase (SOD), and triose phosphate isomerase (TPI) were stained on the lithium-borate system; aspartate aminotransferase (AAT) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were stained on the tris-citrate system. Stain recipes for IDH, MDH, PGI, PGM, SDH, G3PDH, ME and TPI followed Soltis et al. (1983); ADH and PGD followed Gottlieb (1973); and AAT followed Cardy et al. (1981). Minor modifications to recipes were rarely made in order to reduce cost or improve staining.

Standards were selected that, collectively, represented all observed allelic variation, facilitating allele identification. A small number of individuals from *C. viridula* subsp. *viridula* var. *viridula* populations sampled by Kuchel and Bruederle (2000), as well as several individuals collected for this study, were used to identify alleles present in North American populations in relation to the species included here. Individual genotypes were interpreted from allozyme phenotypes as reported in Bruederle and Fairbrothers (1986).

These data were analyzed using the application GDA (Lewis and Zaykin, 2002), generating the following statistics: proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (A_p), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (*f*), Nei's unbiased genetic identity (I) (1978), and Wright's F-statistics (F_{IS} and F_{ST}) (1965). For the F-statistics (F_{IS} and F_{ST}), 95% confidence intervals were produced by bootstrapping across loci and the standard deviation for each locus was calculated by jackknifing across populations (as implemented in GDA). Inbreeding coefficients (*f*) were calculated for those loci that were polymorphic in each population using GDA (method of moments estimate); deviations from Hardy-Weinberg equilibrium were tested for significance by chi-squared analysis. The null hypothesis of no difference between values of genetic diversity measures (P, A_p, H_o, and H_e) was tested using the Wilcoxon rank sum test with continuity correction.

3.3 Nuclear ribosomal DNA sequence data

Sequencing of nuclear ribosomal regions (ITS and ETS) was undertaken to provide insight into the evolutionary history of *Carex* section *Ceratocystis*, particularly *C. lutea* and *C. cryptolepis*. Genomic DNA was extracted from either silica gel dried tissue or pressed specimens using the DNeasy Plant Mini Kit (Qiagen, Valenica, California, USA). Amplification of nrDNA regions was carried out in 25 µl reaction mixtures of: 2.5 µl 10X

reaction buffer; 1.5 µl 25 mM $MgCl_2$; 1.0 µl 25 mM dNTPs, equimolar ratio; 0.25 µl 1U/µl *Taq*; 1.5 µl 10 µM forward primer; 1.5 µl 10 µM reverse primer; 1 µl of approximately 10 ng/µl sample DNA; and 15.75 µl water. Primers for the internal transcribed spacer regions (ITS-1 and ITS-2) and the intervening 5.8S ribosomal subunit were ITS 4i and ITS 5i (Roalson et al., 2001 and references therein); and primers for a portion of the five prime end of the intergenic spacer (ETS 1f) were 18Sr and ETS1f (Starr et al., 2003). Thermal cycler conditions were the same for both regions: an initial denaturation at 95°C for two minutes, followed by 32 cycles of denaturation (95°C for one minute), primer annealing (55°C for one minute), and strand extension (72°C for one minute), and a final extension at 72°C for ten minutes. Amplification products were purified using ExoSAP-IT (USB, Cleveland, OH, USA) according to a modification of the manufacturer's protocol involving reduction in enzyme concentration and increase in incubation time. Sequencing of both forward and reverse strands was performed at the Rocky Mountain Center for Conservation Genetics and Systematics (University of Denver, Denver, Colorado, USA) on a Beckman Coulter CEQ 8000 Genetic Analysis System following cycle-sequencing with the GenomeLab DTCS Quick Start Kit for Dye Terminator Cycle Sequencing (Beckman Coulter, Fullerton, California, USA).

3.4 Phylogenetic analyses

Forward and reverse sequences of each strand were assembled and edited in Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned in ClustalX (Thompson et al., 1997). Sequences from taxa within section *Ceratocystis* available on Genbank were used to determine the extent of the regions. GenBank accessions for species from section *Spirostachyae*, as well as taxa identified by Hendrichs et al. (2004) as being allied with section *Ceratocystis* were included in the ITS data set. For the ETS data set a BLAST search was conducted, and GenBank accessions for taxa identified as having similar sequence were included in analyses. The Φ test for recombination (Bruen et al., 2006), implemented by SplitsTree4 4.6 (Huson and Bryant, 2006), was performed as a means of detecting hybridization or introgression. Alignments were exported to PHYLIP 3.66 (Felsenstein, 2004) as separate ITS and ETS data sets, and as a combined data set; each sequence data set was input into SEQBOOT to produce 1000 bootstrap replicates, and DNADIST, on default settings, was used to produce F84 genetic distances. The sequence based distance matrices were analyzed using NEIGHBOR, an implementation of the Neighbor-Joining distance method. One thousand bootstrap replicates of the allozyme allele frequency data set were produced using SEQBOOT; GENDIST, with the option for all alleles present, was used to produce a matrix of Nei's (1972) genetic

distances. The allozyme allele frequency based distance matrix was analyzed using KITSCH, an implementation of the Fitch-Margoliash distance method assuming a molecular clock. CONSENSE was used to produce a majority-rule (extended) consensus tree for each data set. Trees were viewed and saved as images using the program TreeView 1.6.6 (Page, 2001).

4. Allozyme diversity and phylogenetic observations

4.1 A new species of *Carex* section *Ceratocystis*

Herein is reported population genetic data consistent with a taxon new to science. Putative refugial populations of *C. cryptolepis* (Ankeney Fen, IMI Fen, Cline Road, Lynx Prairie, and Wilderness Road) were fixed at all loci, with no infraspecific variation. These populations are genetically distinct from other North American *Carex* section *Ceratocystis* taxa; multiple fixed differences occur in *C. lutea* and *C. cryptolepis*, as well as North American populations of *C. flava* and *C. viridula* subsp. *viridula* var. *viridula* (Table 3). Additionally, three sterile individuals from the Springville population of *C. cryptolepis* were heterozygous at the loci fixed for unique alleles in "refugial" populations. Motivated by the observed allelic patterns, a thorough morphological inspection revealed a number of subtle characters differentiating "refugial" specimens from the rest of *C. cryptolepis*. Ecology was also unique, including seeps in prairie openings with soils derived from dolomite bedrock. As such, these "refugial" populations were assigned to a new species of *Carex* section *Ceratocystis*, hereafter referred to as "C. viridistellata," and analyzed separately. Mean pairwise genetic identity between populations of *C. cryptolepis* and "*C. viridistellata*" (I = 0.86) was

TABLE 3. Allelic data. Population allele frequency data (variable loci) for *C. cryptolepis, C. flava, C. lutea, "C. viridistellata,"* and *C. viridula* subsp. *viridula* var. *viridula* (standardized data from Kuchel and Bruederle, 2000). Abbreviations follow Table 1.

		AAT		ADH			DIA-1		IDH-1		IDH-2				MDH-2		MDH-3	
		a	b	a	b	c	a	b	a	b	a	b	c	d	a	b	a	b
Carex lutea	HR	0.44	0.56	0.10	0.89	0.01	0.60	0.40	1.00	0.00	0.00	0.98	0.00	0.02	0.00	1.00	1.00	0.00
	NS	0.30	0.70	0.00	1.00	0.00	0.98	0.02	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00
	SRS	1.00	0.00	0.00	1.00	0.00	0.33	0.67	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	1.00	0.00
	SC	0.98	0.02	0.00	1.00	0.00	0.66	0.34	1.00	0.00	0.01	0.99	0.00	0.00	0.00	1.00	1.00	0.00
	WS	0.15	0.85	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.92	0.06	0.01	0.00	1.00	1.00	0.00
C. cryptolepis	CM	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00
	IP	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
	MF	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
	MN02	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.36	0.00	0.64	0.00	1.00	0.00	1.00
	ML	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.76	0.24	0.00	1.00	0.00	1.00
	PR	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
	PC	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.50	0.50	0.00	1.00	0.00	1.00
	SR	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00
	SL	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
	SB	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.97	0.03	0.00	1.00	0.00	1.00
	SV	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	1.00
	TM	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00
	TP	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00
	UW	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00
"C. viridistellata"	AF	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00
	CL	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00
	IMI	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00
	LP	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00
	WR	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00
C. flava	BS	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
	SBF	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
	WFA	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
	WB	0.00	1.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00
C. viridula subsp. *viridula* var. *viridula*	VIR	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00

TABLE 3. Continued.

		PGD			PGI		PGM-1					SDH			SOD		
		a	b	c	a	b	a	b	c	d	e	a	b	c	a	b	c
Carex lutea	HR	1.00	0.00	0.00	0.00	1.00	0.00	0.76	0.24	0.00	0.00	0.00	1.00	0.00	0.09	0.91	0.00
	NS	1.00	0.00	0.00	0.00	1.00	0.02	0.93	0.04	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
	SRS	1.00	0.00	0.00	0.00	1.00	0.00	0.13	0.88	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
	SC	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00
	WS	1.00	0.00	0.00	0.00	1.00	0.00	0.31	0.69	0.00	0.00	0.00	1.00	0.00	0.02	0.98	0.00
C. cryptolepis	CM	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	IP	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	MF	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	MN02	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	ML	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	PR	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	PC	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	SR	1.00	0.00	0.00	0.02	0.98	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	SL	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	SB	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	SV	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	TM	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	TP	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
	UW	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	1.00	0.00
"C. viridistellata"	AF	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	CL	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	IMI	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	LP	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
	WR	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	1.00	0.00
C. flava	BS	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
	SBF	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
	WFA	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
	WB	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00	0.00	0.00	1.00
C. viridula subsp. *viridula* var. *viridula*	VIR	0.00	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	1.00	0.00	0.00

lower than between populations of a species (I > 0.90), although higher than between *C. cryptolepis* and *C. lutea* (I = 0.81); between "*C. viridistellata*" and *C. lutea*, I = 0.69. A more complete investigation is warranted, but it appears that the new species is a relatively rare endemic intermediate in distribution between *C. cryptolepis* and *C. lutea* (in terms of extent as well as geography).

4.2 Allozyme genetic diversity

Of the 14 substrate specific stains, 13 produced interpretable banding patterns. ME could not be consistently resolved for *C. cryptolepis*, and was removed from data analysis. A total of 18 loci was resolved in all taxa: *Aat-1, Adh, Dia-1, G3pdh, Idh-1, Idh-2, Mdh-1, Mdh-2, Mdh-3, Mnr, 6Pgd-1, Pgi-2, Pgm-1, Pgm-2, Sdh, Sod-1, Tpi-1*, and *Tpi-2*.

Six loci were polymorphic (the most common allele occurred at a frequency less than 0.99) in *C. lutea*: *Aat-1, Adh, Dia-1, Idh-2, Pgm-1*, and *Sod-2*; in contrast, only two loci were polymorphic in *C. cryptolepis* (*Idh-2* and *Pgi-2*), while "*C. viridistellata*" maintained no polymorphic loci (Appendix). The limited sampling of North American populations of *C. flava* did not reveal any polymorphic loci, and samples from even great geographic distances were identical at the assayed loci. Descriptive statistics for populations and taxa are reported in Table 4. Proportion of loci polymorphic (P) in populations of *C. lutea* ranged from P = 11.1 – 33.3%, with a mean of P = 20.0%; this was significantly higher than observed in *C. cryptolepis* (P =

2.0% [0.0% – 5.6%]; Wilcoxon Rank Sum test, $p < 0.001$) or "C. viridistellata" (P = 0.0%; Wilcoxon Rank Sum test, $p < 0.01$). Number of alleles per polymorphic locus (A_p) in populations of *C. lutea* ranged from A_p = 2.00 – 2.33, with a mean of A_p = 2.15; at the species level, A_p ranged from A_p = 2.00 – 4.00. Number of alleles per polymorphic locus in populations of *C. cryptolepis* never exceeded A_p = 2.00, although A_p ranged from A_p = 2.00 – 3.00 at the species level.

Observed and expected heterozygosities were both significantly higher (Wilcoxon Rank Sum test, $p < 0.01$) in *C. lutea* (H_o = 0.028 [0.016 – 0.060], H_e = 0.049 [0.028 – 0.098]) than in *C. cryptolepis* (H_o = 0.003 [0.000 – 0.014], H_e = 0.006 [0.000 – 0.028]) or the new species (H_o = 0.000 and H_e = 0.000) (Table 4). Large positive inbreeding coefficients (*f*) were generally correlated with significant deviations (chi-square test, $p < 0.05$) from Hardy-Weinberg equilibrium (Table 5). No locus in any population had a negative inbreeding coefficient that differed significantly from Hardy-Weinberg expectations.

A large amount of genetic diversity was apportioned among populations of *C. lutea*, with an overall F_{ST} = 0.412; population differentiation was greater in *C. cryptolepis*, with an overall F_{ST} = 0.796, although the 95% CI (0.003 – 0.800) overlapped that of *C. lutea* (0.052 – 0.513) (Table 6). Neither *C. flava* nor *"C. viridistellata"* showed population differentiation.

TABLE 4. Summary of genetic diversity statistics. P = proportion of loci polymorphic; A_p = alleles per polymorphic locus expected; H_e = heterozygosity; H_o = observed heterozygosity; N = mean sample size per population (across loci). Mean values reported for populations of *Carex viridula* subsp. *viridula* var. *viridula* and European populations of *C. flava* are from Kuchel and Bruederle (2000) and Bruederle and Jensen (1991), respectively.

		N	P	A_p	H_e	H_o
Carex lutea	Haw's Run Savanna	40.8	0.333	2.167	0.098	0.060
	Neck Savanna	22.9	0.167	2.333	0.033	0.017
	Sandy Run Savanna	12.7	0.111	2.000	0.038	0.023
	Shaken Creek Savanna	45.8	0.167	2.000	0.029	0.016
	Watkins Savanna	38.3	0.222	2.250	0.049	0.025
	mean		**0.200**	**2.150**	**0.049**	**0.026**
C. cryptolepis	Cambridge	19.0	0.000	***	0.000	0.000
	Irwin Prairie	9.0	0.000	***	0.000	0.000
	Marl Flat	22.0	0.000	***	0.000	0.000
	MN02	25.0	0.056	2.000	0.026	0.013
	Muck Lake	48.9	0.056	2.000	0.021	0.009
	Pic River	25.0	0.000	***	0.000	0.000
	Pickerel Creek	24.0	0.056	2.000	0.028	0.014
	Sayles Road	24.9	0.056	2.000	0.002	0.002
	Sloperville Bog	15.0	0.056	2.000	0.004	0.004
	Spring Lake	49.8	0.000	***	0.000	0.000
	Springville Marsh	46.8	0.000	***	0.000	0.000
	Tuttle Marsh	28.0	0.000	***	0.000	0.000
	Tyler Pond	24.8	0.000	***	0.000	0.000
	UW Arboretum	34.2	0.000	***	0.000	0.000
	mean		**0.020**	**2.000**	**0.006**	**0.003**
C. "viridistellata"	Ankeney Fen	25.8	0.000	***	0.000	0.000
	Cline Road	24.0	0.000	***	0.000	0.000
	IMI Fen	25.0	0.000	***	0.000	0.000
	Lynx Prairie	18.0	0.000	***	0.000	0.000
	Wilderness Road	59.0	0.000	***	0.000	0.000
	mean		0.000	***	**0.000**	**0.000**
C. flava	Bergen Swamp	25.0	0.000	***	0.000	0.000
	Sloperville Bog	7.9	0.000	***	0.000	0.000
	Walnut Flat	21.9	0.000	***	0.000	0.000
	Woodbury	25.0	0.000	***	0.000	0.000
	mean		**0.000**	***	**0.000**	**0.000**
C. viridula	North America (529 individuals)		**0.000**	***	**0**	**0**
C. viridula	Europe (47 individuals)		**0.100**	**2.000**	**0.039**	**0.014**
C. flava	Europe (184 individuals)		**0.135**	**2.000**	**0.0248**	**0.0048**

TABLE 5. Measures of inbreeding. Population level inbreeding indices (*f*) at polymorphic loci for five populations of *Carex lutea* Le Blond (narrow endemic) and 14 populations of *C. cryptolepis* Mack. (broad endemic); significant deviations ($p < 0.05$) from Hardy-Weinberg equilibrium are indicated by italicized bold text.

		AAT	ADH	DIA-1	IDH-2	PGI	PGM-1	SOD
Carex lutea	Haw's Run Savanna	***0.416***	0.393	***0.386***	-0.013	***	***0.350***	***0.540***
	Neck Savanna	***0.472***	***	0.000	***	***	***0.662***	***
	Sandy Run Savanna	***	***	0.290	***	***	***0.645***	***
	Shaken Creek Savanna	-0.011	***	***0.503***	0.000	***	***	***
	Watkins Savanna	***0.614***	***	***	0.298	***	***0.528***	0.000
C. cryptolepis	MN02	***	***	***	***0.495***	***	***	***
	Muck Lake	***	***	***	***0.566***	***	***	***
	Pickerel Creek	***	***	***	***0.516***	***	***	***
	Sayles Road	***	***	***	***	0.000	***	***
	Sloperville Bog	***	***	***	0.000	***	***	***

For both *C. lutea* and *C. cryptolepis*, a large amount of genetic diversity was maintained among individuals, rather than within individuals as heterozygosity ($F_{IS} = 0.427$ and $F_{IS} = 0.509$, respectively), but for *C. cryptolepis* the 95% CI (-.003 – 0.520) indicates no difference (Table 5).

4.3 Nuclear ribosomal DNA sequence results

Seventeen individuals representing six taxa were successfully sequenced for both the ITS and ETS nrDNA regions, while a number of individuals were sequenced for only one region or the other; sequence data for additional ingroup taxa as well as outgroup taxa were obtained from GenBank (Table 2). The Φ test failed to detect significant evidence of recombination in the ITS and ETS regions ($p = 0.6412$), and visual inspection of the sequence data did not reveal obvious indications of recombination.

TABLE 6. Population differentiation. Wright's (1965) *F* statistics (F_{IS} and F_{ST}) for five populations of *Carex lutea* Le Blond (narrow endemic) and fourteen populations of *C. cryptolepis* Mack. (broad endemic); standard deviation (sd) for each locus was calculated by jackknifing across populations and 95% confidence intervals (95% CI) were calculated by bootstrapping across loci.

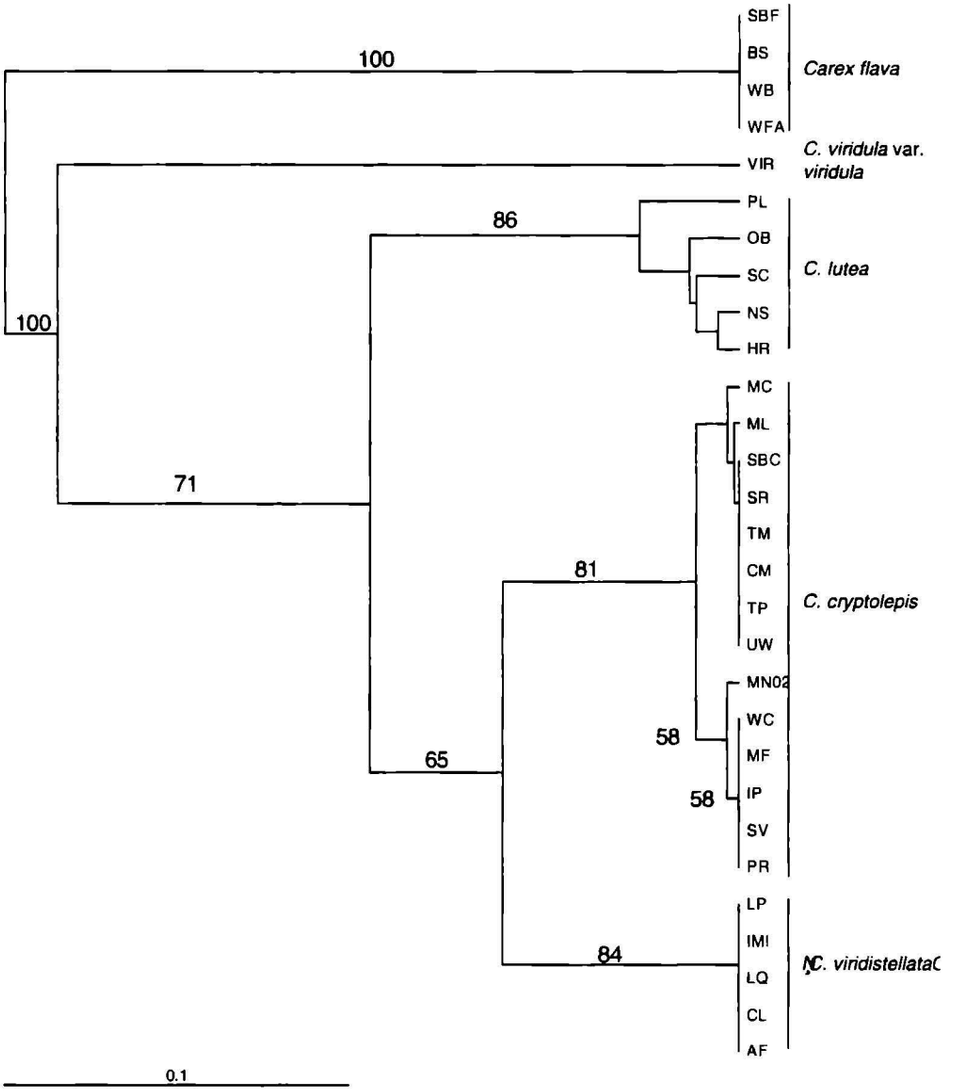
	Carex lutea				*C. cryptolepis*			
Locus	F_{IS}	(sd)	F_{ST}	(sd)	F_{IS}	(sd)	F_{ST}	(sd)
AAT	0.458	0.061	0.534	0.251	***	***	***	***
ADH	0.395	0.002	0.078	0.009	***	***	***	***
DIA-1	0.417	0.066	0.267	0.177	***	***	***	***
G3PDH	***	***	***	***	***	***	***	***
IDH-1	***	***	***	***	***	***	***	***
IDH-2	0.196	0.183	0.022	0.022	0.520	0.038	0.800	0.102
MDH-1	***	***	***	***	***	***	***	***
MDH-2	***	***	***	***	***	***	***	***
MDH-3	***	***	***	***	***	***	***	***
MNR	***	***	***	***	***	***	***	***
PGD	***	***	***	***	***	***	***	***
PGI	***	***	***	***	-0.003	0.002	0.004	0.002
PGM-1	0.475	0.087	0.511	0.166	***	***	***	***
PGM-2	***	***	***	***	***	***	***	***
SDH	***	***	***	***	***	***	***	***
SOD	0.472	0.395	0.042	0.039	***	***	***	***
TPI-1	***	***	***	***	***	***	***	***
TPI-2	***	***	***	***	***	***	***	***
Mean	**0.432**		**0.412**		**0.509**		**0.796**	
95% CI	(0.351 - 0.468)		(0.050 - 0.511)		(-0.005 - 0.531)		(0.006 - 0.737)	

4.4 Phylogenetic Analyses

4.4.1 Allozyme based phylogeny

Species of section *Ceratocystis* occurring in North America, for which allozyme data are available, were well supported as distinct lineages in the Fitch-Margoliash tree (Fig. 2). *Carex viridula* subsp. *viridula* var. *viridula* was analyzed as a single taxonomic unit, rather than as multiple populations, as no populations were actually sampled for this research; consequently that branch does not have bootstrap support. That all other populations cluster as species, however, provides support for the distinctness of *C. viridula* subsp.

FIGURE 2. ALLOZYME BASED PHYLOGENY. Neighbor-joining tree from 1000 bootstrap replicates of Nei's genetic distances (1972) calculated from allozyme allele frequency data; numbers above branches, or to the left of nodes, indicate percent bootstrap support.



viridula var. *viridula*. Weak support is observed for a sister relationship between the new species and *C. cryptolepis*, while moderate support is observed for a monophyletic group of North American endemics. Branch length is scaled to genetic distance, and indicates population level differentiation might be relatively recent in *C. lutea* and *C. cryptolepis*. Weak support for a clade within these recently diverged populations of *C. cryptolepis* is observed.

4.4.2 ITS and ETS based phylogenies

Carex section *Ceratocystis* is strongly supported as a monophyletic group in the ITS tree (Fig. 3). *Carex lutea* and the new species receive weak support as sister taxa, *C. hostiana* received weak support for an ancestral position, and the remaining taxa appear as a moderately supported clade. Relationships within the *C. cryptolepis*, *C. flava* and *C. viridula* clade are generally unresolved. It is surprising to see some specimens of *C. flava* cluster together with such strong support, while other specimens occur in a polytomy with *C. viridula* taxa and *C. cryptolepis*. In the ETS tree (Fig. 4), *C. lutea* and the new species again appear as sister taxa, but with much stronger support than observed in the ITS tree. Relationships among the other taxa are poorly resolved, with *C. cryptolepis* forming an unsupported clade within a weakly supported clade of *C. viridula* taxa and populations of a paraphyletic *C. flava*.

FIGURE 3. NRDNA (ITS) BASED PHYLOGENY. Neighbor-joining tree from 1000 bootstrap replicates of F84 distances of ITS-1, 5.8S, and ITS-2 nrDNA sequence data; numbers indicate percent bootstrap support. *C. cryptolepis* is polyphyletic.

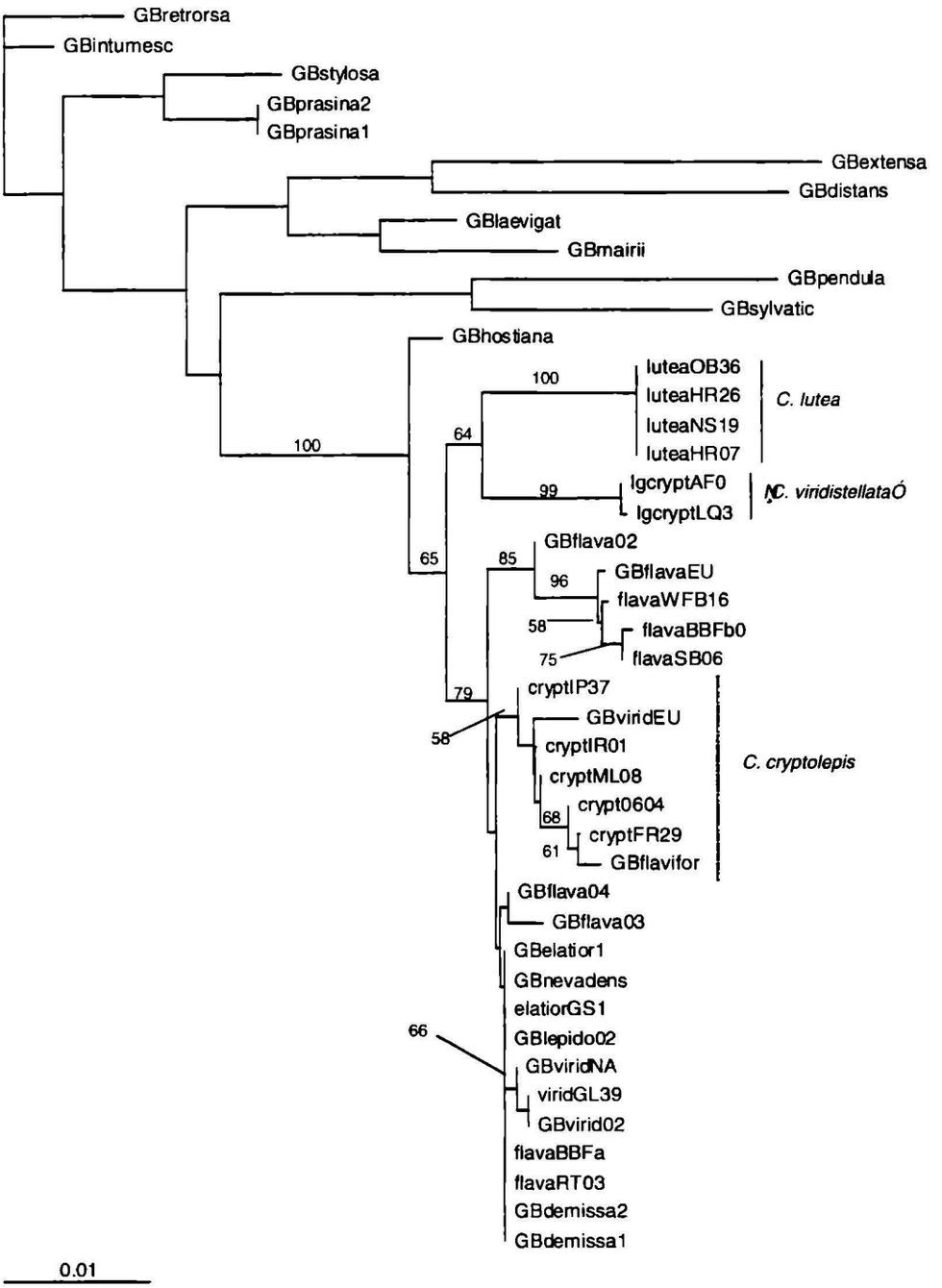
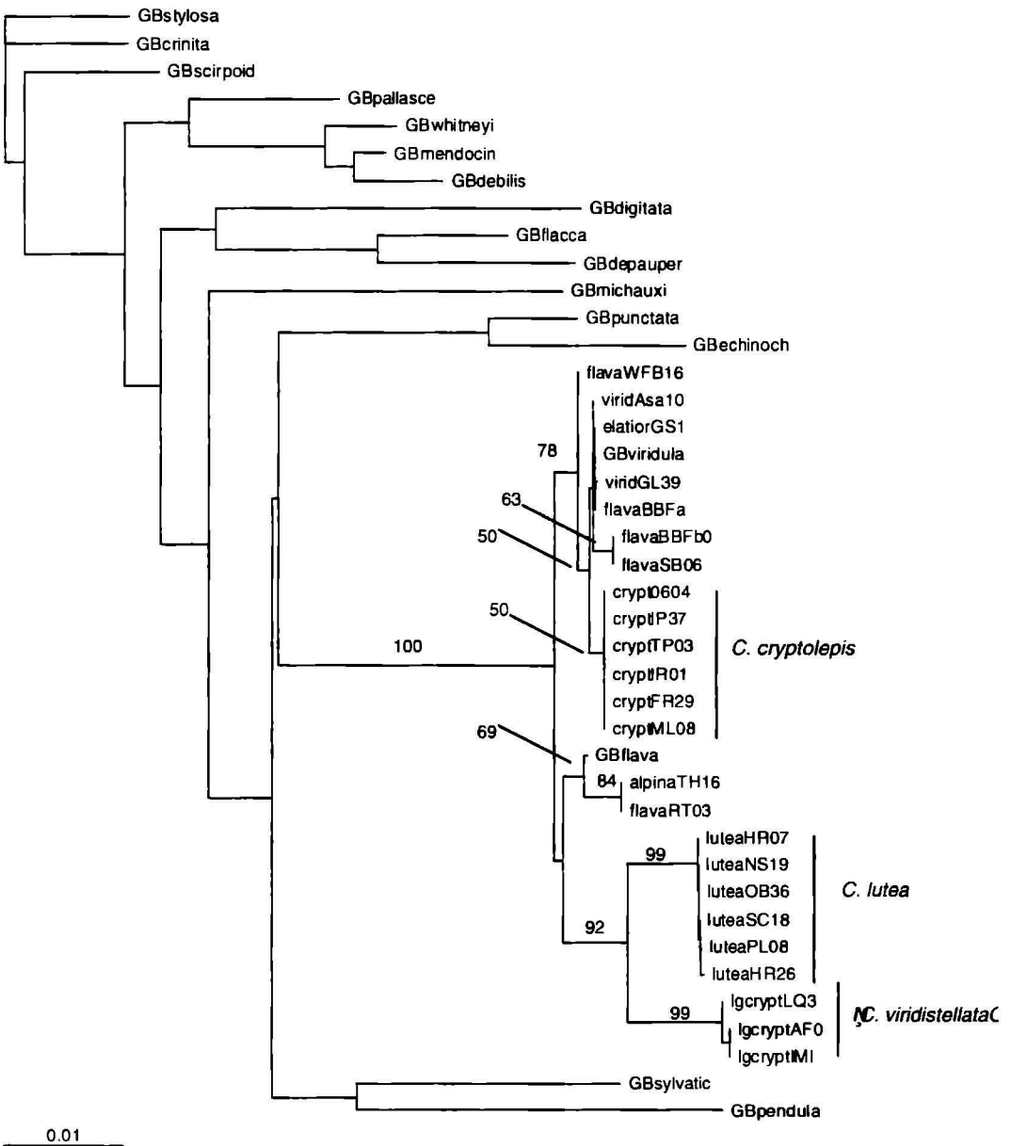
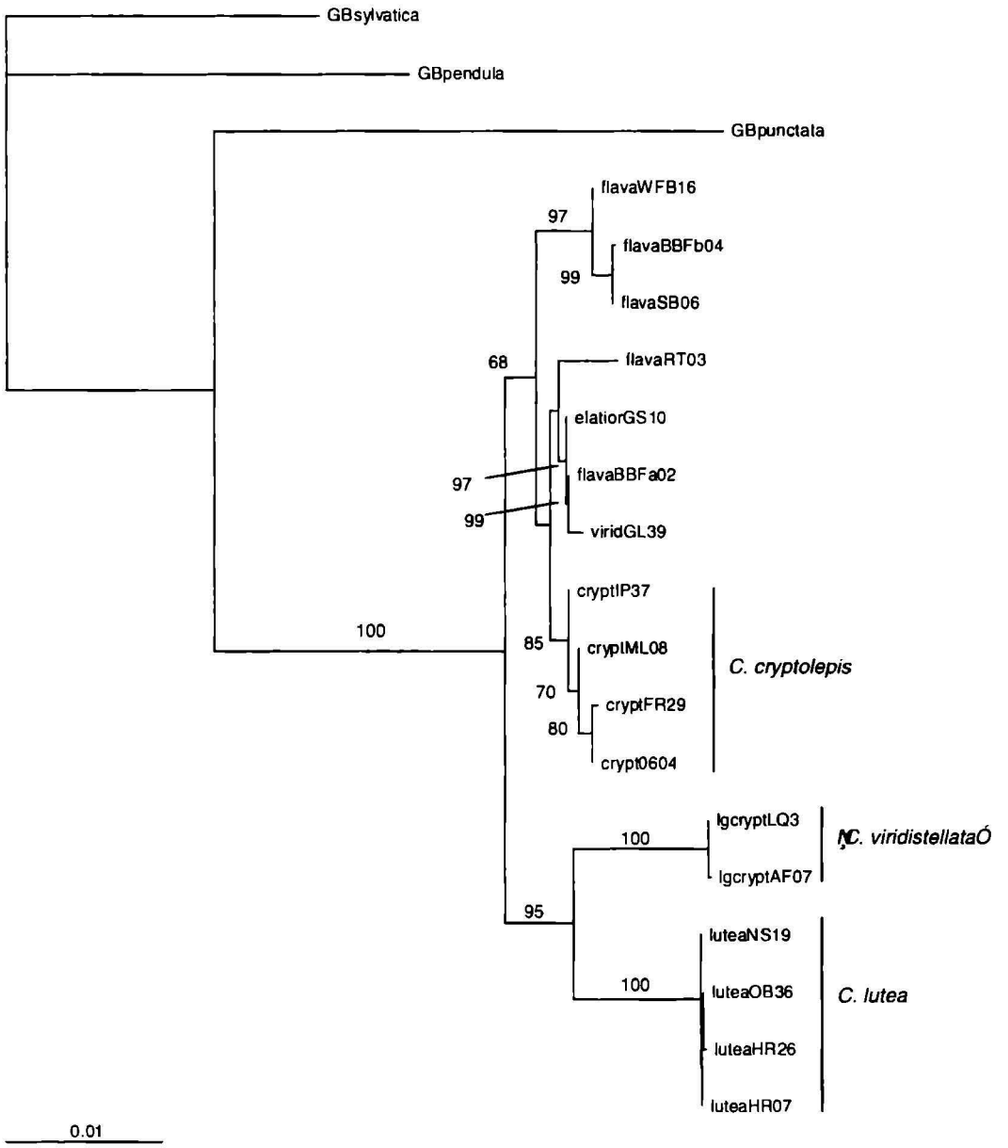


FIGURE 4. NRDNA (ETS) BASED PHYLOGENY. Neighbor-joining tree from 1000 bootstrap replicates of F84 distances of ETS-1f nrDNA sequence data; numbers indicate percent bootstrap support.



The combined ITS/ETS tree provides strong support for *C. lutea* and the new species as sister species, with support for the clade as sister to all other taxa in section *Ceratocystis* (Fig. 5); it should be noted that species considered basal in the group, i.e., *C. hostiana* and *C. durieui*, were not included in the combined analysis, due to a lack of tissue and/or Genbank accessions. Individuals of *C. cryptolepis* cluster together as a well supported clade, though appearing as part of a poorly resolved clade including *C. flava* and *C. viridula*.

FIGURE 5. NRDNA (ETS, ITS) BASED PHYLOGENY. Neighbor-joining tree of the combined nrDNA data sets; percent bootstrap support of 1000 replicates of the F84 distances is indicated above the branch, or to the left of the branch.



5. Distribution, a poor predictor of genetic diversity

Carex section *Ceratocystis* includes three North American endemic species: *C. lutea*, restricted to Pender and Onslow Counties, North Carolina; a previously unrecognized species of unknown extent with populations in Ohio, Indiana, and Southern Michigan (Derieg et al., manuscript in preparation; Reznicek, University of Michigan, personal observation); and *C. cryptolepis*, broadly distributed across the Great Lakes Region and northeastern North America. Despite its narrow endemism, *C. lutea* maintains high levels of genetic diversity relative to the other North American endemics in this section. Summarizing, populations of *C. lutea* exhibited 2 – 6 polymorphic loci, whereas nine of the populations of *C. cryptolepis* and all of the populations of the new species were fixed across all loci. *Carex lutea* also maintains a higher number of alleles per polymorphic locus. Although *C. lutea* is characterized by the persistence of few populations occupying a highly specific habitat within a small region, levels and apportionment of genetic diversity are not remarkably different from other caespitose carices; in fact, the number of polymorphic loci is somewhat higher than average (Bruederle et al., in press). It appears that factors other than relative extent of geographic distribution are responsible for the observed patterns of genetic

diversity; possibly breeding system, hybridization and/or additional aspects of recent evolutionary history, e.g., Pleistocene climate change.

5.1 Breeding system and genetic diversity

Breeding system can influence patterns of genetic diversity (Hamrick et al., 1979; Purdy et al., 1994). Self-compatible species with caespitose habit are expected to experience frequent inbreeding, which can lead to loss of alleles (with fixation across a higher percentage of loci) and increased population differentiation. The primary differences observed between populations of *C. lutea* and *C. cryptolepis* with respect to genetic diversity involved number of loci polymorphic, number of alleles at polymorphic loci, and degree of population differentiation; however, levels of inbreeding did not differ significantly. Additionally, levels and apportionment of genetic diversity in *C. lutea* are not remarkably different from other caespitose carices (higher rates of inbreeding), although they are lower than seen in rhizomatous carices (higher rates of outcrossing) (Bruederle et al., in press). Growth habit and genetic data both point to typical levels of inbreeding in *C. lutea*; as such, it does not appear that a shift toward increased outcrossing is responsible for maintaining genetic diversity in the species.

5.2 A possible history of hybridization?

Gene flow between taxa can have a profound impact on genetic diversity: past introgression or a hybrid origin for *C. lutea* would account for

the species' high genetic diversity relative to other North American *Ceratocystis*. Hybridization is fairly common in section *Ceratocystis*, although hybrids between *C. cryptolepis* and *C. viridula* are reported to be sterile (Crins and Ball, 1989b; Derieg and Bruederle, personal observation), as are hybrids between *C. cryptolepis* and the new species (Derieg and Bruederle, personal observation). Schmid (1984) stated that taxa within section *Ceratocystis* occurring in Switzerland (*C. flava*, *C. viridula* subsp. *brachyrryncha*, *C. viridula* subsp. *oedocarpa*, and *C. viridula* subsp. *viridula*) are able to form variably fertile hybrids and backcrosses. Pleistocene hybridization is described as having "injected high levels of haplotype diversity" into the narrow endemic *Packera sanguisorboides* (Rydb.) W.A. Weber & Á. Löve (Asteraceae), with subsequent genetic drift leading to population differentiation (Bain and Golden, 2003). Furthermore, introgression between subspecies of *Carex curvula* All. has been linked to the exploitation of marginal habitat (Choler et al., 2004).

Allozyme data provide some support for this hypothesis. At a number of loci where fixed allelic differences occur between species pairs, e.g., North American populations of *C. flava* and *C. viridula* subsp. *viridula* var. *viridula*, *C. lutea* maintains both alleles and, occasionally, unique alleles (Derieg and Bruederle, unpublished data). An alternative explanation for shared alleles among species is common ancestry. The combined ITS/ETS data set suggests

that the later is more likely; the position of *C. lutea* and the new species as a sister clade to the other included *Ceratocystis* taxa does not suggest a hybrid origin. Additionally, neither the Φ test nor visual inspection of the sequence data provided evidence of recombination.

5.3 Complex glacial/post-glacial histories

The glacial history of Eastern North America has undoubtedly confounded the relationship between degree of endemism and level of genetic diversity in the North American endemics of *Carex* section *Ceratocystis*. The distribution of *C. cryptolepis* does not extend south of the last glacial maximum (LGM), which occurred during the Wisconsin glaciation roughly 20 000 years before present (ybp). Three sampled populations of the new species, previously thought to be refugial populations of *C. cryptolepis*, occur just south of the LGM, in the Edge of Appalachia Preserve (The Nature Conservancy) and surrounding area. The other two populations sampled are in formerly glaciated regions, though near the southern extent of the ice. Both species would have persisted during Pleistocene glaciations in ice free refugia. Following the LGM, retreat of the ice proceeded from 18 000 ybp until 7000 ybp, with species' distributions expanding into newly available habitat (Hewitt, 2000; Pielou, 1991). Dispersal from refugial populations established new populations expected to possess a subset of the original allelic variation, which subsequently expanded; this "leading-edge" of populations would have

filled available habitat, reducing the ability of other populations to effectively invade (Hewitt, 2000). Modeling of such a dispersal pattern produces low genetic diversity and a large degree of population differentiation (Ibrahim et al., 1996).

The general expectation that post-glacial migration leads to lower levels of genetic diversity and increased population differentiation has been confrimed previously (e.g., Waller et al., 1987; Lewis and Crawford, 1995; Broyles, 1998; Boys et al., 2005), although it is certainly not the rule (e.g., Griffin and Barrett, 2004). While no refugial populations of *C. cryptolepis* were available for this study and no estimate of loss of genetic diversity can be made, it seems likely that post-glacial dispersal of populations led to a loss of alleles (and a consequential increase in the number of fixed loci) through founder effect; the degree of population differentiation observed in *C. cryptolepis* meets expectations of leptokurtic dispersal, as well. Climatic shifts induced other events that conceivably would have resulted in distributional shifts and influenced genetic diversity in these taxa; the formation of the Prairie Peninsula might have been such an event, and likely would have impacted *C. cryptolepis* and the new species differently.

While the Pleistocene histories of *C. cryptolepis* and the new species likely contributed to the loss of genetic diversity in these taxa, the geographic distribution of *C. lutea* may have limited the impact of climate change. The

distribution of *C. lutea* is roughly 700 km south of the main distribution of section *Ceratocystis* in North America, though a disjunct occurrence of *C. flava* is approximately 400 km distant (Le Blond et al, 1994; Wieboldt et al, 1998). This unique distribution is one of the most prominent differences between *C. lutea* and the other North American endemics. *Carex lutea* might at one time have been a broadly distributed species, with Pleistocene climate change effecting a great contraction of the distribution. Given such a scenario, where founder effect and bottlenecks are minimized, it is possible that levels of genetic diversity would have been maintained to some degree. A similar history has been proposed for *Polystichum otomasui* Sa. Kurata (Dryopteridaceae), a narrow endemic perennial fern of Japan with high levels of genetic diversity (Maki and Asada, 1998). Alternatively, populations of the common ancestor to *C. lutea* and the new species could have been isolated in refugia separated by the Appalachian Mountains, and subsequently diverged, as suggested for numerous other organisms (e.g., Church et al., 2003; Parker et al., 1997). As stated earlier, refugial populations can be expected to maintain relatively high levels of genetic diversity. If *C. lutea* diverged locally from a broadly distributed progenitor species during a glacial period, it would be expected to maintain a subset of the genetic variation of the parental species; at one extreme, Linhart and Premoli (1993) observed no major loss of

genetic diversity in *Aletes humilis* Coulter and Rose (Apiaceae) relative to its putative progenitor *A. acaulis* (Torrey) Coulter and Rose.

6. Conclusions

Europe is thought to have been the region of original radiation in the section (Crins and Ball, 1989b), and dispersal into North America seems likely to have involved bottlenecks and founder effect (e.g., no genetic diversity observed in North American populations of *C. viridula* subsp. *viridula* var. *viridula* [Kuchel and Bruederle, 2000]). The level of genetic diversity observed in *C. lutea*, as well as the preliminary phylogenetic position of *C. lutea* and the new species, hint at a more complex phylogeographic history for the section than previous authors have presented, and a more thorough analysis, including additional taxa and markers, will be necessary to resolve the evolutionary history of section *Ceratocystis*.

Carex lutea maintains relatively high levels of genetic diversity compared to the other North American endemics in this section, but about average for species with similar life histories (i.e., caespitose carices). A high degree of population differentiation, about 40% of the genetic diversity maintained by *C. lutea* at the assayed loci, is due to differences among populations, and thus extirpation of even a single population could impact levels of genetic diversity. As such, maintaining habitat quality at the sights with extant populations is likely one of the most critical aspects of managing

the species. Data presented in this study should provide important baseline information for monitoring populations, as well as providing a starting point for additional studies. In particular, investigation of aspects of natural history (e.g., allelic variation maintained in the seedbank, mechanisms of seed dispersal, longevity of individual clumps and importance of vegetative reproduction in population dynamics) will help identify processes influencing levels and apportionment of genetic diversity in the North American endemics of section *Ceratocystis*.

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