GENETIC DIVERSITY IN THE *CAREX JAMESII* COMPLEX (CYPERACEAE: SECT. *PHYLLOSTACHYAE*) WITH INSIGHTS INTO THE EVOLUTION AND ORIGIN OF THE NEWLY DESCRIBED SPECIES *CAREX TIMIDA*

Bruce A. Ford

Department of Botany
University of Manitoba
Winnipeg, Manitoba
R3T 2N2, CANADA
bford@cc.umanitoba.ca

Robert F.C. Naczi

Claude E. Phillips Herbarium
Dept. of Agriculture & Natural Resources
Delaware State University
Dover, DE 19901-2277, U.S.A.
rnaczi@dsc.edu

ABSTRACT

The *Carex jamesii* complex is composed of three species: *C. jamesii*, *C. juniperorum*, and *C. timida*. Despite the morphological similarity of *C. jamesii* and *C. timida*, these two taxa are not closely linked in a cluster analysis using genetic identity values. Rather, *C. timida* is grouped with *C. juniperorum*. The close genetic similarity between these two species confirms the findings of a previous phylogenetic study that showed that *C. juniperorum* and *C. timida* are sister species. *Carex timida* populations from the Ouachita Mountains of Arkansas are genetically unique and blur the distinctiveness between the latter species and *C. juniperorum*. Such a finding could be a sampling artifact. Alternatively, the plants found in the Ouachita Mountains may be closest to the ancestor that gave rise to *C. juniperorum* and *C. timida*. A comparison of genetic, phylogenetic, and phenetic relationships provides insights into the delineation of infraspecific taxa and justification for the recognition of *C. timida* at the species level.

ABSTRACT

El complejo *Carex jamesii* está compuesto por tres especies: *C. jamesii*, *C. juniperorum*, y *C. timida*. A pesar de la semejanza morfológica de *C. jamesii* y *C. timida*, estos dos taxa no están fuertemente unidos en un análisis cluster usando valores de identidad genética. En su lugar, *C. timida* está agrupado con *C. juniperorum*. La fuerte similitud genética de estas dos especies confirma los hallazgos de un estudio filogenético previo que mostró que *C. juniperorum* y *C. timida* son especies hermanas. Las poblaciones de *Carex timida* de la montañas Ouachita de Arkansas son genéticamente singulares y enturbia la distinción entre la última especie y *C. juniperorum*. Tal hallazgo puede ser un artefacto de muestreo. Alternativamente, las plantas que se encuentran en las montañas Ouachita pueden ser más cercanas al ancestro que dio lugar a *C. juniperorum* y *C. timida*. Una comparación de las relaciones genéticas, filogenéticas, y fenéticas proporcionan una mejor comprensión de la delineación de los taxa infraespecíficos y la justificación para el reconocimiento de *C. timida* a nivel específico.

INTRODUCTION

Carex L. section *Phyllostachyae* Tuck. ex Kük. is a well-defined group of 10 species (*C. backii* Boott; *C. basiantha* Steud.; *C. jamesii* Schwein.; *C. juniperorum* Catling, Reznicek, & Crins; *C. latebracteata* Waterfall; *C. saximontana* Mack.; *C. superata*

SIDA 19(4): 885 – 897. 2001

Naczi, Reznicek, & B.A. Ford; *C. timida* Naczi & B.A. Ford; *C. willdenowii* Willd.; *C.* sp. nov. Saarela & B.A. Ford) that is endemic to forested and semi-forested habitats in North America. This section has been the subject of considerable systematic research (Catling et al. 1993; Ford et al. 1998a, 1998b, 1998c; Naczi et al. 1998; Starr et al. 1999; Naczi & Ford 2001; Saarela and Ford in press) with new insights having been gained not only into the taxonomy, phylogeny, and genetic structure of this section, but the genus *Carex* as a whole. One of the most unexpected findings of our research has been the discovery of a proportionately large number of undetected or undescribed species. These new species turn out to be members of widespread species complexes that are masquerading under a single name. Undetected species usually become apparent when plants from the northeastern and central United States and adjacent Canada are compared with specimens from the southeastern United States or western North America. This trend was evident in our earlier study of the *C. willdenowii* complex (*C. willdenowii* s. str.; *C. basiantha*; *C. superata*) (Ford et al. 1998c; Naczi et al. 1998) and most recently in our investigation of *C. jamesii* s. lat. (*C. jamesii* s. str.; *C. juniperorum*; *C. timida*) (Naczi & Ford 2001) and *C. backii* (*C. backii* s. str.; *C. saximontana*; *C.* sp. nov.) (Saarela & Ford in press).

The *C. jamesii* complex is a well-defined, monophyletic assemblage of taxa (Ford et al. 1998b; Starr et al. 1999) distinguished from other species in section *Phyllostachyae* by a combination of filiform stigmas, pistillate scales that do not conceal the perigynium, and perigynium bodies that are abruptly contracted into a beak. Molecular studies indicate that all three species share identical ITS sequences (Starr et al. 1999; Starr pers. comm.). Morphologically, *C. juniperorum* is the most divergent member of this complex being distinguished by its numerous perigynia (4-9 per inflorescence), short culms (< 1/3 the length of the leaves), and lack of hyaline margins on the pistillate scales. *Carex jamesii* and *C. timida* are characterized by their relatively few perigynia (1-3 per inflorescence), elongate culms (subequal to the length of the leaves), and pistillate scales with conspicuously hyaline margins. Both species are superficially similar but can be readily distinguished by differences in cataphyll epidermal cell morphology, sheath color, and proximal staminate scale length.

Isozyme analysis has provided important ancillary data for systematic studies of *Carex* and in particular for recent taxonomic investigations of species complexes in section *Phyllostachyae* (Ford et al. 1998a, 1998b, 1998c). The intent of this study was to: 1) assess the taxonomic status of *C. timida* using isozyme data; 2) determine the degree of genetic divergence within and between species in the *C. jamesii* complex; and 3) compare the phylogenetic inferences from isozyme data to the evolutionary hypothesis proposed by Naczi and Ford (2001).

MATERIALS AND METHODS

A total of 649 individuals were collected from 26 populations (16 of *C. jamesii*, 4 of *C. juniperorum*, and 6 of *C. timida*) (Table 1). Our study included all *C. jamesii* and *C. juniperorum* populations examined by Ford et al. (1998a, 1998b) plus additional populations of *C. jamesii* and *C. timida* that were collected after the completion of these studies. The methodology for field sampling and enzyme analysis follows that of Ford et al. (1998c). Eleven enzymes coded by 15 interpretable, putative loci were included in this study. The 15 loci and their associated alleles were: aspartate aminotransferase, AAT-1 (a); diaphorase DIA-1 (a to e), DIA-2 (a); glucose-6-phosphate isomerase, GPI-2 (a to f); leucine aminopeptidase, LAP-1 (a to c); malate dehydrogenase, MDH-1 (a to b), MDH-2 (a to c); menadione reductase, MDR (a to b); peroxidase, PER-2 (a); phosphoglucomutase, PGM-1 (a to e), PGM-2 (a to e); shikimate dehydrogenase, SKD (a to c, allele c is a null allele observed in population 20 of *C. timida*); superoxide dismutase, SOD (a to b); and triose-phosphate isomerase, TPI-1 (a to d), TPI-2 (a to c). Allele frequencies, Nei's unbiased genetic identities (*I*) (Nei 1978), and an UPGMA (unweighted pair-group method) phenogram were calculated using BIOSYS-1 (Swofford & Selander 1981). Total genetic diversity for each species (H_T), average diversity within (H_S) and among populations (D_{ST}), and the coefficient of genetic differentiation (G_{ST}) were calculated using Nei and Chesser's (1983) procedure, unbiased for sample size, using GENESTAT-PC v. 2.1 (Lewis & Whitkus 1989). These analyses included both monomorphic and polymorphic loci in their calculations.

RESULTS

A total of 15 putative loci were surveyed in this study with all loci, expect AAT-1, DIA-2, and PER-2 being polymorphic in one or more populations. *Carex jamesii* had 12 polymorphic loci, while 9 variable loci were found in *C. juniperorum* and *C. timida*. *Carex jamesii* also had the greatest allelic diversity with 42 alleles identified, while 30 and 31 different alleles were observed in *C. juniperorum* and *C. timida*, respectively. With the exception of unique alleles for PGM-1, the allozymes found in *C. timida* and *C. juniperorum* were a subset of those found in *C. jamesii* (Table 2).

Genetic variability statistics fell within the range previously reported for *C. jamesii* and *C. juniperorum* (Ford et al. 1998a) (Table 3). The mean number of alleles per locus (K) ranged from 1.2 in *C. juniperorum* and *C. timida* (population 52, Hastings Co., Ontario and population 53, Monroe Co., Kentucky, respectively) to 2.1 in the Campbell Co., Kentucky population of *C. jamesii* (population 2). The percentage of polymorphic loci (P) was variable and ranged from 20.0 in the Monroe Co., Kentucky population of *C. timida* (population 53) to

TABLE 1. Collection data for populations of the *Carex jamesii* complex. Population codes are referred to parenthetically following each citation. Vouchers are deposited in WIN except where noted.

Carex jamesii Schweinitz

CANADA. ONTARIO. **Essex Co.:** Anderdon Twp., 5 km NE of Amherstburg, 22 May 1994, *Ball 940526* (22). **Niagara Regional Mun.:** Louth Twp., Twenty Mile Creek, Jordan, 13 Jun 1979, *Ball 79039* (PWB in TRTE (31). **Waterloo Co.:** Wilmot Twp., 8 km W of New Dundee on the Nith River, 3 Jun 1982 *Ball 82074* (PWB in TRTE) (32). **U.S.A. ARKANSAS. Franklin Co.:** ca. 1 mi N of Cecil, Citadel Bluff Army Corps of Engineers Park, 19 May 1994, *Naczi 3923 & Ford* (21). **Newton Co.:** ca. 3 mi NE of Boxley, Lost Valley Recreation Area of Buffalo National River, 19 May 1994, *Naczi 3917 & Ford* (13). **Scott Co.:** ca. 2 mi N of Y City, W of route 71 and S of Fourche La Fave River, 20 May 1994, *Naczi 3939 & Ford* (18). **INDIANA**. **Grant Co.:** Taylor University Arboretum, SW edge of Upland, 17 May 1994, *Rothrock 3255* (9); Stellers Road, 1.3 mi N of Matthews, 17 May 1994, *Rothrock 3254* (8). **KENTUCKY. Boone Co.:** 3 air mi S of Petersburg, ca. 0.3 mi W of route 20 along S side of Woolper Creek, 12 Jun 1994, *Naczi 4096* (39). **Campbell Co.:** Highland Heights, 10 May 1994, *Naczi 3826* (2); Silver Grove, N of route 8, floodplain of Ohio River, opposite St. Anne's Convent, 12 May 1995, *Naczi 4575 & Ganss* (51). **Mason Co.:** ca. 2 air mi W of Dover, along S side of route 8, 29 May 1994, *Naczi 4027 & Flynn* (33); ca. 2 air mi W of Dover, along S side of route 8, 29 May 1994, *Naczi 4028 & Flynn* (34). **MISSISSIPPI. DeSoto Co.:** ca. 2 mi N of Walls, along E side of route 61, 25 May 1994, *Naczi 4026 et al.* (24). **OHIO. Montgomery Co.,** SW of Farmersville, E side of Anthony Road, 0.3 mi SW of its junction with Manning Road, 29 May 1998, *Ford 98152 & Naczi* (57). **VIRGINIA. Bath Co.:** ca. 0.4 mi S of Healing Springs, along W side of route 220, 23 Jun 1994, *Naczi 4482 & Thieret* (41).

Carex juniperorum Catling, Reznicek, & Crins

CANADA. ONTARIO. Hastings Co.: Tyendinaga Twp., E side of Salmon River, ca. 15 km W of Napanee, 24 Jul 1995, *Ford 9566 et al.* (52). **U.S.A. KENTUCKY. Bath Co.:** ca. 5 air mi ESE of Owingsville, 16 May 1994, *Naczi 3890* (5). **Lewis Co.:** ca. 3.5 air mi ESE of Trinity, 5 May 1994, *Naczi 3808 et al.* (1). **OHIO. Adams Co.:** ca. 3 air mi NE of Peebles, 16 May 1994, *Naczi 3878* (7).

Carex timida Naczi & B.A. Ford

U.S.A. ARKANSAS. Polk Co.: SW of town of Rich Mountain on summit of Rich Mountain, Queen Wilhelmina State Park, along N side of route 88, in vicinity of trailhead of Spring Trail, 20 May 1994, *Naczi 3940 & Ford* (20); ca. 8 mi E of Vandervoort, N of route 246 and E of Cossatot River, 20 May 1994, *Naczi 3949 & Ford* (16). **KENTUCKY. Monroe Co.:** SE of Tomkinsville, along the W side of route 216, 6 road mi E of its junction with route 163, along McFarland Creek, 23 May 1998, *Ford 98100 & Naczi* (53). **Rowan Co.:** ca. 6.5 air mi S of center of Morehead, ca. 0.25 mi down slope from W side of route 1274, upslope from Sugar Camp Branch, ca. 1.3 road mi N of junction of routes 1274 and 801, 28 May 1998, *Ford 98145 & Naczi* (55). **OHIO**. **Montgomery Co.:** SW of Farmersville, E side of Anthony Road, 0.3 mi SW of its junction with Manning Road, 29 May 1998, *Ford 98153 & Naczi* (56). **TENNESSEE**. **Franklin Co.:** S of Huntland, along E side of route 97, 2.6 road mi S of its junction with route 122, 24 May 1998, *Ford 98108 & Naczi* (54).

73.3 in the Grant Co., Indiana population of *C. jamesii* (population 8). The average observed heterozygosity within populations (H_{obs}) ranged from 0.137 in the Bath Co., Virginia population of *C. jamesii* (population 41) to 0.336 in the Grant Co., Indiana population of this same species (population 8). The expected heterozygosity in each population based upon Hardy-Weinberg expectations (H_{exp}) was less than that observed in each population with values ranging from

TABLE 2. Allozyme frequencies for polymorphic loci in *Carex jamesii*, *C. juniperorum*, and *C. timida* as averages for each species (except where noted). *N* = number of individuals used in the calculation of averages.

Locus	Allele	*C. jamesii* (*N* = 383)	*C. juniperorum* (*N* = 112)	*C. timida* Pop. 53–56 (*N* = 98)	*C. timida* Pop. 16 (*N* = 24)	*C. timida* Pop. 20 (*N* = 32)
DIA-1	A	0.360	0.509	0.500	0.500	0.484
	B	0.026	–	–	–	0.016
	C	0.391	0.246	0.398	0.500	0.484
	D	0.001	0.009	–	–	–
	E	0.221	0.237	0.102	–	0.016
GPI-2	A	0.297	–	–	–	–
	B	0.124	0.500	0.500	0.500	0.500
	C	0.159	–	–	–	–
	D	0.409	–	–	–	–
	E	0.009	0.500	0.372	0.500	0.500
	F	–	–	0.128	–	
LAP-1	A	0.008	0.019	–	–	–
	B	0.987	0.972	1.000	1.000	1.000
	C	0.005	0.009	–	–	–
MDH-1	A	0.537	0.960	1.000	0.979	0.984
	B	0.463	0.040	–	0.021	0.0.16
MDH-2	A	0.026	0.054	1.000	1.000	0.938
	B	0.026	0.009	–	–	0.031
	C	0.948	0.938	–	–	0.031
MDR	A	0.057	–	–	–	–
	B	0.943	1.000	1.000	1.000	1.000
PGM-1	A	0.020	–	–	–	–
	B	0.095	–	–	–	–
	C	0.885	–	–	1.000	–
	D	–	–	1.000	–	–
	E	–	1.000	–	–	1.000
PGM-2	A	0.054	0.063	–	–	–
	B	0.534	–	–	–	–
	C	0.003	0.938	–	0.625	1.000
	D	0.363	–	–	–	–
	E	0.047	–	1.000	0.375	–
SOD	A	0.979	0.977	1.000	1.000	1.000
	B	0.021	0.023	–	–	–
SKD	A	0.005	–	0.194	1.000	–
	B	0.995	1.000	0.806	–	–
	C (null allele)	–	–	–	–	1.000

TABLE 2. cont.

Locus	Allele	*C. jamesii* (*N* = 383)	*C. juniperorum* (*N* = 112)	*C. timida* Pop. 53–56 (*N* = 98)	*C. timida* Pop. 16 (*N* = 24)	*C. timida* Pop. 20 (*N* = 32)
TPI-1	A	0.009	0.004	0.372	0.167	0.469
	B	0.337	0.991	0.628	0.833	0.469
	C	0.631	0.004	–	–	–
	D	0.023	–	–	–	–
TPI-2	A	0.004	0.504	0.372	0.500	0.484
	B	0.449	0.004	0.128	–	0.016
	C	0.547	0.491	0.500	0.500	0.500

0.082 in the Bath Co., Virginia population of *C. jamesii* (population 41) to 0.261 in the Mason Co., Kentucky population of this same species (population 34).

Gene diversity statistics (Table 4) indicated that *C. juniperorum* had the lowest total gene diversity (H_T) (0.135), while that for *C. jamesii* (0.265) and *C. timida* (0.247) was almost twice as great. A similar trend was seen within-populations (H_S), with the lowest value found in *C. juniperorum* while much higher values were observed in *C. jamesii* (0.188) and *C. timida* (0.141).

The gene diversity among populations (D_{ST}) and coefficient of genetic differentiation (G_{ST}) were also variable with extremely low values found in *C. juniperorum* (D_{ST} = 0.007, G_{ST} = 0.049), while relatively high values were associated with *C. jamesii* (D_{ST} = 0.077, G_{ST} = 0.290) and *C. timida* (D_{ST} = 0.106, G_{ST} = 0.429). Taken together these numbers indicate that species within the *C. jamesii* complex harbor as little as 57.1% (*C. timida*) to as much as 95.1% (*C. juniperorum*) of their genetic diversity within populations.

Intra-specific genetic identity values were variable, and in some instances surprisingly low (Table 5). Average values ranged from 0.990 for *C. juniperorum* to 0.852 in *C. timida*. The low value found in this latter species was largely the result of the presence of unique alleles for the isozymes PGM-1, PGM-2, and SKD in the Arkansas populations (populations 16 and 20) of this taxon (Table 5). No activity was observed for SKD in population 20 suggesting that these plants may have lost the ability to express this enzyme phenotype.

Inter-specific genetic identity values were lower than those observed within species and ranged from 0.655 (*C. jamesii* and *C. timida*) to 0.760 (*C. juniperorum* and *C. timida*) (Table 5). These lower values can be attributed to the presence of diagnostic or high frequency alleles in all species. Alleles for the isozymes MDH-2, PGM-1, and PGM-2 helped to differentiate *C. jamesii* from *C. timida* and/or *C. juniperorum* (Table 2). The high genetic identity between *C. juniperorum* and *C. timida* was the result of the presence of similar alleles for PGM-1 and/or PGM-2 in Arkansas populations of *C. timida* and populations of *C. juniperorum*. All

TABLE 3. Genetic variability in 26 populations of the *Carex jamesii* complex: sample size (*N*), mean number of alleles per locus ± SE (*k*), percentage of polymorphic loci ± SE (*P*) (a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99), observed heterozygosity ± SE (H_{obs}), expected heterozygosity ± SE (H_{exp}) (Unbiased estimate Nei [1978]).

Population #	*N*	*k*	*P*	H_{obs}	H_{exp}
Carex jamesii					
2	38	2.1 ± 0.3	66.7	0.211 ± 0.095	0.195 ± 0.061
8	31	1.9 ± 0.2	73.3	0.336 ± 0.123	0.250 ± 0.069
9	30	1.9 ± 0.3	53.3	0.329 ± 0.121	0.253 ± 0.073
13	30	1.7 ± 0.2	53.3	0.264 ± 0.116	0.168 ± 0.058
18	26	1.5 ± 0.2	33.3	0.262 ± 0.116	0.149 ± 0.060
21	34	1.5 ± 0.2	40.0	0.259 ± 0.114	0.147 ± 0.060
22	13	1.3 ± 0.1	33.3	0.333 ± 0.126	0.173 ± 0.066
24	31	1.9 ± 0.3	46.7	0.252 ± 0.111	0.181 ± 0.065
31	24	1.9 ± 0.3	53.3	0.331 ± 0.125	0.198 ± 0.066
32	19	1.7 ± 0.2	60.0	0.270 ± 0.114	0.163 ± 0.058
33	20	1.7 ± 0.2	53.3	0.333 ± 0.124	0.217 ± 0.064
34	9	1.8 ± 0.2	60.0	0.341 ± 0.118	0.261 ± 0.069
39	18	1.4 ± 0.2	33.3	0.333 ± 0.126	0.179 ± 0.068
41	34	1.4 ± 0.2	26.7	0.137 ± 0.090	0.082 ± 0.082
51	12	1.4 ± 0.2	33.3	0.333 ± 0.126	0.177 ± 0.067
57	14	1.6 ± 0.2	40.0	0.310 ± 0.118	0.216 ± 0.072
Carex juniperorum					
1	34	1.7 ± 0.2	46.7	0.214 ± 0.106	0.164 ± 0.058
5	27	1.7 ± 0.2	53.3	0.208 ± 0.103	0.131 ± 0.052
7	29	1.4 ± 0.2	33.3	0.205 ± 0.105	0.115 ± 0.054
52	22	1.2 ± 0.1	20.0	0.200 ± 0.107	0.102 ± 0.055
Carex timida					
16	24	1.4 ± 0.1	40.0	0.231 ± 0.105	0.156 ± 0.059
20	32	1.7 ± 0.3	40.0	0.265 ± 0.116	0.153 ± 0.062
53	25	1.2 ± 0.1	20.0	0.200 ± 0.107	0.102 ± 0.055
54	20	1.3 ± 0.1	26.7	0.267 ± 0.118	0.137 ± 0.061
55	26	1.3 ± 0.1	26.7	0.267 ± 0.118	0.136 ± 0.060
56	27	1.3 ± 0.1	33.3	0.267 ± 0.118	0.164 ± 0.062

populations of *C. timida* could be distinguished from *C. juniperorum* by the presence of the allele MDH-2a (Table 2).

A cluster analysis of populations using Nei's (1978) unbiased genetic identity values indicated the presence of two distinct groups, one corresponding to *C. jamesii* and a second to *C. juniperorum/timida*. Syntopic populations of *C. jamesii* and *C. timida* (population 56 and 57) were separated with no intermediates being detected. Within the second cluster, *C. juniperorum* and *C. timida* formed indistinct groups owing to the allelic similarity of the two Arkansas populations of *C. timida* with *C. juniperorum* (Fig. 1).

TABLE 4. Gene diversity statistics for the *Carex jamesii* complex. H_T = total gene diversity, H_S = within population gene diversity, D_{ST} = gene diversity among populations, G_{ST} = coefficient of genetic differentiation.

Species	H_T	H_S	D_{ST}	G_{ST}
Carex jamesii	0.265	0.188	0.077	0.290
Carex juniperorum	0.135	0.128	0.007	0.049
Carex timida	0.247	0.141	0.106	0.429

TABLE 5. Matrix of genetic identity coefficients (range) for all pairwise comparisons of sampled populations (*N*) of the *Carex jamesii* complex.

Species	*N*	*C. jamesii*	*C. juniperorum*	*C. timida*
Carex jamesii	16	0.901 (0.697–1.000)		
Carex juniperorum	4	0.744 (0.683–0.834)	0.990 (0.985–1.000)	
Carex timida	6	0.655 (0.545–0.779)	0.760 (0.695–0.845)	0.852 (0.717–0.984)

DISCUSSION

Taxonomic and Phylogenetic Implications

Our isozyme study provides allelic data that supports the recognition of three species in the *C. jamesii* complex (cf. Naczi & Ford 2001). Each species is distinguished by at least one unique or high frequency allele, and with one exception (see below), each forms a distinctive group in the cluster analysis of genetic identity values. Despite the morphological similarity of *C. jamesii* and *C. timida*, these two species were consistently separated in the cluster analysis. Even when these species occur in mixed populations (e.g., populations 56 and 57), no intermediates were detected.

Phylogenetic relationships within section *Phyllostachyae* have been explored in a number of papers, with Naczi and Ford (2001) having investigated the relationship of the newly described *C. timida* to other members of the section. Despite the morphological similarity of *C. jamesii* and *C. timida*, this study suggests that *C. timida* and *C. juniperorum* are sister species and that *C. jamesii* is basal to this clade. Our genetic distance analysis of isozyme data substantiates this hypothesis.

The discovery of genetically unique populations of *C. timida* from the Ouachita Mountains of Arkansas, which blur the distinctiveness between the latter species and *C. juniperorum*, was surprising, especially since morphologically these plants have been shown to be *C. timida* (Naczi and Ford 2001). There

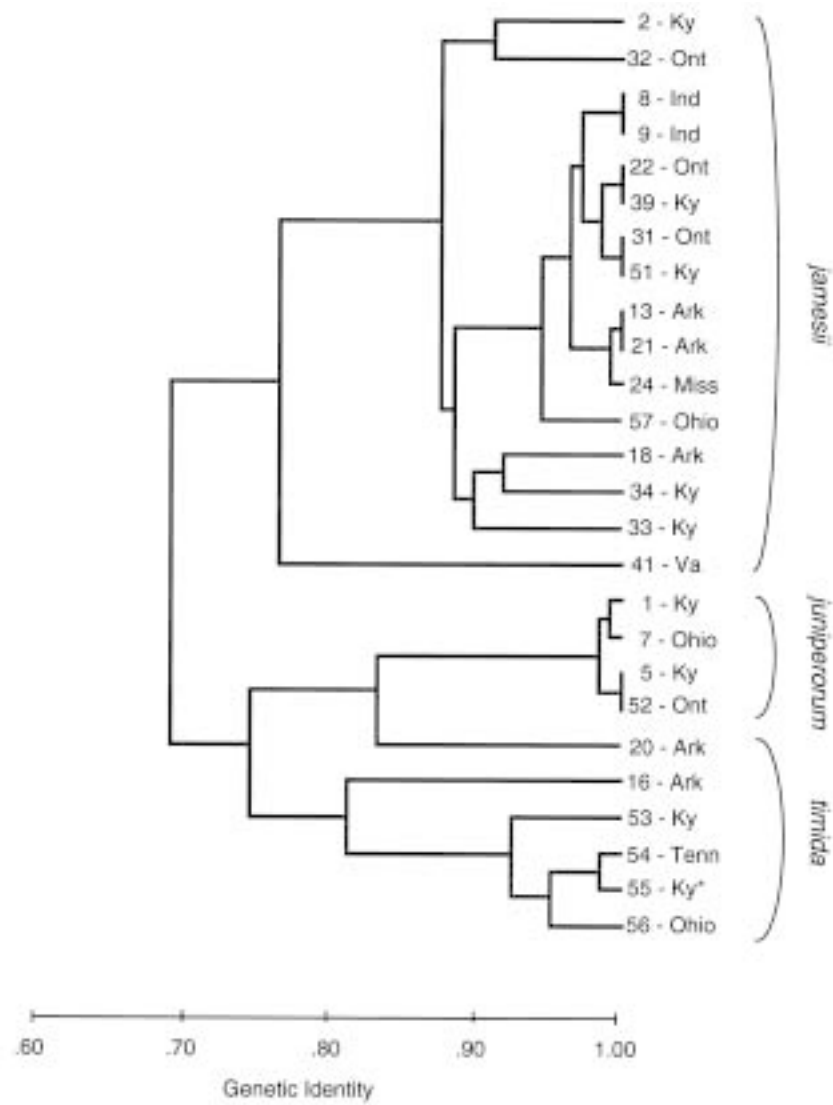

FIG. 1. Phenogram of 26 populations of the *Carex jamesii* complex using Nei's (1978) unbiased genetic identity values and UPGMA cluster analysis. * = type locality for *C. timida*. Cophenetic correlation coefficient = 0.915.

are, however, a number of possible explanations for this finding. One possibility is that the lack of distinct groups is a sampling artifact. If more loci had been surveyed, other diagnostic alleles, such as MDH-2a, might have been found

resulting in the formation of more distinctive clusters. Another possibility is that the *C. timida* populations sampled from the Ouachita Mountains (especially population 20) are closest to the ancestor that gave rise to *C. juniperorum* and *C. timida,* accounting for their unique positions in the cluster analysis. Alternatively, *C. juniperorum* could have arisen from a population, or populations, of *C. timida* similar to those found in western Arkansas. The Ouachita Mountains are a recognized glacial refugium and a center of endemism (Robinson & Allen 1995); a number of narrowly distributed plant and animal species occur in this region (Kral & Bates 1991; Robinson & Allen 1995). Many species that found refuge here are thought to have moved northward following the events of Pleistocene glaciation (Robinson & Allen 1995). In some instances, these more northerly populations became isolated from populations in the south resulting in the formation of new species (Ross & Ricker 1971; Robinson & Allen 1995). This phenomenon could account for the origin of *C. juniperorum* and the occurrence of genetically unique populations of *C. timida* in the Ouachita Mountains.

Genetic Variability and Diversity

Table 3 and 4 indicate that *C. juniperorum* possesses about half the genetic variability and diversity found in *C. timida* and *C. jamesii*. This pattern is similar to that found in our study of *C. willdenowii* s. lat., where *C. superata* was thought to possess half of the variation/diversity found in the other species of this complex due to its very short culms (could restrict pollen and seed movement) and restricted distribution (limited gene flow, selection due to environmental homogeneity (Ford et al. 1998c). Like *C. superata, C. juniperorum* has extremely short culms, with inflorescences that are crowed in the base of the plant. Furthermore, this species is rare and occurs in disjunct regions in southern Ontario, Kentucky, Ohio, and Virginia (Naczi and Ford 2001). The combined evidence suggests that factors similar to those operating in *C. superata* may be influencing the genetic structure of *C. juniperorum.*

At the opposite end of the spectrum is *C. jamesii*. This is the widest ranging species in the *C. jamesii* complex and is sympatric with both *C. timida* and *C. juniperorum. Carex jamesii* has the highest number of polymorphic loci (12 out of 15), the greatest number of alleles, and the highest genetic variability and diversity values (H_T and H_S) for any species in this clade. In addition, with the exception of unique alleles for PGM-1, the allozymes found in *C. timida* and *C. juniperorum* are a subset of those found in *C. jamesii*. The widespread nature of *C. jamesii*, and its adaptation to a variety of climatic and ecological conditions, may be factors contributing to the high levels of genetic diversity and variability found in this species. Being only one node away from the ancestor that gave rise to the *C. jamesii* clade (cf. Naczi & Ford 2001), it is also possible that *C. jamesii* retains much of the variation found in the ancestor to this group accounting for this species extensive allelic diversity.

Insights into the Delineation of Infraspecific Taxa and Justification for Recognizing *C. timida* as a Distinct Species

A comparison of the evolutionary hypothesis proposed in this paper with the superficial similarity found between *C. timida* and *C. jamesii* allows us to explore issues surrounding the recognition of infraspecific taxa and justification for recognizing *C. timida* at the species level. Infraspecific categories are frequently used by taxonomists as a means of recognizing poorly differentiated taxa or taxa distinguished by seemingly minor morphological differences. Current phylogenetic methods may not be appropriate for determining relationships at this level since these relationships are not necessarily hierarchical and the characters used to define taxa are not always discrete. This fact, along with problems associated with outgroup selection, has led most caricologists to develop infraspecific classifications using phenetic methods (e.g., Murray 1969; Reznicek & Ball 1980; Crins & Ball 1983; Reznicek 1987; Standley 1985; Crins & Ball 1989a, 1989b; Ball & Zoladz 1994; Dunlop & Crow 1999). One might wish to consider *C. timida* as a subspecies of *C. jamesii* because of the close morphological similarity of these two taxa. Indeed, an evaluation of the results of our phenetic study might have made this a tenable conclusion (cf. Naczi & Ford 2001). However, when the results of our phylogenetic and genetic research are considered it is clear that the recognition of *C. timida* as a subspecies of *C. jamesii* would have created a clade composed entirely of artificial taxa. While infraspecific relationships are not necessarily hierarchical, infraspecific classifications do represent explicit phylogenetic hypotheses. This study shows that genetic and phylogenetic divergence is not necessarily correlated with striking morphological differences: morphologically similar taxa are not necessarily closely related. We submit that infraspecific classifications based entirely on grouping morphologically similar taxa can lead to the recognition of artificial species. Further, we suggest that if taxa possess clear-cut differences, no matter how narrow, it is best to recognize these taxa as distinct species. Infraspecific taxa should exhibit some degree of intergradation thus making the identification of a significant number of individuals impossible even under the most perfect circumstances. Using this criterion, *C. timida* is best recognized as a distinct species.

ACKNOWLEDGMENTS

We greatly appreciate the assistance of D.A. Ross McQueen and Julian R. Starr in our earlier studies of *Carex* section *Phyllostachyae*. A special thanks to Anton A. Reznicek for sharing his thoughts on the recognition of infraspecific taxa. We are also grateful to Patrick Calie and Charles Bryson for their careful review of the manuscript. Peter Ball, Wasyl Bakowsky, Charles Bryson, Mike Oldham, Paul Rothrock, and Donald Sutherland supplied key population samples and/or assisted with various aspects of our fieldwork. Piggly Wiggly provided victuals

and inspiration. This research was supported by a Natural Sciences and Engineering Research Council of Canada operating and equipment grants to B.A.F.

REFERENCES

BALL, P.W. and M. ZOLADZ. 1994. The taxonomy of *Carex petricosa* (Cyperaceae) and related species in North America. Rhodora 96:295–310.

CATLING, P.M., A.A. REZNICEK, and W.J. CRINS. 1993. *Carex juniperorum* (Cyperaceae), a new species from northeastern North America, with a key to *Carex* sect. *Phyllostachys*. Syst. Bot. 18:496–501.

CRINS, W.J. and P.W. BALL. 1983 The taxonomy of the *Carex pensylvanica* complex (Cyperaceae) in North America. Canad. J. Bot. 61:1692–1717.

CRINS, W.J. and P.W. BALL. 1989a. Taxonomy of the *Carex flava* complex (Cyperaceae) in North America and northern Eurasia. I. Numerical taxonomy and character analysis. Canad. J. Bot. 67:1023–1047

CRINS, W.J. and P.W. BALL. 1989b. Taxonomy of the *Carex flava* complex (Cyperaceae) in North America and northern Eurasia. II. Taxonomic treatment. Canad. J. Bot. 67:1048–1065

DUNLOP, D.A. and G.E. CROW. 1999. The taxonomy of *Carex* section *Scirpinae* (Cyperaceae). Rhodora 101:163–199.

FORD, B.A., D.A.R. MCQUEEN, J.R., STARR, and R.F.C. NACZI. 1998a. The impact of species-specific traits and phylogenetic relatedness on allozyme diversity in *Carex* sect. *Phyllostachys* (Cyperaceae). Pl. Syst. & Evol. 212:13–29.

FORD, B.A., J.R., STARR, D.A.R. MCQUEEN, and R.F.C. NACZI. 1998b. Relationships among species in *Carex* sect. *Phyllostachys* (Cyperaceae) based on allozyme divergence. Pl. Syst. & Evol. 212:31–51.

FORD, B.A., D.A.R. MCQUEEN, R.F.C. NACZI, and A.A. REZNICEK. 1998c. Allozyme variation and genetic relationships among species in the *Carex willdenowii* complex (Cyperaceae). Amer. J. Bot. 85:546–552.

KRAL, R. and V. BATES. 1991. A new species of *Hydrophyllum* from the Ouachita Mountains of Arkansas. Novon 1:60–66.

LEWIS, P. and R. WHITKUS. 1989. GENESTAT-PC V. 2.1. Department of Botany, Ohio State University, Columbus.

MURRAY, D.F. 1969. Taxonomy of *Carex* sect. *Atratae* (Cyperaceae) in the southern Rocky Mountains. Brittonia 21:55–76.

NACZI, R.F.C., A.A. REZNICEK, and B.A. FORD. 1998. Morphological, geographical, and ecological differentiation in the *Carex willdenowii* complex (Cyperaceae). Amer. J. Bot. 85:434–447.

NACZI, R.F.C. and B.A. FORD. 2001. Systematics of the *Carex jamesii* complex (Cyperaceae: sect. *Phyllostachyae*). Sida 19:853–884.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

NEI, M. and R.K. CHESSER. 1983. Estimation of fixation indices and gene diversities. Ann. Human Genet. 47:253–259.

REZNICEK, A.A. and P.W. BALL. 1980. The taxonomy of *Carex* section *Stellulatae* in North America north of Mexico. Contr. Univ. Michigan Herb. 14:153–203.

REZNICEK, A.A. 1987. *Carex* sect. *Stellulatae* (Cyperaceae) in the neotropics. Contr. Univ. Michigan Herb. 16:201–210.

ROBINSON, H.W. and R.T. ALLEN. 1995. Only in Arkansas: a study of the endemic plants and animals of the state. The University of Arkansas Press, Fayetteville.

ROSS, H.H. and W.E. RICKER. 1971. The classification, evolution, and dispersal of the winter stonefly genus *Allocapnia*. Illinois Biol. Monogr 45:1–166.

SAARELA, J.M. and B.A. FORD. In press. Taxonomy of the *Carex backii* complex (section *Phyllostachyae*, Cyperaceae). Syst. Bot.

STANDLEY, L.A. 1985. Systematics of the *Acutae* group of *Carex* (Cyperaceae) in the Pacific Northwest. Syst. Bot. Monogr. 7:1–106.

STARR, J.R., R.J. BAYER, and B.A. FORD. 1999. The phylogenetic position of *Carex* section *Phyllostachys* and its implications for phylogeny and subgeneric circumscription in *Carex* (Cyperaceae). Amer. J. Bot. 86:563–577.

SWOFFORD, D.L. and R.B. SELANDER. 1981. Biosys-1: computer program for the analysis of allelic variation in genetics. User's manual. University of Illinois, Urbana.