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Clarification of the hybrid origin of carex x deamii Herm. (Cyperaceae) based on macro and micro morphological characters

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Clarification of the Hybrid Origin of Carex x deamii Herm. (Cyperaceae) based on Macro and Micro Morphological Characters.

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TABLE OF CONTENTS

PAGE

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LIST OF TABLES

LIST OF FIGURES

Abstract

The origin of *Carex* x *deamii* Herm. has been in question ever since it was named to science in 1938. Collections of the hybrid have always been associated with *C. shortiana* Dewey and either *C. typhina* Michx. or *C. squarrosa* L.. *C. typhina* and *C. squarrosa* are closely related taxa (Section Squarrosae) and often are morphologically similar. Because of this similarity, determining the correct parental species to the hybrid has been extremely difficult. It is known that the hybrid is sterile and only reproduces asexually. Pollen was analyzed to ascertain the viability of all four taxa. This study utilizes both macro and micro morphological characters in a numerical taxonomic analysis to determine the parental species of *C.* x *deamii*. Macromorphological characters include vegetative and reproductive parts. Micromorphological characters focused on surface features of leaves, perigynia, and pistillate scales. In addition, achenes were analyzed for distinctive features and X-Ray analysis was done on all plant parts to determine the presence and distribution of silica for these taxa. Data was analyzed using SAS and HYWIN (Hypothesizing hybrids using Weighted Intermediacy). Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA) were done using the SAS program. The three putative parental species were all had ~90 % viable pollen. The hybrid, while sterile, did have 2.3 % of its pollen viable. All statistical analyses showed *C. shortiana* to be the most likely parent to the hybrid. Likewise, all analyses showed *C. typhina* to be the other parent species. The most important characters in both macro and micro morphological analysis were perigynia features (perigynium beak length in macromorphological analysis and perigynium epidermal cell length/width ratio in the micromorphological analysis.

CHAPTER I.

INTRODUCTION

The Cyperaceae or Sedge Family is one of the largest families of flowering plants with more than 4,000 species in approximately 90 genera (Woodland 1991). The family exhibits a worldwide distribution and occurs primarily in moist temperate habitats. Sedges are almost exclusively herbaceous in habit, with the exception of some tropical species.

General Taxonomy and Features of the Cyperaceae

Superficially, the Cyperaceae resemble the Poaceae (Grass Family) with their long, narrow, parallel-veined leaves. However, sedges differ morphologically in many ways. For example, leaves are three ranked in the Cyperaceae and only two-ranked in the Poaceae, the Cyperaceae have closed sheaths in contrast to the open sheaths of grasses, and the stems of sedges are often triangular in cross section while those of grasses are either rounded or somewhat flattened. Metcalfe points out that there are, in fact, some anatomical similarities between the two families. Both possess similar prickle hairs as well as some similarities in general vascular organization. However, he also states that there are clearly many anatomical differences between grasses and sedges. According to Metcalfe (1969), " while the Cyperaceae and Poaceae might have evolved from a very remote common ancestor they must have pursued independent phylogenetic courses for a very long time ". Furthermore, recent

evidence suggests that the Juncaceae or Rush Family are probably the most closely related family to the Cyperaceae in spite of the fact that they exhibit quite different flower structures. They do, however, share many embryological and anatomical similarities (Metcalfe 1969) as well having flavanoid pigments and pseudomonad pollen in common (Standley 1985). They also possess similar chromosomal features. Both families characteristically have very small chromosomes with diffuse or non-localized centromeres in which there is not one point of attachment for the spindle fibers during mitosis and meiosis. As a result, chromosome breakage or fragmentation is very common in the Cyperaceae and irregular chromosome numbers are found in many species (Stace 1989).

The Cyperaceae family is thought to have originated as forest-floor plants in the late Cretaceous or early Tertiary Period (Ball 1990). Earliest fossil records are of fruit from the Paleocene Epoch approximately 60 million years ago.

The subfamily Caricoideae Pax includes only one tribe, the *Cariceae* Kunth ex Dumort (authors for suprageneric names follow Goetghebeur 1985). The *Cariceae* consists of five genera (*Carex* L., *Cymophyllus* Mackenzie, *Kobresia* Willd., *Schoenoxiphium* Nees., and *Uncinia* Pers.) and about 2,000 species characterized by unisexual flowers with female flowers subtended by a partially or wholly closed perigynium (Blaser 1944). *Carex*, *Cymophyllus*, and *Uncinia* have completely closed perigynia while *Kobresia* and *Schoenoxiphium* have more or less open perigynia (Reznicek 1990).

Carex is by far the largest genus with nearly 2,000 species distributed worldwide. The genus *Carex*, like others in the Cyperaceae, is characterized by mostly long, narrow, parallel-veined leaves which are three-ranked with closed leaf sheaths. Flowers in *Carex* are unisexual and members of the genus are monoecious with their inflorescence of three types: **1.** with staminate flowers at the apex and pistillate flowers at the base of each spike (androgynous), **2.** with pistillate flowers at the apex and staminate flowers at the base of each spike (gynecandrous) or **3.** with staminate and pistillate flowers on separate spikes (Steyermark 1963). Flowers of *Carex*, like most of the Cyperaceae, are very much reduced. They lack the "showy" inflorescences of most Angiosperms. Instead, their flowers possess only the "essential" flower parts (stamens and pistils), those necessary for reproduction. Staminate flowers consist basically of three stamens subtended by a scale. Pistillate flowers consist of a pistil with two or three stigmas surrounded by a perigynium (a sac-like structure characterisic of the genus). Pistillate flowers are also subtended by a scale. Achenes of *Carex* are either lenticular or more commonly three-angled.

Because of the very reduced floral structure and the tremendous number of species of sedges, distinction between taxa has often been difficult. For these reasons, the Cyperaceae family and the genus *Carex* were often neglected for research in the past. However, in recent years sedges have been the subject of increasingly more research. This is due, in part, to the increased awareness of the values of wetlands (Mitsch and Gosselink 1993) as well as the realization that

our knowledge level of this family had fallen far behind that of many other Angiosperm families.

The genus *Carex* has been further subdivided into 3 subgenera (Reznicek 1990) and 72 sections (Standley 1985). The subgenera are:

1. *Indocarex* (Mainly a tropical group which contains no species in North America north of Mexico.)
2. *Vignea* (Includes 42.8 % of *Carex* species in North America north of Mexico (Cayouette and Catling 1992).)
3. *Eucarex* (Includes 57.1 % of *Carex* species in North America north of Mexico (Cayouette and Catling 1992).)

Carex, is one of the most important genera in North America with nearly 600 species (Bernard 1990). Although it is of relatively little economic importance, the genus *Carex* is extremely valuable ecologically. Deam illustrated this point very well in his Flora of Indiana (1940) when he described the value of *Carex* to native wildlife. He wrote of deer and rabbits grazing on leaves of sedges and birds eating the achenes and nesting in sedges which form mounds. Some species of *Carex* are also of great importance ecologically because of their ability to effectively control soil erosion " ... in natural rangelands, along riverbanks, and on dunes and highway verges" (Catling et al. 1990).

Hybridization in the Genus Carex

Hybridization is very common in the genus *Carex*. This fact, along with the reasons mentioned previously, are responsible for much of the taxonomic difficulties in the genus. The first record of a *Carex* hybrid from North America came in 1842 from Francis Boote, when he described *Carex sullivantii* as a sterile hybrid between *Carex pubescens* Muhlenb. (now *Carex hirtifolia* Mackenzie) and *Carex gracillima* Schwein (Boote 1842). Since then, a total of 253 *Carex* hybrids have been described for North America. Most *Carex* hybrids are found in the subgenus *Eucarex* with 78.8 % compared to only 21.7 % for the subgenus Vignea (Cayouette and Catling 1992).

It is often difficult to distinguish between a new hybrid and intergrading species in *Carex*. Attributes often used to determine hybrid origin include sterility, both in fruit and pollen, character intermediacy (morphological, anatomical, and cytological), habitat intermediacy, and the presence of the putative parents (Stace 1989). Macromorphological character intermediacy is most often used by taxonomist in studying closely related and complex groups of sedges (Catling 1993; Catling 1996; Crins 1990; Crins and Ball 1988; Crins and Ball 1989; Reznicek 1987; Reznicek and Catling 1985; Standley 1983) and it is the primary focus of this study. Micromorphological features of the achenes and perigynia of members of the genus *Carex* have also been useful in past studies (Evans 1976; Olgun and Beyazoglu 1997; Rettig 1986; Rettig 1990; Standley

1987a; Standley 1987b; Waterway 1990; Wujek and Menapace 1986) and will be included in this analysis.

One characteristic often used to describe hybrids is their sterility. Hybrids often exhibit very reduced fertility of both pollen and fruit production. Some hybrids, however, do not exhibit a noticeable degree of sterility. Stace (1989) states that interspecific hybrids can be as fertile as either parent or they can be totally sterile. Most hybrids do exhibit very noticeable sterility, but very few exhibit 100 % sterility (Stace 1989).

Pollen sterility of hybrids has been found to be related to the levels of meiotic anomalies that are present (Cayouette and Morisset 1985). In the genus *Carex*, hybrids are largely sterile with hybrids of more distantly related groups exhibiting greater levels of meiotic anomalies and therefore greater sterility. Intersectional hybrids in the genus *Carex* , such as the case of C. x *leamii*, have been found to be almost 0 % fertile based on the few studies available (Moore and Calder 1964; Toivonen 1981; Cayouette 1990).

Sterility of hybrids in the genus *Carex* is based largely on abnormalities associated with the achenes. These abnormalities may include the presence of thin walled or empty achenes, achenes bearing embryos without endosperm or the complete absence of achenes (Toivonen 1981; Standley 1983; Reznicek and Catling 1985; Reznicek and Catling 1986; Crins and Ball 1987; Catling et al. 1989).

This study will focus on the hybrid origin of *Carex* x *deamii* F. J. Hermann (Figure 1a), which was first collected in Pike County, Indiana in 1926 by Charles C. Deam. In 1938 the taxon was recollected and described by Frederick J. Hermann as a sterile hybrid between *Carex shortiana* Dewey (Figure 1b) and *Carex typhina* Michx. (Figure 1c) (Hermann 1938). Since then, however, another possible parent species has been suggested. Steyermark (1958) discovered populations of the hybrid in Howell and Adair Counties in Missouri. In Adair County, Steyermark found the hybrid to be present with *C. shortiana*, *C. typhina*, and *Carex squarrosa* L. (Figure 1d), but in Howell County, *C. squarrosa* was present while *C. typhina* was absent. Shildneck (Hess and Shildneck 1982) made similar findings when he discovered *C.* x *deamii* in Macon County, Illinois. The putative parents *C. shortiana* and *C. squarrosa* were present, but once again, *C. typhina* was absent.

C. shortiana is thought to be the maternal contributor to the hybrid based on past morphological studies (Puckett 1994). The putative paternal contributors, *C. typhina* and *C. squarrosa*, are closely related taxa (both in Section Squarrosae) and therefore very similar. Because of this fact, it has been extremely difficult to make a judgement as to which are the actual parental species of *C.* x *deamii*. This study will employ macromorphological characters as well as the use of micromorphological characters in attempting accurate determination of the correct paternal contributor.

Figure 1. Images of the four taxa in this study.

A. *Carex* x *deamii* Herm.

B. *Carex shortiana* Dewey (photo by USDA no date)

C. *Carex typhina* Michx.

D. *Carex squarrosa* L.

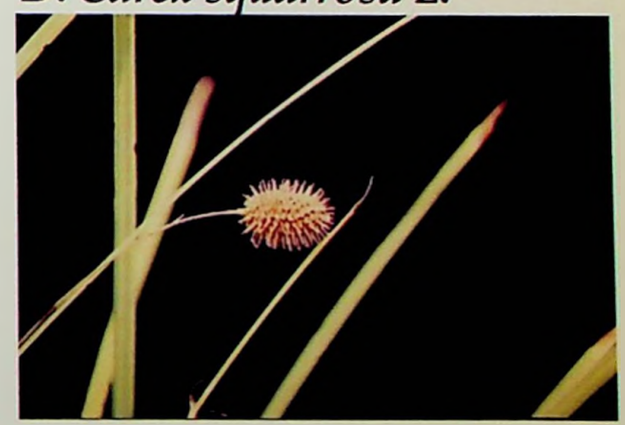
A. *Carex* x *deamii* Herm.

B. *Carex shortiana* Dewey

(USDA No Date)

C. *Carex typhina* Michx.

D. *Carex squarrosa* L.



Taxonomy of the Study Species

A number of changes have occurred in the taxonomy of these species and the following list of synonyms from *North American Flora: Cyperaceae* (Mackenzie 1935) reflects those changes:

Carex shortiana Dewey	1836
Carex Shortii Torr.	1836
Carex formosa Dewey	1837
Carex ohiotica Hochst. & Steud.	1840 (Name only)
Carex oposandra Kunze	1855 (Name only)
Carex aporandra Kunze	1860 (Name only)
Carex typhina Michx.	1803
Carex squarrosa var. *typhina* Nutt. (Based on *C. typhina* Michx.)	1818
Carex typhinoides Schw.	1824
Carex squarrosa var. *typhinoides* Dewey (Based on *C. typhinoides* Schw.)	1826
Carex squarrosa L.	1860
Carex squarrosa L.	1753
Carex squarrosa f. *robusta* Peck	1896

C. shortiana Dewey was first collected in Lexington, Kentucky by Dr. Charles Short and later named in his honor by Dewey (1836). This taxon, as listed above, has numerous synonyms.

The taxonomic status of *C. typhina* Michx. has been much more complicated. *C. typhina* was first described to science in 1803 by Michaux in his work, *Flora Boreali-Americana.* Michaux's type collection is from " Hab. in regione Illinoensi " (1803). Nuttall, in 1818, reduced this species to variety status of *C. squarrosa* saying that it represented a multi-spiked variety of the typically monostachyous *C. squarrosa.*

Schweinitz (1824) named a new species, *Carex typhinoides*, but a very inadequate description was given for this new taxon. So inadequate was the description that it later became difficult to determine whether this species was distinct from *C. squarrosa* L. or merely the same multi-spiked variety described by Nuttall. Dewey, in 1826, reduced *C. typhinoides* to a variety of *C. squarrosa* saying that it differed from the common variety in having one, two, or three spikes.

Later, this variety would receive species status again and was designated *C. typhinoides* Schweinitz. This, however, would not last long. Fernald (1909) reexamined Michaux's type material of *C. typhina* and stated that it was the same as *C. typhinoides*. He based this on the pistillate scales being blunt. *C. typhina* Michx. became the correct name since it was the first to be described.

This confusion and difficulty in defining *C. typhina* shows the great similarity that sometimes exists between *C. typhina* and *C. squarrosa* specimens.

The classification of the species involved in this study follows Kartesz (1994a, 1994b) and Reznicek (1990).

Kingdom	Plantae
Division	Spermatophyta
Subdivision	Angiospermae
Class	Monocotyledoneae
Order	Commenlinales
Suborder	Juncianeae
Family	Cyperaceae
Subfamily	Caricoideae
Tribe	Cariceae
Genus	*Carex*
Subgenus	*Eucarex*
Section	Shortianae
Species	*shortiana* Dewey.
Hybrid	x *deamii* Herm.
Section	Squarrosae
Species	*squarrosa* L.
Species	*typhina* Michx.

Geographic Distribution

The hybrid, *Carex* x *deamii*, is the most limited of the four taxa in its distribution. The taxon has been found at a total of nine sites in four states (Figure 2):

Indiana

Pike Co:	30 May, 1926, *Deam 43,090* (MICH); 5 June, 1934, *Deam 55,011* (MICH); 5 June, 1934, *Hermann 6147*(MICH) (Isotypes, 4 specimens).

Missouri

Adair Co.:	19 September, 1955, *Steyermark 79705* (F).
Barton Co.:	20 May, 1952, *Palmer s.n.* (MUHW).
Howell Co.:	25 June, 1955, *Steyermark 78724* (F).
St. Louis Co.:	30 July, 1887, *Letterman s.n.* (Originally described as C. shortiana Annotated by J. A. Steyermark, 1957).

Illinois

Macon Co.:	14 June, 1976, *Shildneck C-7917* (SIU); 7 July, 1976, *Shildneck C-7976* (SIU); 12 June, 1977, *Shildneck C-8189* (SIU); 14 June, 1979, *Shildneck C-11117* (SIU); 4 July, 1979, *Shildneck C-11204* (SIU).
Shelby Co.:	14 June, 1981, *Shildneck C-12897* (SIU); 19 July, 1981, *Shildneck C-12931* (SIU); 19 July 1982, *Shildneck C-13193* (SIU).

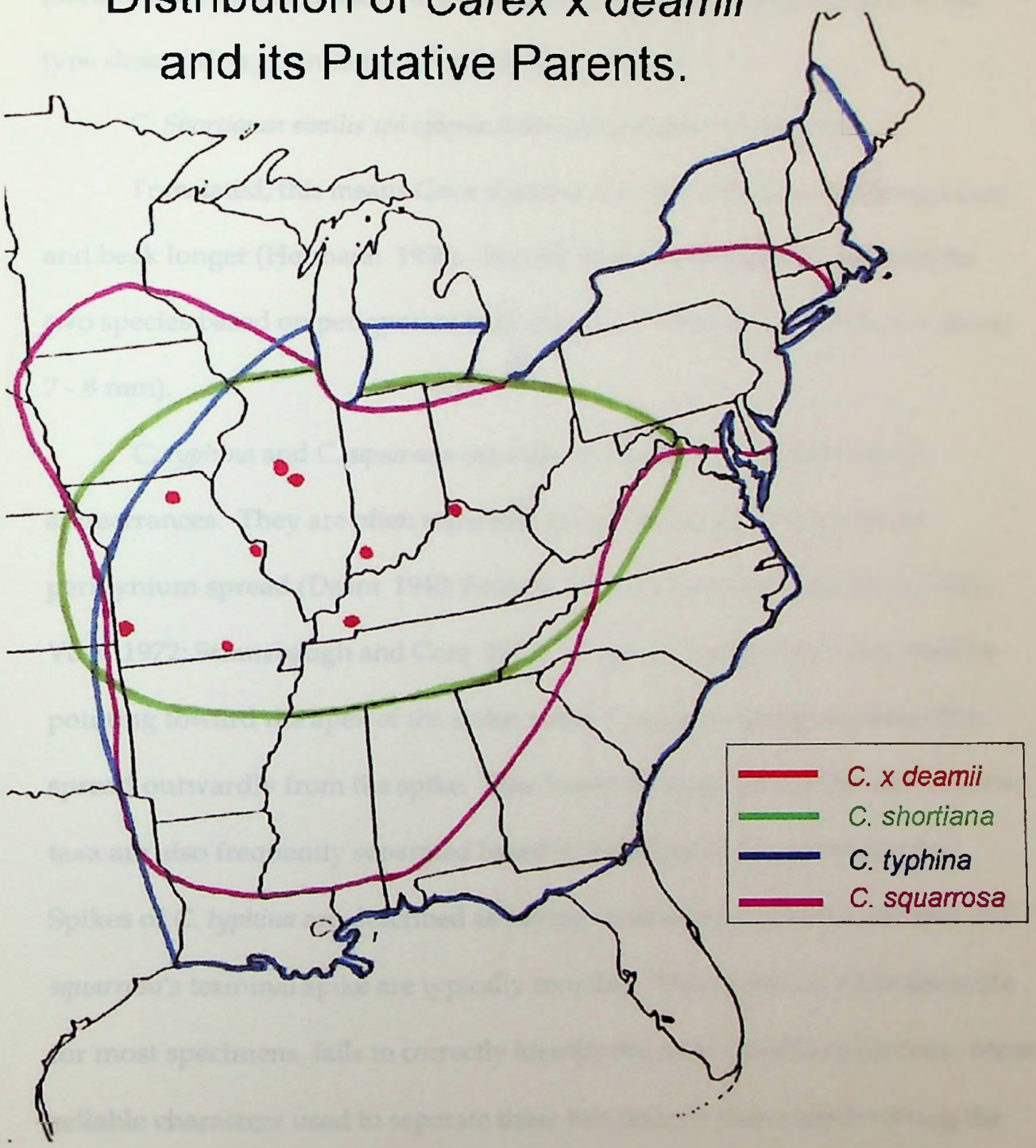
Kentucky

Kenton Co.:	1943, *Braun* (US?) (Beal and Thieret 1987).
Trigg Co.:	*Athey* (D. K. Evans pers. com.).

The remaining three species have a much broader distribution throughout the eastern portion of North America (Figure 2). *C. shortiana* is found from Pennsylvania to southern Ontario and Iowa, and southward to Virginia, Tennessee and Oklahoma. *C. typhina* is distributed from western Quebec and western New England, westward to Wisconsin and Iowa, and southward to Georgia and Louisiana. *C. squarrosa* is found from western Quebec and western New England to Wisconsin and Nebraska, and southward to North Carolina and Arkansas (Mackenzie 1935).

Figure 2. Distribution map of the *C.* x *deamii* Herm.(red), *C. shortiana* Dewey (green line), *C. typhina* Michx. (blue), and *C. squarrosa* L. (purple).

Distribution of *Carex* x *deamii* and its Putative Parents.



Morphological Description

C. shortiana and *C.* x *deamii* are placed in the same section taxonomically (Section Shortianae) and are very similar in appearance. This is evident by the type description given to the hybrid by Hermann:

C. Shortianae similis sed spicula latiora perigyniaque rostris longis.

Translated, this means *Carex shortiana* like, but spike broader, perigynium and beak longer (Hermann 1938). The key in Steyermark (1963) separates the two species based on perigynium beak length (*C. shortiana* 3.5 - 6 mm, *C.* x *deamii* 7 - 8 mm).

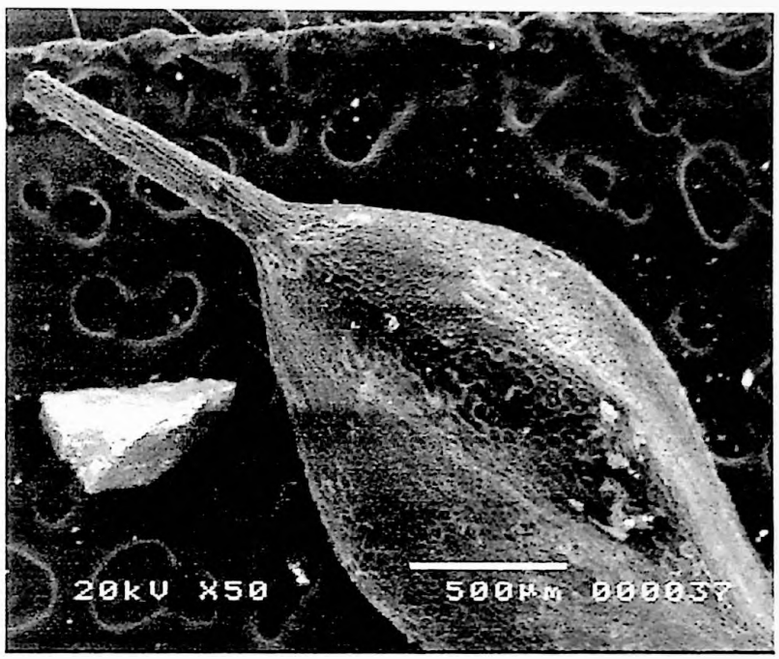
C. typhina and *C. squarrosa* can take on very similar morphological appearrances. They are often separated in keys based on the angle of the perigynium spread (Deam 1940; Fernald 1950; Steyermark 1963; Braun 1967; Voss 1972; Strausbaugh and Core 1977). *C. typhina* perigynia are described as pointing toward the apex of the spike, while *C. squarrosa* perigynia most often spread outwardly from the spike. This, however, is not always the case. The two taxa are also frequently separated based on the shape of the terminal spike. Spikes of *C. typhina* are described as having an acute apex whereas the apex of *C. squarrosa*'s terminal spike are typically rounded. This character, while adequate for most specimens, fails to correctly identify the more variable collections. More reliable characters used to separate these two taxa are characters involving the perigynium and achene. In Deam's *Flora of Indiana* (1940), *C. typhina* and *C. squarrosa* are separated by the shape of their achenes (*C. typhina* is broadly

ellipsoid, half to three-fifths as wide as long while *C. squarrosa* is oblong-ellipsoid, a third to two-fifths as wide as long). Perhaps the most reliable character in separating these two sometimes similar taxa is the shape of the style. The style of *C. typhina* (Figure 3a) extends straight while the style of *C. squarrosa* (Figure 3b) has a characteristic large bend.

Table 1 gives morphological descriptions for *C. shortiana, C. typhina,* and *C. squarrosa* according to K. K. Mackenzie's *Flora of North America: Cyperaceae* (1935).

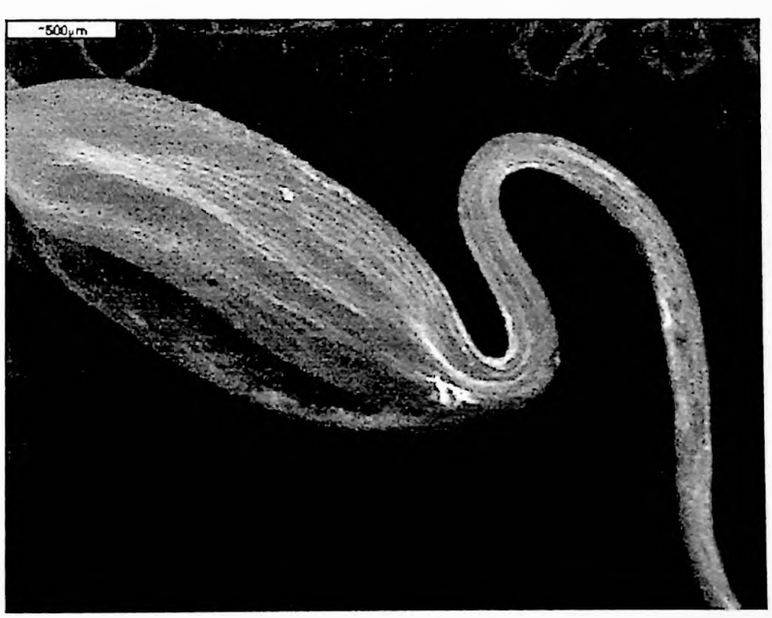
Figure 3. Images of a.) *Carex typhina* Michx. and b.) *Carex squarrosa* L. showing differences in their styles.

A.



Carex typhina Michx.

B.



Carex squarrosa L.

Table 1. Morphological Descriptions of *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L. according to K. K. Mackenzie's Flora of North America: Cyperaceae 1935.

Table 1. Description of Morphological Characters for *Carex shortiana*, *Carex typhina* and *Carex squarrosa*. Taken from North American Flora (Mackenzie 1935).

Character/Species	*Carex shortiana*	*Carex typhina*	*Carex squarrosa*
Culms	3 - 9 dm high	3 - 9 dm high	3 - 9 dm high
Leaves	4 - 8 mm wide	3.5 - 10 mm wide	2 - 6 mm wide
Spikes	5 - 6 10 - 35 mm long. 4 - 5 mm wide.	1-6 (mostly 3), Conical apex. 20 - 45 mm long (pistillate) 8 - 16 mm wide	1 (occasionally 2-4), Rounded at the top. 10 - 30 mm long (pistillate) 15 - 22 mm wide
Perigynia	Beak very minute. 0.2 mm long. Bodies very broadly obovoid, compressed triangular. 2.5 - 3 mm long. 2 mm wide.	Beaks Appressed-Ascending. 2.5 - 3 mm long. Bodies broadly obovoid-cuneate. 4 - 5 mm long. 3 mm wide.	Beaks squarrose, radiating in all directions. 2.5 - 3.5 mm long. Bodies broadly obovoid-cuneate. 4 - 5 mm long. 3 mm wide.
Pistillate Scales	Ovate, mucronate or acute. 3-nerved green center. Much narrower than and from somewhat shorter to somewhat longer than the perigynia.	Lanceolate. 3- nerved center. Narrower than and about the length of the perigynium.	Oblong- lanceolate. 3-nerved center. Narrower and much shorter than the perigynium.
Achene	Oblong-obovoid. 1.75 mm long. 1 mm wide. Triangular	Obovoid. 2.5 mm long. 1.5 mm wide. Triangular.	Linear-oval. 3 mm long. 1.5 mm wide. Triangular.

Taxonomic Importance of Macromorphological Characters

Analysis of macromorphology has been the primary mode in which plants have been studied in the past. In fact, most classification schemes (especially in the Cyperaceae) are still based primarily on macromorphological characters. The characters that are used, however, vary from family to family and from genus to genus. Floral characters have been, and still are, the most used features in classifying angiosperms. Nonetheless, vegetative characters have been found useful for many groups and Reznicek (1986a) suggests they may be useful in classifying the genus *Carex*.

Floral characters in the genus *Carex* are very reduced and conserved throughout the genus. Therefore, they are of very little help in the classification of sedges. While flower structure is very conservative, the shape and size of the inflorescence are quite variable within the genus. Consequently, these features, as well as features of the perigynium and pistillate scale, have been used in classifying and identifying members of the genus *Carex*.

A number of techniques have been developed to draw valuable taxonomic information from morphological data. These techniques can be as simple as comparing only one or two characters at a time or as complex as multivariate analyses that simultaneously compare many characters. A number of statistical programs have been developed to utilize multivariate data sets. These programs have been been a great tool in classifying difficult groups or complexes

of plants which cannot be reliably separated based on one or two characters. Many of these difficult complexes are present in the genus *Carex*.

Taxonomic Importance of Micromorphological Characters

Most existing taxonomic treatments of plant families are based largely on analysis of macromorphological characters. Micromorphological characters, however, have also contributed greatly to plant systematics. Features commonly used include leaf and seed surface anatomy.

An abundance of useful features can be found on leaf surfaces. Shapes and sizes of epidermal cells can sometimes be important taxonomic characters (Stace 1989). Likewise, stomata shape and distribution patterns are often observed. Any epidermal projections could possibly be an identifiable attribute. Stace (1965, 1981) found that trichome structure was useful at all levels of taxonomy in the family Combretaceae Presence of prickles and papillae on leaf surfaces in the genus *Carex* have sometimes been found to be significant (Mallory 1979).

The surfaces of seeds have also garnered much attention by plant taxonomist using anatomy as a tool (Stace 1989). Seeds often exhibit unique features which may allow them to germinate more readily, stave off herbivory, or in some way benefit the species. These same features can frequently be used by taxonomists to identify specific groups of plants. In the genus *Carex*, for example, conical silica bodies are found underneath the outer cell wall. These silica bodies provide members of the genus *Carex* a natural scarification process.

Taxonomist have also found these silica bodies useful. Their shape, number, size, etc... can all be characterized and classified (Walter 1979).

Other micromorphological features of the genus Carex have been useful in taxonomic studies. Presence or absence of papillae on the perigynium surface, perigynia epidermal cell size, and perigynia epidermal cell shape have been used in many Carex micromorphological taxonomy studies (Standley 1985).

Objectives

(1.) The primary objective of this study is to determine the correct parentage of the hybrid, *Carex* x *deamii* Herm. Specifically, both, macromorphological and micromorphological features (surface anatomy features as seen under SEM) will be used in this analysis. Many techniques for determining the parents of hybrids will be used and compared. These techniques include bivariate character analysis, Principal Components Analysis (PCA), Canonical Discriminant Analysis (CDA), and HYWIN (Hypothesizing hybrids based on Weighted Intermediacy).

(2.) In addition to this primary objective, a number of other goals have been set for this study. Among these are the determination of the hybrids viability (both in fruit and in pollen).

(3.) Also, since numerous measurements will be made on the four taxa, a characterization of their variability will be a very useful result of this study. (4.) This variability will be used to determine characters which prove most reliable in determining the identity of each species.

Studies on the surface anatomy features of *Carex* are ever increasing in frequency and this will be the first time, to my knowledge, that any of these taxa have been examined for distinctive surface anatomy features. Therefore, a complete description of their surface anatomy may prove useful in later studies involving the phylogeny of the genus.

CHAPTER II.

METHODS

Analysis of Pollen and Achene Viability

Viability of pollen was analyzed for all four taxa from collections made in the field and from collections grown in the Marshall University greenhouse. Specimens of *C. shortiana*, *C. typhina*, and *C. squarrosa* were collected during their pollen dispersal stage from three sites in the West Virginia and Ohio area (Appendix 1). *C.* x *deamii* pollen viability was observed from live greenhouse material (originally collected in Illinois).

In the lab, numerous anthers were taken from fresh material of each specimen and the pollen deposited onto a microscope slide containing one drop of acetocarmine stain. The slides were examined under a Baush and Lomb Compound Light Microscope. Approximately 200 pollen grains were counted from each collection and determined to be sterile or fertile. Any misshapen pollen grains were deemed sterile. Only pollen grains that took up the acetocarmine stain and were plump were considered to be fertile (Pereira et al. 1997).

Achene sterility of the hybrid was analyzed by examining all available herbarium specimens (n=21). Any achenes found to be plump were considered viable while shriveled and distorted achenes were determined to be nonviable.

Analysis of Macromorphological Characters

Selection and Measurement of Morphological Characters

Initially, twenty-seven morphological characters were selected based on information that proved to be useful in previous taxonomic keys (Deam 1940; Fernald 1950; Steyermark 1963; Braun 1967; Voss 1972; Strausbaugh and Core 1977). Also, previously published (Standley 1983; Reznicek and Catling 1985; Reznicek 1986b) taxonomic papers on *Carex* complexes were used in character selection. These characters included both reproductive (ex. spike length (SL), spike width (SW), etc. . .) and vegetative characters (ex. leaf width (LW), etc. . .). Table 2 provides a list of all macromorphological characters selected and explains how they were measured.

Larger characters such as leaf length (LL) and culm length (CL) were measured to the nearest millimeter using a clear plastic ruler. Characters such as spike length (SL), spike width (SW), pistillate length (PL), and staminate length (STL) were measured to the nearest 0.01 mm with a 150 mm Mitutoyo dial caliper under a Leica stereo microscope. Smaller characters such as those for the perigynia and pistillate scales were measured to the nearest 0.01 mm using an ocular micrometer on a Leica stereo microscope set at 10X. When possible, three measurements of each character were taken from each herbarium collection. A mean was then calculated from this data and used as the value of that character for that particular collection. Characters such as spike length (SL) and spike width (SW) could not always be done in this fashion depending on the number

Table 2. List of all macromorphological characters along with measurement techniques.

Table 2. List of morphological characters and description of measurement procedure.

Character (Abbreviation)	Description
Leaf Width (LW)	-Width of leaf blade at its widest point.
Vein Number (VN)	-Number of veins across leaf. Measured from the widest point of leaf.
Spike Number (SN)	-Number of spikes per fertile culm.
Spike Length (SL)	-Length of spike from base of first staminate scale to tip of uppermost perigynium.
Spike Width (SW)	-Width at the widest point. Measured from perigynium on one side to perigynium on the opposite side.
Spike Ratio (SR)	-Ratio derived by dividing spike length (SL) x spike width (SW). A value which depicts the general shape of the uppermost spike.
Pistillate Length (PL)	-Length from the lowest pistillate scale to the tip of the uppermost perigynium.
Pistillate Length/Width Ratio (PR)	-Ratio derived by dividing the pistillate length (PL) by the spike width (SW). A value that depicts the shape of the pistillate portion of the uppermost spike.
Staminate Length (STL)	-Length from the lowest staminate scale to the junction between staminate and pistillate flowers.

Table 2(continued)

Pistillate/Staminate Ratio (PSR)	-Ratio derived by dividing the pistillate length (PL) x the staminate length (STL).
Perigynium Length (PEL)	-Length of the perigynium from base to apex of the beak.
Perigynium Width (PEW)	-Width of the perigynium measured at its widest point.
Perigynium Length/Width Ratio (PER)	-Ratio derived by dividing the perigynium length (PEL) by the perigynium width (PEW). A value which depicts the overall shape of the perigynium.
Perigynium Body Length (PBL)	-Length of the perigynium from the base to the beginning of the beak.
Perigynium Body Ratio (PBR)	-Ratio derived from dividing perigynium body length (PBL) by perigynium width (PEW). A value which depicts the general shape of the perigynium body.
Perigynium Beak Length (PKL)	-Length of the perigynium beak from the apex of the beak to the point of expansion of the perigynium body.
Perigynium Beak/Body Ratio (PBB)	-Ratio derived by dividing the perigynium beak length (PKL) by the perigynium body length (PBL).
Pistillate Scale Length (SCL)	-Length of pistillate scales from base to apex. Include any awns or tips present.
Pistillate Scale Width (SCW)	-Width of pistillate scales at their widest point.

Table 2 (continued)

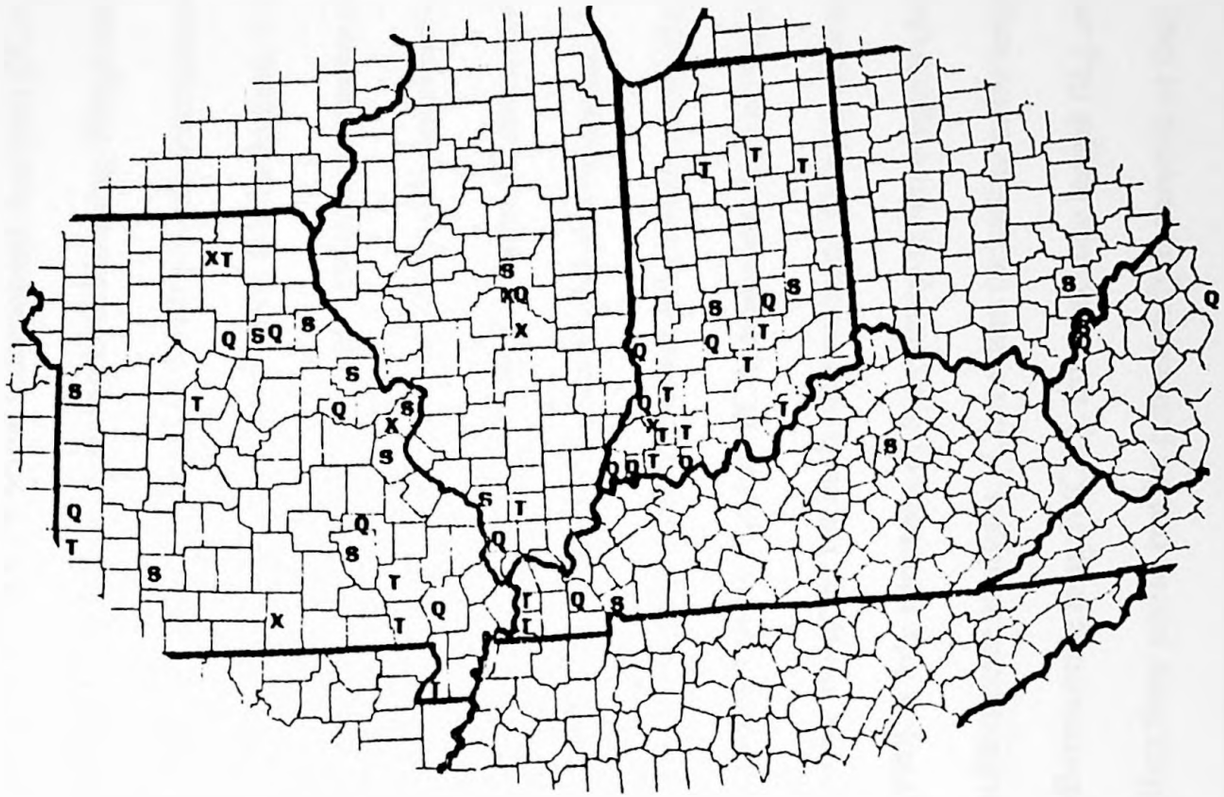
Pistillate Scale Ratio (SCR)	-Ratio derived by dividing the pistillate scale length (PSL) x the pistillate scale width (PSW). Value depicts the general shape of the pistillate scales.
Staminate Scale Length (SSL)	-Length of the staminate scales from base to apex.
Staminate Scale Width (SSW)	-Width of staminate scales at their widest point.
Staminate Scale Ratio (SSR)	-Ratio derived by dividing staminate scale length (SSL) x staminate scale width (SSW). Value depicts the general shape of the staminate scales.

of fertile culms on the herbarium sheet. Herbarium specimens for this study came from the Marshall University Herbarium (MUHW), The Ohio State University (OS), and the University of Michigan (MICH), and the United States National Herbarium, Smithsonian Institute (US) as well as from harbaria in the geographical area of the hybrid. These latter herbaria included the Chicago Field Museum of Natural History (F), Indiana University (IND), Missouri Botanical Garden (MO), Southern Illinois University (SIU), and the University of Cincinnati (CINC). Herbarium abbreviations follow Index Herbarium (Holmgren et al. 1990).

A total of 75 herbarium specimens were measured from the four taxa. Only fifteen of the nineteen *Carex* x *deamii* Herm. specimens obtained were used due to the incompleteness of four collections. Twenty specimens each were measured for *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L. Geographic distribution of these specimens was primarily restricted to the range of the hybrid (Figure 4).

Figure 4. Geographic distribution of herbarium specimens measured for macromorphological characters. X = *Carex* x *deamii* Herm., S = *Carex shortiana* Dewey, T = *Carex typhina* Michx., and Q = *Carex squarrosa* L. Some marks may represent more than one specimen.

Distribution of Carex x deamii Herm. (X), Carex shortiana Dewey (S), Carex typhina Michx. (T), and Carex squarrosa L. (Q)



Statistical Analysis

Data collected from herbarium specimens were subjected to Analysis of Variance (ANOVA), Duncan's Multiple Range Test, Canonical Discriminant Analysis (CDA), Principal Component Analysis (PCA), Discriminant Analysis, and HYWIN (Hypothesizing hybrids using Weighted Intermediacy). Character measurements were used to construct full descriptions of each taxa. This represents the first such description for the hybrid, *C.* x *deamii*.

Duncan's Multiple Range Test groups species for each character selected based on whether there is a significant difference between the mean values. CDA is a multivariate analysis that takes all characters selected into consideration and produces a scatterplot of results. PCA is also a multivariate analysis that takes all characters selected into consideration and produces a scatterplot of its results. PCA, however, does not take into consideration the species assigned for each observation. These tests were done using SAS/STAT (1990). Only nineteen of the twenty-seven original characters were used in these multivariate analyses. Characters including culm length (CL), culm plus inflorescence length (CIL), inflorescence length (IL), etc. were not used because either they could not be consistently measured or they were not useful in separating taxa.

Discriminant analysis produces a generalized squared distance to species that allows for comparison of how close each species is to one another.

Discriminant analysis also produces a classification for all specimens examined. This helps in determining if any specimens were identified incorrectly. HYWIN, developed by Estabrook, Gil-ad, and Reznicek (1986), is useful in hypothesizing which specimens might be of hybrid origin, as well as determining which other two specimens would represent the most likely parent species. Analysis done by the HYWIN program considers each specimen separately and produces a "triple" (one prospective hybrid and the two probable parents). It checks every combination of specimens that has been entered and ranks the triples in order of likelihood. Three indicators are used to establish these "triples": 1.) Hybrid Intermediacy Score (IN), 2.) Distance between possible parents (PD), and 3.) Measure of equality of the two distances between the possible hybrid and each of the two possible parents (EQ). A subset consisting of ten perigynia and pistillate scale characters were used in this analysis. In the past these characters proved to be best in separating these taxa.

Analysis of Micromorphological Characters

Achene Micromorphology

Achene material for micromorphological analysis was obtained from herbarium specimens and from greenhouse grown specimens. Specimens were selected based on quality of material, age of specimen, and maturity. Only fully mature specimens were used for data collection. Numerous achenes of each taxa were collected for micromorphological analysis. All specimens used were of a similar geographic distribution.

Once collected, achenes from all four taxa were subjected to acetolysis treatment as described by Walter (1975). This treatment is necessary to remove the outer epidermal cell wall which masks the taxonomically important silica bodies below. In this study several acetolysis treatments were used to determine the best method for all taxa involved. These treatments were as follows:

1.) No treatment. Achenes viewed without being subjected to acetolysis treatment.

2.) Water treatment. Achenes subjected to submersion in water for 36 hours.

3.) Acid 1. Achenes soaked in a solution of 1 part concentrated sulfuric acid (H_2SO_4) and 9 parts acetic anhydride ($(CH_3CO)_2O$) for 12 hours.

4.) Acid 2. Achenes soaked in a solution of 1 part concentrated sulfuric acid (H_2SO_4) and 9 parts acetic anhydride ($(CH_3CO)_2O$) for 24 hours.

5.) Acid 3. Achenes soaked in a solution of 1 part concentrated sulfuric acid (H_2SO_4) and 9 parts acetic anhydride ($(CH_3CO)_2O$) for 36 hours.

After each treatment the achenes were washed with distilled water and sonicated in a small vial for approximately five minutes to remove any remaining surface debris. After sonication the achenes were desiccated using a drying oven at low temperature.

After collection and treatment, the achene material was adhered to aluminum SEM stubs using double-sided carbon tape. Achenes were coated with gold/palladium for five minutes using a Hummer 6.2 Sputtering System by

Anatech Limited. The sputtering system was maintained at 15 millamperes plasma discharge current and 60-80 millitorr vacuum level. The specimens were viewed as soon after coating as possible with a JEOL JSM-5310LV Scanning Electron Microscope. Achenes were usually viewed immediately after coating and observed for any identifiable features. Images with scale marker bars included were saved as TIF image files for later observation. Achene cells were measured using SigmaScan/Image Measurement Software for Windows developed by Jandel Scientific (1993).

Perigynium, Pistillate Scale, and Leaf Epidermal Micromorphology

Selection and Measurement of Micromorphological Characters

Material from 16 herbarium specimens, four per taxon, were examined for perigynium, pistillate scale, and leaf features (Appendix 3).

Perigynia and pistillate scales were removed from the middle portion of the uppermost spike. Two to four perigynia and pistillate scales were usually viewed for each herbarium specimen. Two small leaf segments approximately 1 cm in length were removed from the middle portion of the widest leaf. One segment was observed for adaxial surface features while the other segment was observed for abaxial surface features.

Acetolysis treatment was not necessary prior to viewing perigynium, pistillate scale or leaf material. SEM preparation for achene material was also used with perigynium, pistillate scale, and leaf material. Specimens were examined for various leaf, perigynium, and pistillate scale features. Features

observed were chosen because of their use in previous *Carex* micromorphology papers (Standley 1985; Walter 1979).

Initially, fifteen characters were selected for this study (Table 3). Measurement of characters was accomplished on computer using the SigmaScan/Image Measurement Software for Windows developed by Jandel Scientific (1993). Five measurements were made for each character and the mean of these measurements was used as the value for that specimen. All quantitative characters measurements were rounded to the nearest .01 micrometer. Qualitative characters (LfPap, LfPrk, BkPap, BkPrk, and ScPrk) were given a value of 1 or 2 indicating the feature was not present (1) or present (2).

Statistical Analysis

Data collected from micromorphological measurements was subjected to the same statistical analysis as macromorphological data (ANOVA, Duncan's Multiple Range Test, CDA, PCA, and HYWIN).

X-ray Analysis

Elemental compositions of a sampling of various plant parts (leaves, achenes, perigynia, pistillate scales, stigma lobes) surfaces were analyzed to show the distribution of silica and other elements in *Carex* specimens (Goldstein et al. 1992). This analysis was accomplished with an Oxford Link ISIS X-ray analysis system attached to the the JEOL scanning electron microscope. All x-ray analyses were done with the scanning electron microscope set at 0 degrees tilt and 20 kV accelerated voltage.

Table 3. List of all micromorphological characters selected along with their abbreviation.

Abaxial Leaf Features

- Cell length (LCL)
- Cell Width (LCW)
- Cell Length/Width Ratio (LCR
- Stomata Length (SL)
- Stomata Width (SW)
- Stomata Length/Width Ratio (SR)
- Leaf Prickle (LFPRK)

Perigynium Surface Features

- Perigynium Cell Length (PCL)
- Perigynium Cell Width (PCW)
- Perigynium Cell Length/Width Ratio (PCR)

Pistillate Scale Feature

- Pistillate Scale Prickle (SCPRK)

CHAPTER III.

RESULTS

Analysis of Pollen and Achene Viability

Pollen was analyzed for the four taxa to determine percent stainability (%stainability ~= %viability). *C.* x *deamii* was found to be 2.3 % viable on average (N=514) while the three possible parent species always exceeded 90 % viability. *C. typhina* had the greatest mean viability with 94.9 % (N=473) followed by *C. shortiana* with 92.8 % (N=402) and *C. squarrosa* with 90.8 % (N=572). Table 4 shows values for pollen viability counts.

Table 4. Pollen Viability Counts for *C.* x *deamii*, *C. shortiana*, *C. typhina*, and *C. squarrosa* along with collection locations.

Species	% Viability	Collection Site
C. x *deamii*	2.5 (7/281)	Marshall Univ. greenhouse (Transplanted from Illinois).
	2.1 (5/233)	Marshall Univ. greenhouse (transplanted from Illinois).
C. shortiana	91.9 (181/197)	Guyan Cr. Oxbow, Mason Co., WV
	93.7 (192/205)	Crab Creek Lagoon, Mason Co., WV
C. typhina	95.9 (232/242)	Guyan Cr. Oxbow, Mason Co., WV
	93.9 (217/231)	Crab Cr. Lagoon, Mason Co., WV
C. squarrosa	88.2 (179/203)	Guyan Cr. Oxbow, Mason Co., WV
	91.4 (159/174)	Crab Cr. Lagoon, Mason Co., WV
	92.8 (181/195)	Morris Lane Rd., Scioto Co., OH

Means

***Carex* x *deamii* Herm.**	**2.3 %**	***Carex typhina* Michx.**	**94.9%**
***Carex shortiana* Dewey.**	**92.8 %**	***Carex squarrosa* L.**	**90.8%**

All available specimens of *C.* x *deamii* were examined (N = 19) for fertile achenes and there were none found on any specimens. All achenes, when found, were only slightly developed (~ 1 mm long; much smaller than normal achenes) and clearly misshapen.

Analysis of Macromorphological Characters

Univariate Analysis

Analysis of Variance (ANOVA)

According to the ANOVA procedure of SAS/STAT (1990) twenty of the twenty-seven morphological characters proved to be significantly different among taxa to at least the .01 level. All nineteen characters used for multivariate analysis showed a significant difference to the .01 level (Table 5). Best among these were all perigynia characters (PEL, PEW, PER, PBL, PBR, PKL, and PBB) with p values of .0001. Two of the pistillate scale characters (SCL and SCR) also had p values of .0001.

Duncan's Multiple Range Test

The Duncan's Multiple Range Test groups the species for each morphological character based on whether they are significantly different. Taxa with the same letter are not significantly different while those with different letters are significantly different.

Since character intermediacy is an important factor in determining hybrid origin, Duncan groupings were used to determine those characters for which C. x *deamii* was immediately intermediate between C. *shortiana* and C.*typhina* and between C. *shortiana* and C. *squarrosa*. The results of the Duncan groupings places C. x *deamii* immediately between C. *shortiana* and C. *typhina* for thirteen characters (SL, SW, SR, PR, SN, PEL, PEW, PER, PBL, PBR, PKL, PBB, and SCR) and between C. *shortiana* and C. *squarrosa* for only four characters (LW, PL, SCL,

and SCW). Table 6 provides the Duncan groupings for all characters as well the means +/- one standard deviation.

Hybrids often exhibit morphological similarity to their parent species. *C.* x *deamii* is no different and is very similar in appearance to the putative maternal parent *C. shortiana*. In fact, in the analysis of macromorphological characters, *C.* x *deamii* was closest to *C. shortiana* for fifteen of nineteen characters used in the multivariate analysis. Only SL, PL, PR, and PBB were closer to either *C. typhina* or *C. squarrosa*. For each macromorphological character, *C.* x *deamii* was also evaluated to see if it was closest to *C. typhina* or *C. squarrosa*. *C.* x *deamii* was closest to *C. typhina* for fourteen characters (LW, SW, SR, PR, SN, PEL, PEW, PER, PBL,PBR, PKL, PBB, SCW, and SCR) and to *C. squarrosa* for only five characters (SL, PL, STL, PSR, and SCL).

Table 5. Table of characters used in macromorphological multivariate analysis along with their F and p values according to ANOVA.

Character	F Value	P Value
LW	42.02	.0001
SL	16.30	.0001
SW	460.98	.0001
SR	91.78	.0001
PL	45.78	.0001
PR	65.36	.0001
STL	6.52	.0006
PSR	37.82	.0001
SN	334.88	.0001
PEL	414.23	.0001
PEW	13.97	.0001
PER	112.77	.0001
PBL	175.63	.0001
PBR	60.95	.0001
PKL	571.57	.0001
PBB	181.52	.0001
SCL	123.68	.0001
SCW	4.41	.0067
SCR	75.39	.0001

*LW=Leaf Width(mm)
*SL=Spike Length(mm)
*SW=Spike Width(mm)
*SR=Spike L/W Ratio
*PL=Pistillate Length(mm)
*PR=Pistillate L/W Ratio
*STL=Staminate Length(mm)
*PSR=Pistillate/Staminate Ratio
*SN=Spike Number
*PEL=Perigynium Length(mm)
*PEW=Perigynium Width(mm)
*PER=Perigynium L/W Ratio
*PBL=Perigynium Body Length(mm)
*PBR=Perigynium Body L/W Ratio
*PKL=Perigynium Beak Length(mm)
*PBB=Perigynium Beak/Body Ratio
*SCL= Pistillate Scale Length(mm)
*SCW=Pistillate Scale Width(mm)
*SCR=Pistillate Scale L/W Ratio

Table 6. Mean values +/- one standard deviation for macromorphological characters used in the assessment of the hybrid origin of *Carex* x *deamii* Herm. The letter following these values depicts the grouping assigned by Duncan's Multiple Range Test (Unlike letters indicate a significant difference). Characters followed by an asterisk were used in multivariate analysis.

Character	*C.* x *deamii*	*C. shortiana*	*C. typhina*	*C. squarrosa*
LW(mm)*	5.57+/-0.69B	6.35+/-0.58A	6.45+/-1.02A	4.12+/-0.59C
SL(mm)*	36.01+/-6.75B	30.03+/-5.09C	41.92+/-5.01A	33.83+/-5.36B
SW(mm)*	8.04+/-0.93C	5.22+/-0.43D	13.14+/-1.34B	17.54+/-1.45A
SR*	4.55+/-1.06B	5.77+/-1.00A	3.24+/-0.56C	1.93+/-0.25D
PL(mm)*	22.05+/-3.82B	18.68+/-3.75C	32.84+/-4.20A	23.24+/-4.14B
PR*	2.77+/-0.55B	3.59+/-0.75A	2.53+/-0.45B	1.31+/-0.18C
STL(mm)*	13.96+/-4.00A	11.37+/-3.93B	9.09+/-2.14C	10.97+/-2.73B,C
PSR*	1.70+/-0.41C	1.86+/-0.71B,C	3.88+/-1.00A	2.20+/-0.52B
SN*	3.28+/-0.38B	4.90+/-0.21A	1.68+/-0.65C	1.11+/-0.26D
PEL(mm)*	3.52+/-0.33C	2.31+/-0.17D	6.76+/-0.60B	8.28+/-0.93A
PEW(mm)*	2.06+/-0.35B	2.03+/-0.17D	2.51+/-0.51A	2.68+/-0.40A
PER*	1.68+/-0.44C	1.15+/-0.09D	2.79+/-0.50B	3.13+/-0.40A
PBL(mm)*	2.61+/-0.25C	2.10+/-0.14D	4.42+/-0.47B	5.23+/-0.78A
PBR*	1.34+/-0.26C	1.04+/-0.08D	1.82+/-0.30B	1.98+/-0.28A
PKL(mm)*	0.91+/-0.17C	0.21+/-0.05D	2.34+/-0.32B	3.05+/-0.30A
PBB*	0.34+/-0.06C	0.10+/-0.02D	0.54+/-0.09B	0.59+/-0.09A
SCL(mm)*	3.22+/-0.48C	2.46+/-0.42D	4.79+/-0.41A	4.49+/-0.44B
SCW(mm)*	1.36+/-0.46A	1.38+/-0.18A	1.45+/-0.24A	1.15+/-0.17B
SCR*	2.37+/-0.59C	1.81+/-0.36D	3.39+/-0.54B	3.95+/-0.47A
VN	27.81+/-3.57A	26.73+/-2.92A	27.17+/-1.34A	22.43+/-2.72B
LN	6.54+/-1.23A	6.11+/-1.38A	6.57+/-0.53A	5.57+/-0.86A
LL(cm)	75.51+/-16.86A	69.04+/-20.66A	73.21+/-10.68A	74.80+/-15.63A
CL(cm)	57.48+/-14.48A	52.57+/-20.50A	48.49+/-6.85A	55.07+/-11.00A
CWB(mm)	3.82+/-0.31A,B	3.95+/-0.75A,B	4.26+/-0.69A	3.65+/-0.65B
CWT(mm)	1.07+/-0.12B	1.33+/-0.20A	1.02+/-0.13B	0.82+/-0.12C
CIH(cm)	63.78+/-14.70A	64.16+/-20.58A	53.07+/-7.86A	58.47+/-11.51A

*LW=Leaf Width(mm)
*SL=Spike Length(mm)
*SW=Spike Width(mm)
*SR=Spike L/W Ratio
*PL=Pistillate Length(mm)
*PR=Pistillate L/W Ratio
*STL=Staminate Length(mm)
*PSR=Pistillate/Staminate Ratio
*SN=Spike Number
*PEL=Perigynium Length(mm)
*PEW=Perigynium Width(mm)
*PER=Perigynium L/W Ratio
*PBL=Perigynium Body Length(mm)
*PBR=Perigynium Body L/W Ratio
*PKL=Perigynium Beak Length(mm)
*PBB=Perigynium Beak/Body Ratio
*SCL= Pistillate Scale Length(mm)
*SCW=Pistillate Scale Width(mm)
*SCR=Pistillate Scale L/W Ratio
VN=Vein Number
LN=Leaf Number
LL=Leaf Length(cm)
CL=Culm Length(cm)
CWB=Culm Width at Base(mm)
CWT=Culm Width at Top(mm)
CIH=Culm+Inflorescence Height(mm)

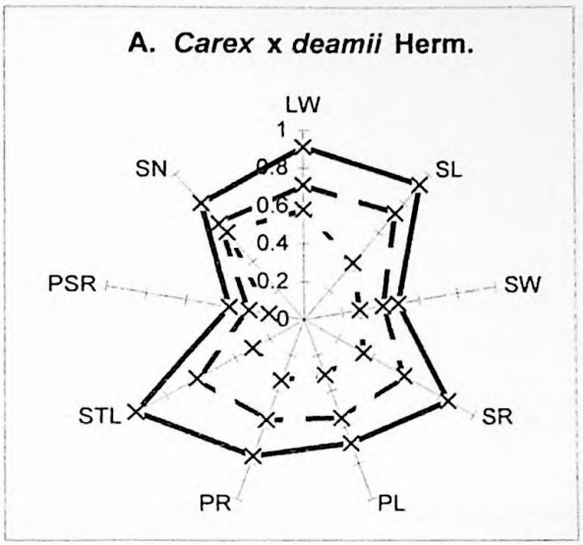
Polygonal Graph Analysis

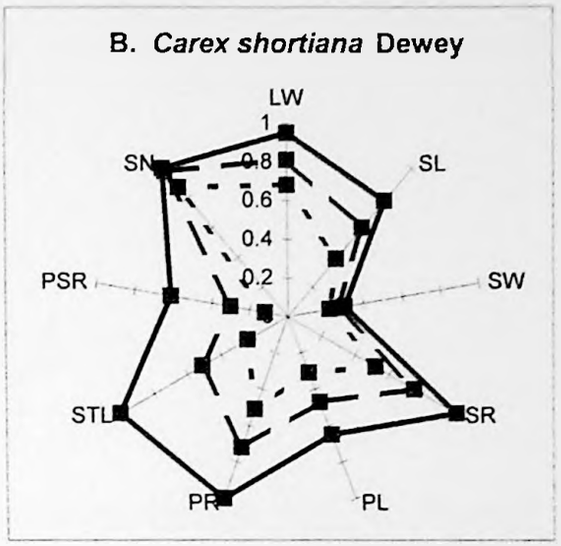
Polygonal graphs of the relative values for each character were created to visually compare the variability of morphological characters between and among taxa. Two sets of polygonal graphs were constructed. The first set (Figure 5) shows each taxa for the first nine characters used in multivariate analysis (LW, SL, SW, SR, PL, PR, STL, PSR, and SN). The actual and relative values of these characters are given in Table 7. In this set of polygonal graphs, staminate length (STL) and spike length (SL) were the most variable characters among the four taxa (Figure 5, Table 6). The least variable characters were spike width (SW), spike number (SN), and spike length/width ratio (SR) (Figure 5, Table 6).

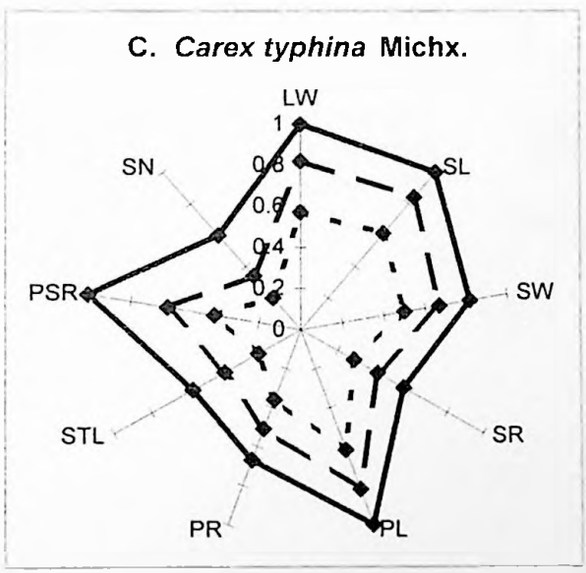
When the mean values are superimposed on the same graph (Figure 6), . x *deamii* and *C. shortiana* show a similar pattern as do *C. typhina* and *C. squarrosa*. The two groups differ primarily by spike width (SW) and spike number (SN). Spike widths of *C. typhina* and *C. squarrosa* are about twice that of *C.* x *deamii* and *C. shortiana* and *C. shortiana* and *C.* x *deamii* clearly possess a greater number of spikes per fertile culm than *C. typhina* and *C. squarrosa*. *C. squarrosa* is the least variable taxa for this set of characters and the polygonal graph pattern reveals a smaller form of *C. typhina* for all characters except spike width (SW) and staminate length (STL).

Figure 5. Polygonal graph of maximum, mean, and minimum values of Macromorphological Characters for A.) *Carex* x *deamii* Herm., B.) *Carex shortiana* Dewey, C.) *Carex typhina* Michx., and D.) *Carex squarrosa* L..

LW=Leaf Width(mm)
SL=Spike Length(mm)
SW=Spike Width(mm)
SR=Spike L/W Ratio
PL=Pistillate Length(mm)
PR=Pistillate L/W Ratio
STL=Staminate Length(mm)
PSR=Pistillate/Staminate Ratio
SN=Spike Number


A. Carex x deamii Herm.
LW
SL
SW
SR
PL
PR
STL
PSR
SN
1
0.8
0.6
0.4
0.2
0


B. Carex shortiana Dewey
LW
SL
SW
SR
PL
PR
STL
PSR
SN
1
0.8
0.6
0.4
0.2
0


C. Carex typhina Michx.
LW
SL
SW
SR
PL
PR
STL
PSR
SN
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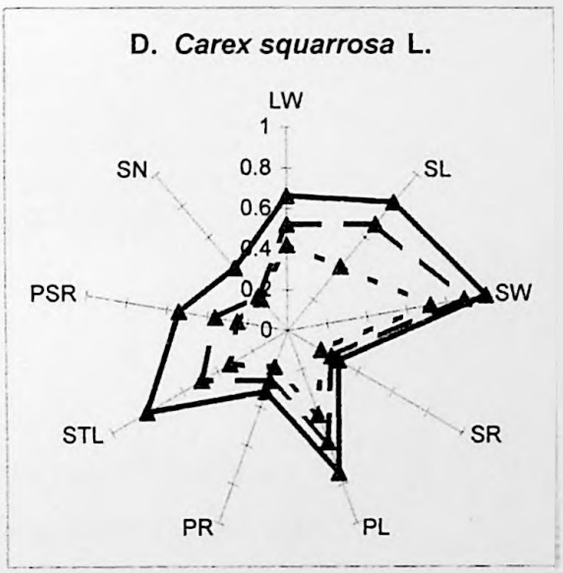
0.8
0.6
0.4
0.2
0


D. Carex squarrosa L.
LW
SL
SW
SR
PL
PR
STL
PSR
SN
1
0.8
0.6
0.4
0.2
0

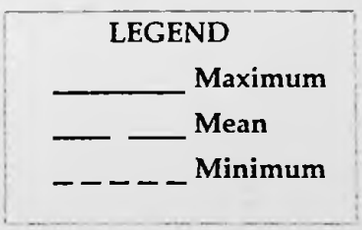
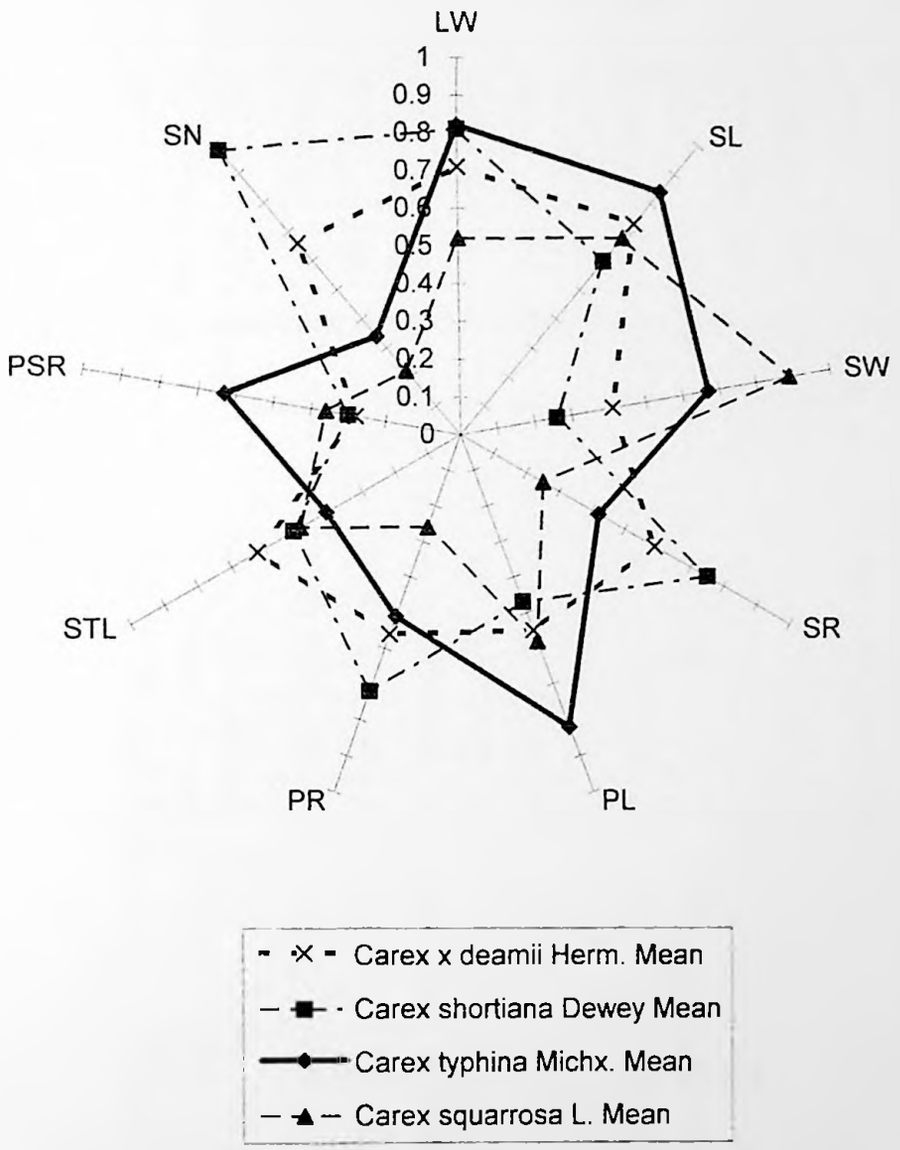

LEGEND
Maximum
Mean
Minimum

Figure 6. Polygonal graph of mean values of Macromorphological Characters for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L..

LW=Leaf Width(mm)
SL=Spike Length(mm)
SW=Spike Width(mm)
SR=Spike L/W Ratio
PL=Pistillate Length(mm)
PR=Pistillate L/W Ratio
STL=Staminate Length(mm)
PSR=Pistillate/Staminate Ratio
SN=Spike Number


LW
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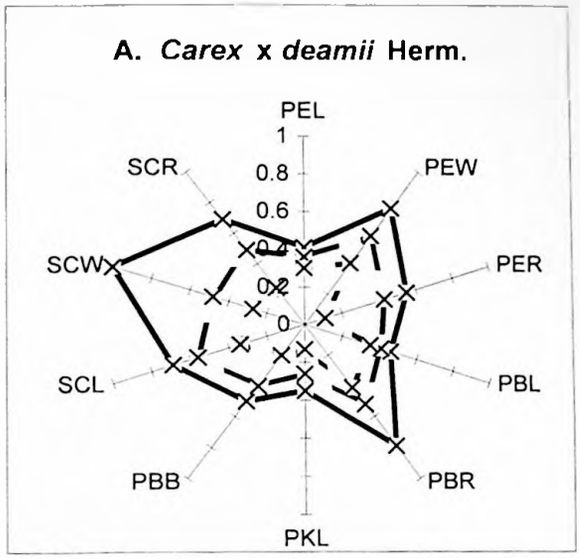
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SL
SW
SR
PL
PR
STL
PSR
SN
Carex x deamii Herm. Mean
Carex shortiana Dewey Mean
Carex typhina Michx. Mean
Carex squarrosa L. Mean

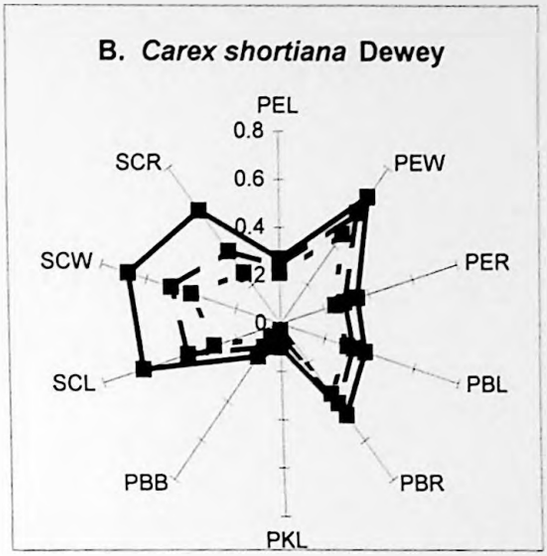
Table 7. Actual and relative values for macromorphological characters of *Carex* x *deamii* and its putative parents.

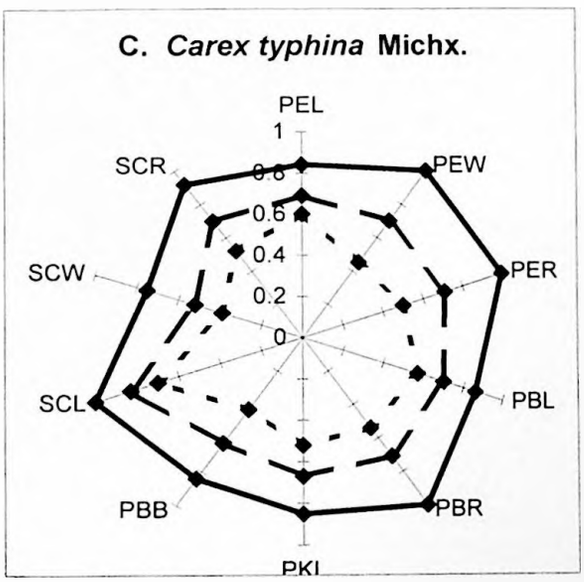
		LW		SL		SW		SR		PL		PR		STL		PSR		SN	
		A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
Carex x deamii Herm.	Maximun	7.14	0.91	46.09	0.93	9.66	0.49	6.57	0.85	27.50	0.69	3.77	0.76	21.73	0.97	2.35	0.38	4.00	0.80
	Mean	5.57	0.71	36.01	0.73	8.04	0.41	4.55	0.59	22.05	0.55	2.77	0.56	13.96	0.62	1.70	0.28	3.28	0.66
	Minimun	4.58	0.58	19.27	0.39	5.74	0.29	2.66	0.35	12.48	0.31	1.72	0.34	6.79	0.30	1.13	0.18	3.00	0.60
Carex shortiana Dewey.	Maximun	7.45	0.95	38.92	0.78	5.96	0.30	7.69	1.00	26.08	0.65	4.99	1.00	22.42	1.00	3.71	0.61	5.00	1.00
	Mean	6.35	0.81	30.03	0.60	5.22	0.26	5.77	0.75	18.68	0.47	3.59	0.72	11.37	0.51	1.86	0.30	4.90	0.98
	Minimun	5.30	0.68	19.49	0.39	4.43	0.22	3.98	0.52	12.29	0.31	2.54	0.51	5.46	0.24	0.74	0.12	4.33	0.87
Carex typhina Michx.	Maximun	7.85	1.00	49.65	1.00	16.20	0.82	4.33	0.56	39.86	1.00	3.32	0.67	13.10	0.58	6.12	1.00	3.00	0.60
	Mean	6.45	0.82	41.92	0.84	13.14	0.67	3.24	0.42	32.84	0.82	2.53	0.51	9.09	0.41	3.88	0.63	1.68	0.34
	Minimun	4.47	0.57	30.50	0.61	9.87	0.50	2.22	0.29	24.77	0.62	1.81	0.36	5.07	0.23	2.51	0.41	1.00	0.20
Carex squarrosa L.	Maximun	5.15	0.66	40.92	0.82	19.70	1.00	2.26	0.29	29.37	0.74	1.58	0.32	17.99	0.80	3.28	0.54	2.00	0.40
	Mean	4.12	0.52	33.83	0.68	17.54	0.89	1.93	0.25	23.24	0.58	1.31	0.26	10.97	0.49	2.20	0.36	1.11	0.22
	Minimun	3.32	0.42	20.54	0.41	14.10	0.72	1.46	0.19	13.13	0.44	0.93	0.19	7.41	0.33	1.51	0.25	1.00	0.20
	LW = Leaf Width (mm)					SR = Spike L/W Ratio						STL = Staminate Length (mm)							
	SL = Spike Length (mm)					PL = Pistillate Length (mm)						PSR = Pistillate/Staminate Ratio							
	SW = Spike Width (mm)					PR = Pistillate L/W Ratio						SN = Spike Number							

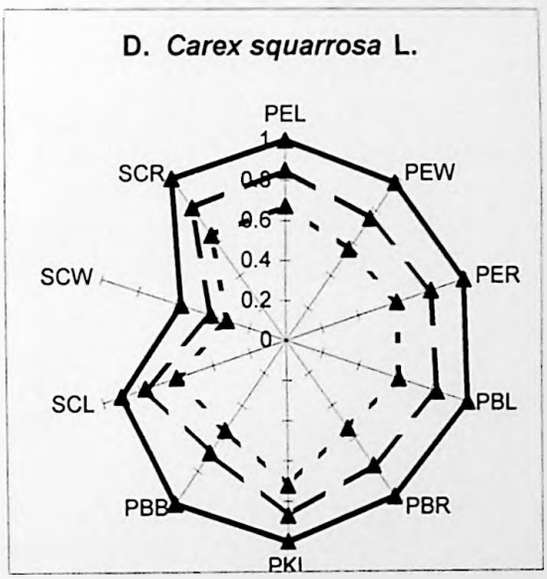
The second set of polygonal graphs (Figure 7) show perigynium characters (PEL, PEW, PER, PBL, PBR, PKL, and PBB) and pistillate scale characters (SCL, SCW, and SCR) for each taxon. The actual and relative values of these characters are given in Table 8. The most variable characters in this set of polygonal graphs were pistillate scale width (SCW) and perigynium width (PEW) (Figure 7, Table 6). The least variable and probably the best characters for separation among the four taxa were perigynium beak length (PKL) and perigynium length (PEL) (Figure 7, Table 6).

Figure 8 shows the mean values for the ten perigynium and pistillate scale characters for all four taxa. From this figure, it is clear that C. *typhina* and C. *squarrosa* are morphologically much larger than C. x *deamii* and C. *shortiana* for all characters except pistillate scale width (SCW). C. *squarrosa* and C. *typhina* show the same pattern for this set of characters, but C. *squarrosa* is repeatedly larger than C. *typhina* for all characters except pistillate scale length (SCL) and pistillate scale width (SCW). Likewise, C. x *deamii* and C. *shortiana* show a similar polygonal pattern with C. x *deamii* being larger for all characters except for pistillate scale width (SCW).









LEGEND

_____ Maximum

_ _ _ _ Mean

_ _ _ _ _ Minimum

Figure 7. Polygonal graph of maximum, mean, and minimum values of Macromorphological Characters for A.) *Carex* x *deamii* Herm., B.) *Carex shortiana* Dewey, C.) *Carex typhina* Michx., and D.) *Carex squarrosa* L..

PEL = Perigynium Length (mm)
PEW = Perigynium Width (mm)
PER = Perigynium L/W Ratio
PBL = Perigynium Body Length (mm)
PBR = Perigynium Body L/W Ratio
PKL = Perigynium Beak Length (mm)
PBB = Perigynium Beak/Body Ratio
SCL = Pistillate Scale Length (mm)
SCW = Pistillate Scale Width (mm)
SCR = Pistillate Scale L/W Ratio

Figure 8. Polygonal graph of mean values of Macromorphological Characters for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L..

PEL = Perigynium Length (mm)
PEW = Perigynium Width (mm)
PER = Perigynium L/W Ratio
PBL = Perigynium Body Length (mm)
PBR = Perigynium Body L/W Ratio
PKL = Perigynium Beak Length (mm)
PBB = Perigynium Beak/Body Ratio
SCL = Pistillate Scale Length (mm)
SCW = Pistillate Scale Width (mm)
SCR = Pistillate Scale L/W Ratio

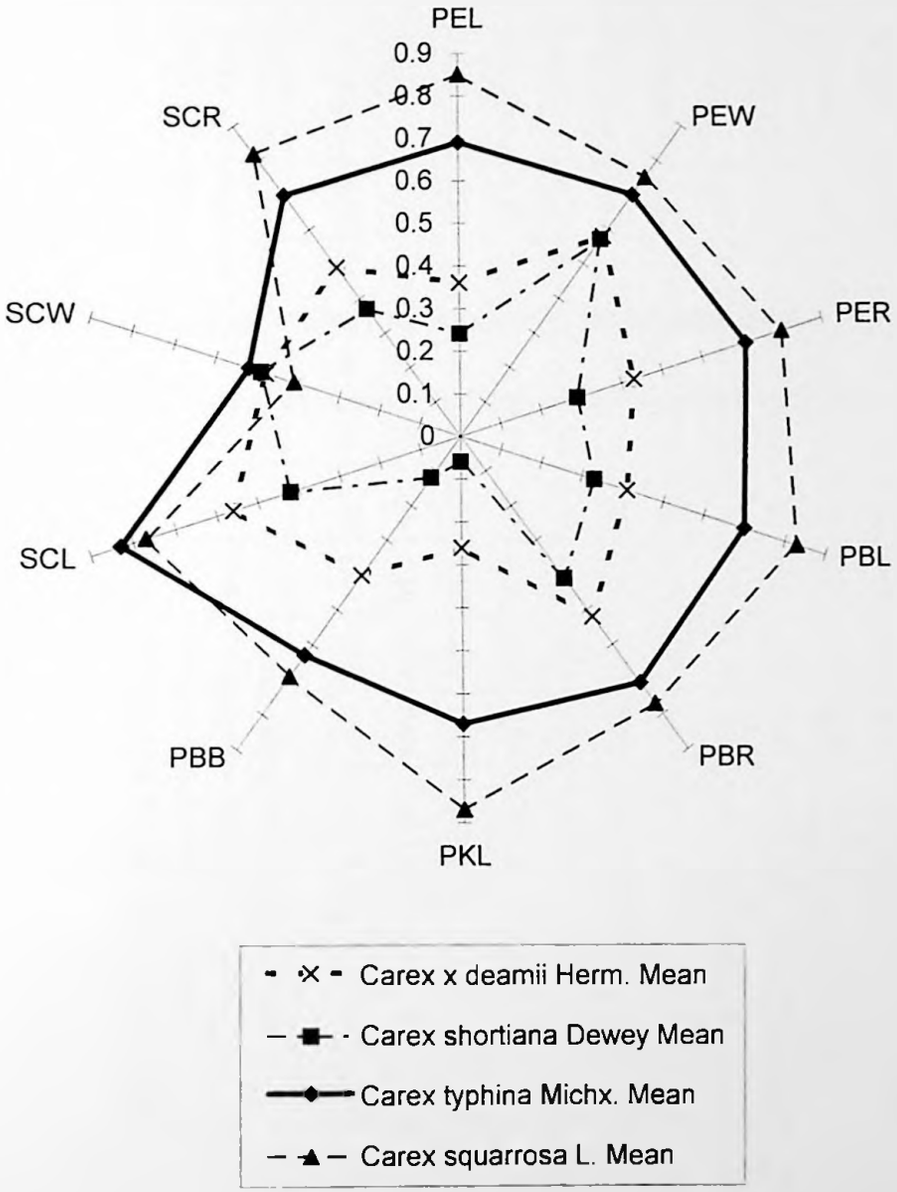

PEL
PEW
PER
PBL
PBR
PKL
PBB
SCL
SCW
SCR
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Carex x deamii Herm. Mean
Carex shortiana Dewey Mean
Carex typhina Michx. Mean
Carex squarrosa L. Mean

Table 8. Actual and relative values for macromorphological characters of *Carex* x *deamii* and its putative parents (polygonal set #2).

		PEL		PEW		PER		PBL		PBR		PKL		PBB		SCL		SCW		SCR	
		A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R	A	R
Carex x deamii Herm.	Maximun	4.01	0.41	2.71	0.76	2.15	0.55	2.91	0.46	2.03	0.79	1.21	0.35	0.43	0.50	4.00	0.69	2.81	1.00	3.35	0.69
	Mean	3.52	0.36	2.06	0.58	1.68	0.43	2.61	0.41	1.34	0.52	0.91	0.26	0.34	0.40	3.22	0.56	1.36	0.48	2.37	0.49
	Minimun	2.90	0.30	1.44	0.40	0.44	0.11	2.18	0.35	1.04	0.41	0.47	0.13	0.17	0.20	2.00	0.34	0.75	0.27	1.18	0.24
Carex shortiana Dewe	Maximun	2.60	0.27	2.31	0.65	1.36	0.35	2.38	0.38	1.20	0.47	0.34	0.10	0.15	0.17	3.61	0.62	1.90	0.68	2.82	0.58
	Mean	2.31	0.24	2.03	0.57	1.15	0.29	2.10	0.33	1.04	0.41	0.21	0.06	0.10	0.12	2.46	0.42	1.38	0.49	1.81	0.37
	Minimun	2.02	0.21	1.66	0.46	0.98	0.25	1.89	0.30	0.91	0.36	0.12	0.03	0.06	0.07	1.75	0.30	1.13	0.40	1.26	0.26
Carex typhina Michx.	Maximun	8.22	0.84	3.58	1.00	3.92	1.00	5.42	0.86	2.56	1.00	2.98	0.85	0.72	0.84	5.80	1.00	2.12	0.75	4.45	0.92
	Mean	6.76	0.69	2.51	0.70	2.79	0.71	4.42	0.70	1.82	0.71	2.34	0.67	0.54	0.63	4.79	0.83	1.45	0.52	3.39	0.70
	Minimun	5.82	0.60	1.60	0.45	1.97	0.50	3.57	0.57	1.39	0.54	1.80	0.52	0.39	0.43	4.07	0.70	1.10	0.39	2.53	0.52
Carex squarrosa L.	Maximun	9.77	1.00	3.47	0.97	3.83	0.98	6.30	1.00	2.47	0.96	3.49	1.00	0.86	1.00	5.24	0.90	1.60	0.57	4.84	1.00
	Mean	8.28	0.85	2.68	0.75	3.13	0.80	5.23	0.83	1.98	0.77	3.05	0.87	0.59	0.69	4.49	0.77	1.15	0.41	3.95	0.82
	Minimun	6.52	0.67	2.00	0.56	2.39	0.61	3.88	0.62	1.37	0.54	2.52	0.72	0.47	0.55	3.46	0.60	0.90	0.32	3.15	0.65

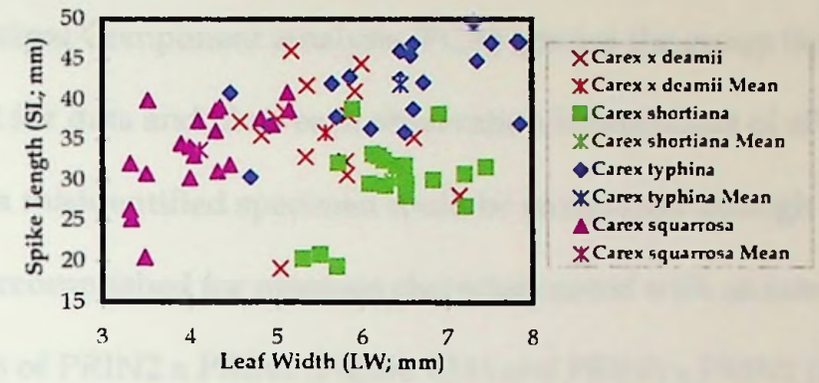
PEL = Perigynium Length (mm)
PEW = Perigynium Width (mm)
PER = Perigynium L/W Ratio
PBL = Perigynium Body Length (mm)
PBR = Perigynium Body L/W Ratio
PKL = Perigynium Beak Length (mm)
PBB = Perigynium Beak/Body Length Ratio
SCL = Pistillate Scale Length (mm
SCW = Pistillate Scale Width (mm
SCR = Pistillate Scale L/W Ratio

Bivariate Analysis

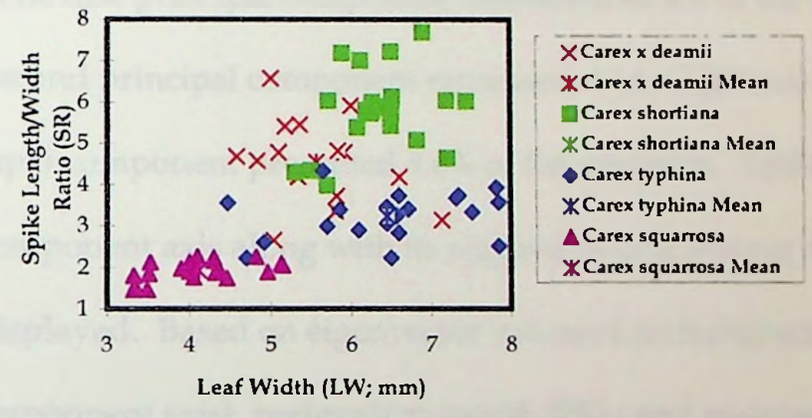
Bivariate scatterplots were created in an attempt to show relationships between the hybrid and the other taxa based on comparisons of two morphological characters at a time. Figure 9A plots spike length (SL) on the y-axis and leaf width (LW) on the x-axis. As a result of this plotting, *C.* x *deamii* overlaps heavily with both *C. shortiana* and *C. typhina* while *C. squarrosa* is clustered somewhat apart from the other taxa. In Figure 9B, the spike length/width ratio (SR) was plotted against leaf width (LW). In this graph, *C.* x *deamii* is clustered directly between *C. shortiana* and *C. typhina* with *C. squarrosa* separated from the other taxa. Pistillate Scale Width (SCW) and pistillate length/width ratio (PR) are plotted against each other in Figure 9C. Once again, *C.* x *deamii* clustered very closely with both *C. shortiana* and *C. typhina* while *C. squarrosa* was separated from all other taxa.

Figure 9. Bivariate Scatterplots for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Spike Length (SL) vs. Leaf Width (LW), B.) Spike length/width Ratio (SR) vs. Leaf Width (LW), and C.) Pistillate Scale Width (SCW) vs. Pistillate spike length/width Ratio (PR).

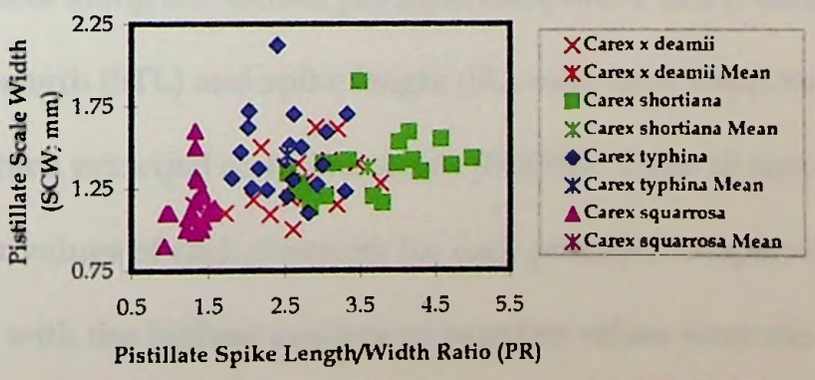
A. Bivariate Scatterplot of Spike Length (SL; mm) vs. Leaf Width (LW; mm) for *Carex* x *deamii* Herm. and its Putative Parents.



B. Bivariate Scatterplot of Spike Length/Width Ratio (SR) vs. Leaf Width (LW; mm) for *Carex* x *deamii* Herm. and its Putative Parents.



C. Bivariate Scatterplot of Pistillate Scale Width (SCW; mm) vs. Pistillate Spike Length/Width Ratio (PR) for *Carex* x *deamii* Herm. and its Putative Parents.



Multivariate Analysis

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) ignores the group (species) assignment for data and plots each observation independent of all others. Therefore, a misidentified specimen could be recognized through this analysis. PCA was accomplished for nineteen characters noted with an asterisk in Table 6. Scatterplots of PRIN2 x PRIN1 (Figure 10A) and PRIN3 x PRIN1 (Figure 10B) were created to graphically portray any macromorphological relationships among individuals. The plot of PRIN2 x PRIN1 summarizes 76.4% of the total variation. The first principal component represents 60.4% of the total variation while the second principal component represented an additional 16.0%. The third principal component presented 8.6% of the variation. Table 9 shows each principal component axis along with its eigenvalue and percent of the total variation displayed. Based on eigenvector values of each character (for each principal component axis), perigynium length (PEL) and perigynium beak length (PKL) were the most valuable characters in separating elements along the first principal component axis (PRIN1). Pistillate length (PL) and leaf width (LW) separated taxa along the second principal component axis (PRIN2) while staminate length (STL) and spike length (SL) were most important in separating along the third principal component axis (PRIN3). Table 10 shows the eigenvector values of each character for each principal component axis. Characters with the highest positive or negative values were most important in

separating elements along each respective axis. Both plots showed *C.* x *deamii* clustered near *C. shortiana* and *C. typhina* clustered near *C. squarrosa*. In both plots *C.* x *deamii* was placed between *C. shortiana* and both putative paternal parent species.

Table 9. Eigenvalues and percent variation represented by each principal component axis.

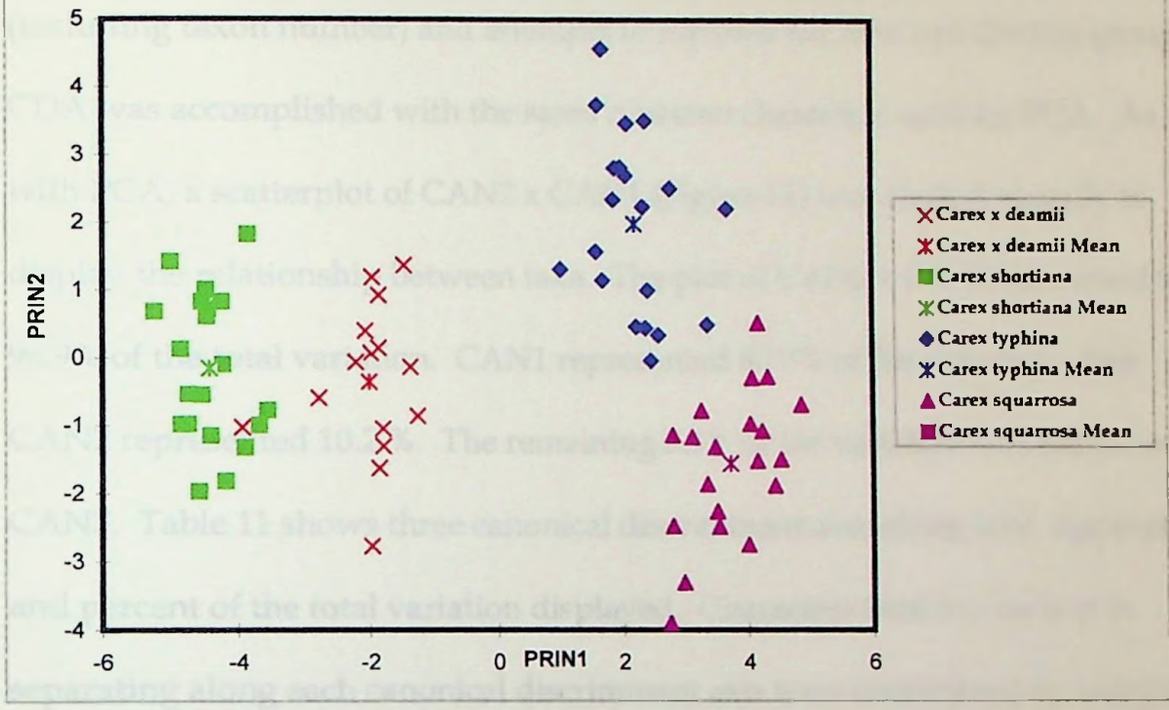
	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	11.4843	8.4473	0.6044	0.6044
PRIN2	3.0370	1.4094	0.1598	0.7643
PRIN3	1.6276	0.3875	0.0857	0.8499
PRIN4	1.2401	NA	0.0653	0.9152

Table 10. Eigenvector values of each character for each principal component axis. The five most valuable characters in separating the four taxa are highlighted for each axis.

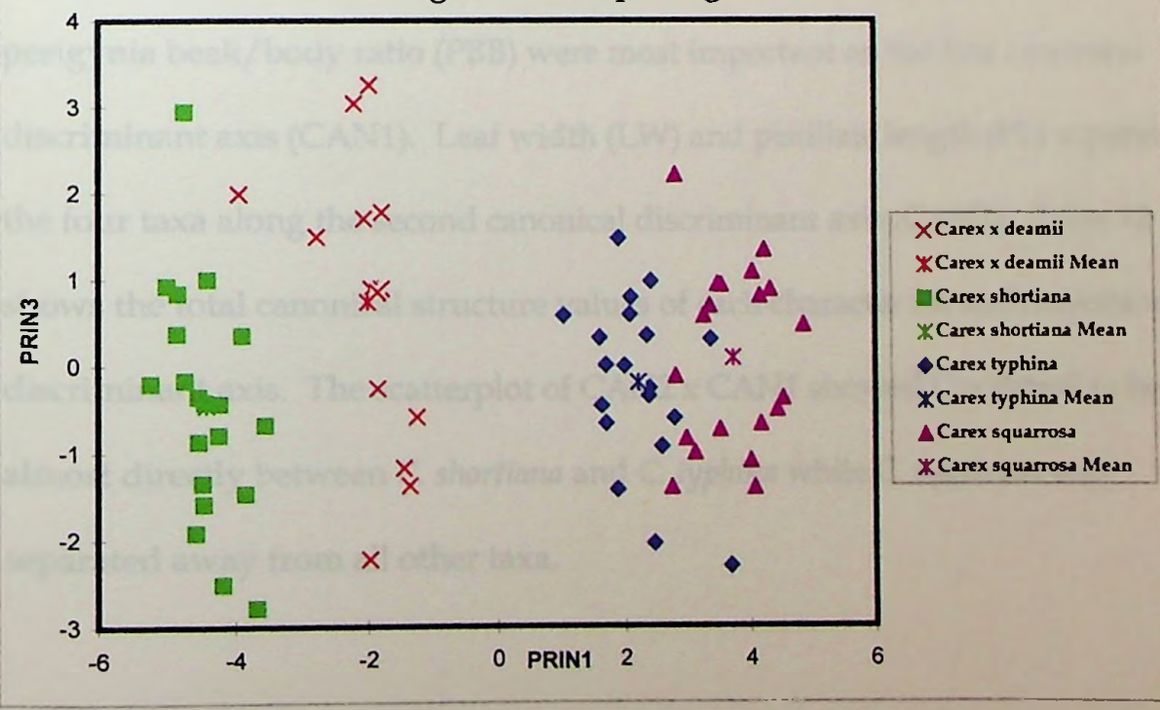
Character	PRIN1	PRIN2	PRIN3	PRIN4
LW	-0.135488	**0.443401**	-0.063087	-0.042248
SL	0.100675	**0.394994**	**0.490107**	-0.059253
SW	**0.286325**	-0.042256	0.018913	0.120278
SR	-0.266611	0.128745	**0.207057**	-0.101042
PL	0.152814	**0.460202**	0.152019	-0.145011
PR	-0.237180	0.264770	0.059407	-0.193682
STL	-0.081941	-0.098701	**0.708938**	0.184699
PSR	0.136646	**0.384469**	**-0.339280**	**-0.243987**
SN	**-0.284258**	0.017842	-0.044950	0.039592
PEL	**0.289107**	-0.002040	0.010984	0.093417
PEW	0.175436	0.106949	-0.026691	**0.626087**
PER	0.274220	-0.027800	0.018664	**-0.236360**
PBL	**0.280737**	0.013366	-0.027080	0.137811
PBR	0.258654	-0.019531	-0.048630	-0.182332
PKL	**0.288999**	-0.019276	0.054311	0.036498
PBB	0.267545	-0.007296	0.139427	-0.084189
SCL	0.267920	0.169902	0.023976	0.027242
SCW	-0.044939	**0.369521**	**-0.179342**	**0.492195**
SCR	0.261677	-0.103133	0.085176	**-0.236735**

Figure 10. Principal Component Analysis (PCA) plots of macromorphological data for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Scatterplot of PRIN1 and PRIN2, B.) Scatterplot of PRIN1 and PRIN3.

A. Principal Component Analysis (PCA) plot of PRIN1 and PRIN2 for *Carex* x *deamii* Herm. and its Putative Parents using Macromorphological Characters.



B. Principal Component Analysis (PCA) plot of PRIN1 and PRIN3 for *Carex* x *deamii* Herm. and its Putative Parents using Macromorphological Characters.



Canonical Discriminant Analysis (CDA)

Canonical Discriminant Analysis (CDA) takes the information given to it (including taxon number) and attempts to separate the taxa into distinct groups. CDA was accomplished with the same nineteen characters used for PCA. As with PCA, a scatterplot of CAN2 x CAN1 (Figure 11) was created visually to display the relationship between taxa. The plot of CAN2 x CAN1 accounted for 96.9% of the total variation. CAN1 represented 86.7% of the variation while CAN2 represented 10.2 %. The remaining 3.1% of the variation was found on CAN3. Table 11 shows three canonical discriminant axes along with eigenvalues and percent of the total variation displayed. Characters found to be best in separating along each canonical discriminant axis were determined by using the values for total canonical structure. Values closest to positive or negative 1.0 were more important in separating along each respective axis. Spike number (SN), perigynia beak length (PKL), perigynia length (PEL), spike width (SW), and perigynia beak/body ratio (PBB) were most important on the first canonical discriminant axis (CAN1). Leaf width (LW) and pistillate length (PL) separated the four taxa along the second canonical discriminant axis (CAN2). Table 12 shows the total canonical structure values of each character for each canonical discriminant axis. The scatterplot of CAN2 x CAN1 showed C. x *deamii* to be almost directly between C. *shortiana* and C. *typhina* while C. *squarrosa* was separated away from all other taxa.

Figure 11. Canonical Discriminant Analysis (CDA) plot of macromorphological data for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Scatterplot of CAN1 and CAN2.

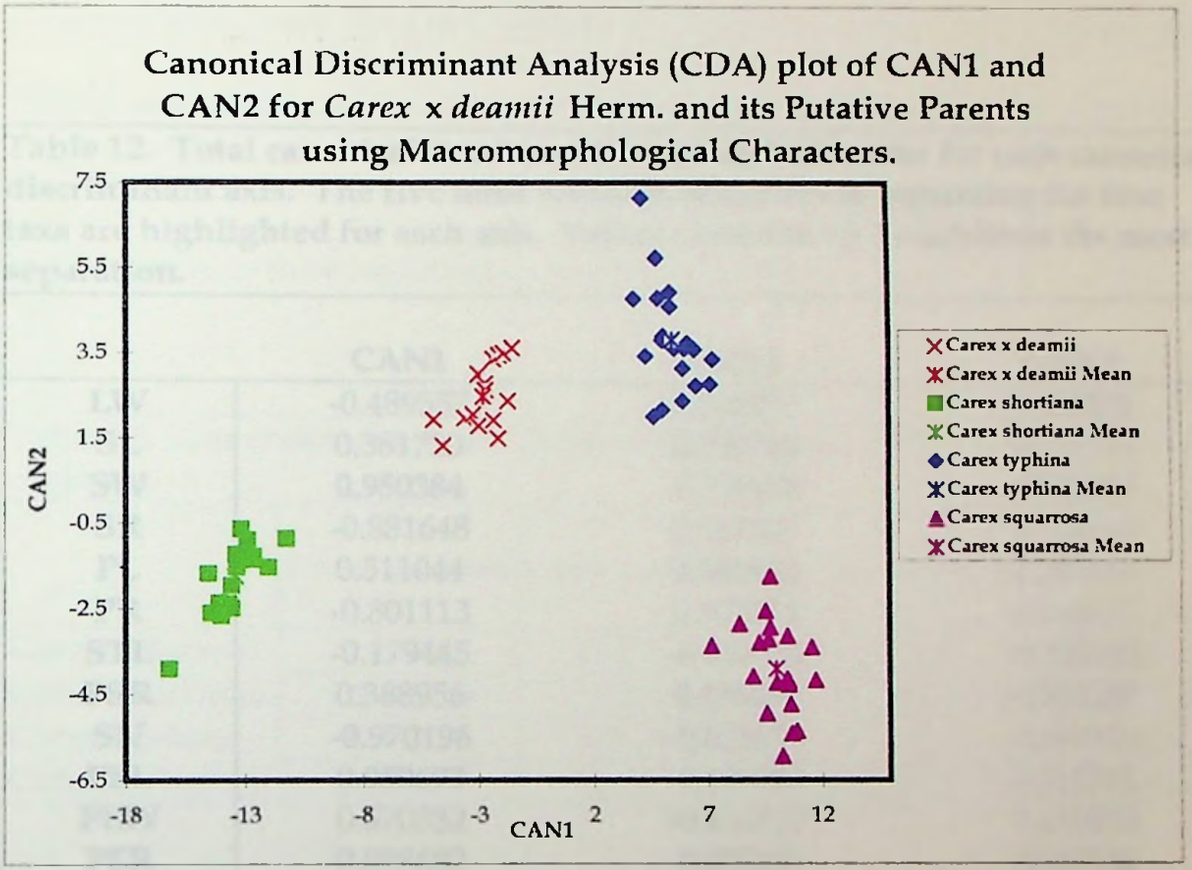

Canonical Discriminant Analysis (CDA) plot of CAN1 and CAN2 for Carex x deamii Herm. and its Putative Parents using Macromorphological Characters.
7.5
5.5
3.5
1.5
-0.5
-2.5
-4.5
-6.5
CAN2
-18
-13
-8
-3
2
7
12
CAN1
Carex x deamii
Carex x deamii Mean
Carex shortiana
Carex shortiana Mean
Carex typhina
Carex typhina Mean
Carex squarrosa
Carex squarrosa Mean

Table 11. Eigenvalues and percent variation represented by each canonical discriminant axis.

	Eigenvalue	Difference	Proportion	Cumulative
CAN1	89.0173	78.5265	0.8670	0.8670
CAN2	10.4909	7.3252	0.1022	0.9692
CAN3	3.1656	NA	0.0308	1.0000

Table 12. Total canonical structure values of each character for each canonical discriminant axis. The five most valuable characters in separating the four taxa are highlighted for each axis. Values closest to +/- 1 contribute the most to separation.

	CAN1	CAN2	CAN3
LW	-0.489557	**0.562357**	**0.387276**
SL	0.381720	**0.525760**	0.122536
SW	**0.950384**	-0.229878	0.113127
SR	-0.881648	0.167607	-0.030065
PL	0.511044	**0.560892**	**0.386810**
PR	-0.801113	0.302755	0.144271
STL	-0.179445	-0.057042	**-0.488203**
PSR	0.388956	**0.476089**	**0.583287**
SN	**-0.970196**	-0.025093	-0.058423
PEL	**0.950673**	-0.148789	0.205216
PEW	0.570332	-0.131812	0.210220
PER	0.898682	-0.052891	0.182538
PBL	0.909017	-0.160179	0.231405
PBR	0.844632	-0.038166	0.133359
PKL	**0.966044**	-0.129981	0.169921
PBB	**0.943120**	0.073726	0.011480
SCL	0.872706	0.195243	**0.260131**
SCW	-0.184357	**0.346388**	0.134770
SCR	0.861874	-0.122651	0.129581

Discriminant Analysis

The Discriminant Analysis procedure of SAS/STAT (1990) produces a generalized squared distance to species (Table 13). This distance shows a very close relationship between both *C. shortiana* and *C. typhina* to *C.* x *deamii*. *C. squarrosa* is also very close to *C. typhina*, as would be expected, but *C. squarrosa* is clearly separated from *C.* x *deamii*. Comparison of predetermined identification of each specimen with the classification suggested by discriminant analysis showed all taxa to be identified correctly (Table 14).

Table 13. Generalized squared distance to species derived from Discriminant Analysis.

	Carex x deamii	*Carex shortiana*	*Carex typhina*	*Carex squarrosa*
Carex x deamii	0.00000	148.15234	93.13218	211.74876
Carex shortiana	148.15234	0.00000	388.74957	554.83928
Carex typhina	93.13218	388.74957	0.00000	83.48150
Carex squarrosa	211.74876	554.83928	83.48150	0.00000

Table 14. Classification results based upon discriminant analysis (PROC DISCRIM of SAS/STAT 1990).

	Percent Correct	Number of specimens Classified into Group			
		C. x deamii	*C. shortiana*	*C. typhina*	*C. squarrosa*
C. x deamii	100	15	0	0	0
C. squarrosa	100	0	20	0	0
C. typhina	100	0	0	20	0
C. squarrosa	100	0	0	0	20
Total	100	15	20	20	20

HYWIN Analysis (Hypothesizing Hybrids using Weighted Intermediacy)

HYWIN is a program specifically designed to determine the presence of hybrids in a population and to determine which species would represent the most likely parental species. A number of "triples" are created to show the most likely hybrid specimens along with the two specimens that would best represent parents to this hybrid. In this analysis, the highest ranking 539 triples out of a possible 202,575 triples were reported to achieve a .95 probability level. Ten of the fifteen *C.* x *deamii* specimens were revealed as hybrids in the top 539 triples. *C.* x *deamii* was determined to be a hybrid in 352 triples. Each time *C.* x *deamii* was reported as a hybrid, *C. shortiana* was determined to be the closest parent. In 202 of these triples, *C. typhina* was revealed as the most likely second parent while the other 150 triples *C. squarrosa* was selected as the most likely second parent species. *C. typhina* was found to be the most likely parent for 8 out of 10 *C.* x *deamii* specimens while *C. squarrosa* was a most likely parent for 2 of 10 specimens (Table 15).

Table 15. *Carex* x *deamii* specimens and the most likely parent species as determined by HYWIN. Highest rank and scores for the determining criteria are also given.

Hybrid	Parent	Parent	Highest Rank	IN	EQ	PD	NP	HS
DEAM1	NA	NA	NA	NA	NA	NA	NA	NA
DEAM2	SHOR6	TYPH2	8	0.740	-0.002	0.494	0.246	2.2324
DEAM3	NA	NA	NA	NA	NA	NA	NA	NA
DEAM4	SHOR17	TYPH16	260	0.720	-0.135	0.462	0.200	2.0474
DEAM5	SHOR6	TYPH8	7	0.729	-0.097	0.600	0.271	2.2326
DEAM6	SHOR6	TYPH8	13	0.737	-0.121	0.600	0.264	2.2168
DEAM7	SHOR6	TYPH8	4	0.710	-0.054	0.600	0.289	2.2567
DEAM8	SHOR6	TYPH15	177	0.760	-0.134	0.449	0.194	2.0749
DEAM9	SHOR6	TYPH8	45	0.678	-0.114	0.600	0.266	2.1648
DEAM10	SHOR18	SQUA8	73	0.813	-0.201	0.529	0.211	2.1405
DEAM11	SHOR6	TYPH8	340	0.545	-0.116	0.600	0.271	2.0288
DEAM12	NA	NA	NA	NA	NA	NA	NA	NA
DEAM13	NA	NA	NA	NA	NA	NA	NA	NA
DEAM14	NA	NA	NA	NA	NA	NA	NA	NA
DEAM15	SHOR17	SQUA8	1	0.849	-0.021	0.546	0.267	2.3747

LEGEND

IN = Hybrid Intermediacy Score
EQ = Measure of Equality of the two distances between the possible hybrid and each of the two possible parents
PD = Distance between possible parents
NP = Nearest Parent
HS = Hybrid Score

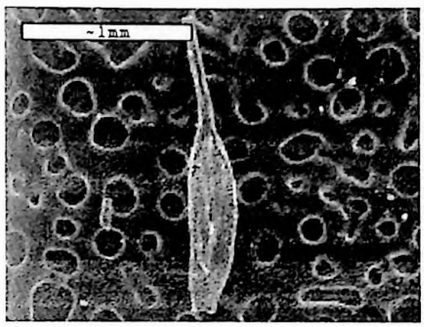
Analysis of Micromorphological Characters

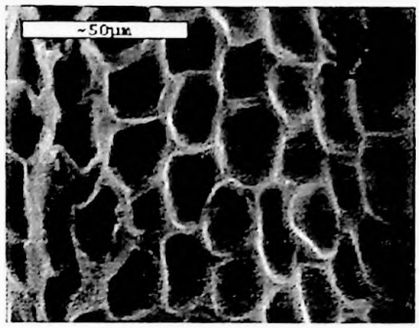
Achene Micromorphology

Achenes were examined primarily for characteristic silica bodies in the epidermal cells. *C. shortiana* (Figure 12 C-E), *C. typhina* (Figure 12 F-H), and *C. squarrosa* (Figure 12 I-K) all had one centrally located conical silica body in most of their epidermal cells (The epidermal cells on the angles of the achenes didn't contain silica bodies). *C.* x *deamii*, however, didn't have any silica bodies at all on the achene surface (Figure 12 A,B).

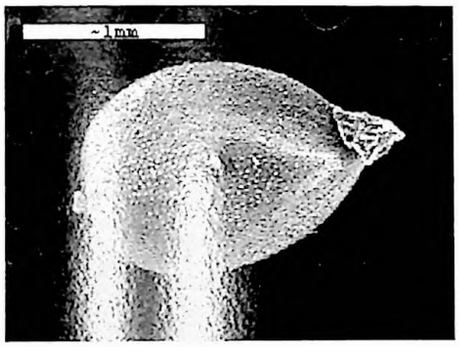
Achene measurements were taken in order to fully describe the surface anatomy of the four taxa (Table 16). However, these measurements were not included in any multivariate analysis because of the abortive and distorted nature of *C.* x *deamii*'s achenes. Only minor differences were found for achene micromorphology measurements. Achene cell widths were significantly different between *C. typhina* and *C. squarrosa* to $p>0.05$ (ANOVA, Tukey Test). Also, *C. shortiana* had consistently larger silica bodies than *C. typhina* and *C. squarrosa*.

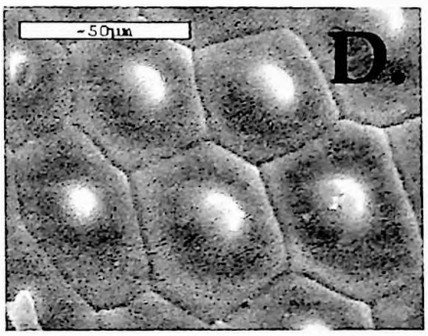
Figure 12. Images of Achene Micromorphology for *Carex* x *deamii* Herm. and its putative parental taxa. A.) *C.* x *deamii* whole achene, B.) *C.* x *deamii* achene cells, C.) *C. shortiana* whole achene, D.) *C. shortiana* achene w/o acetolysis treatment, E.) *C. shortiana* achene after acetolysis, F.) *C. typhina* whole achene, G.) *C. typhina* achene w/o acetolysis treatment, H.) *C. typhina* H.) after acetolysis, I.) *C. squarrosa* whole achene, J.) *C. squarrosa* achene w/o acetolysis treatment, and K.) *C. squarrosa* achene after acetolysis.

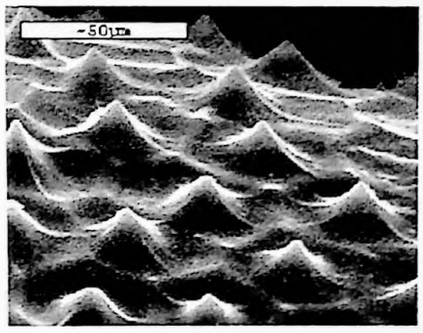




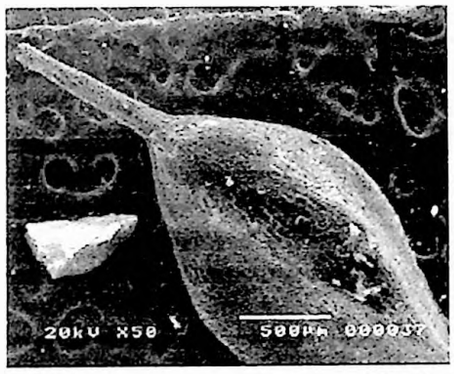
Carex x *deamii* Herm.

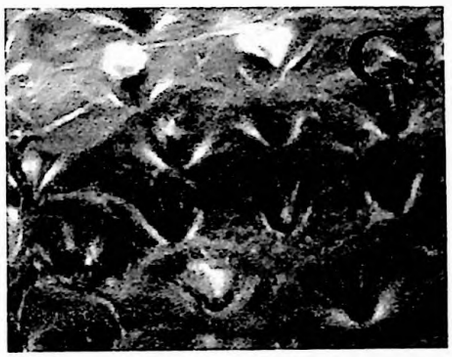


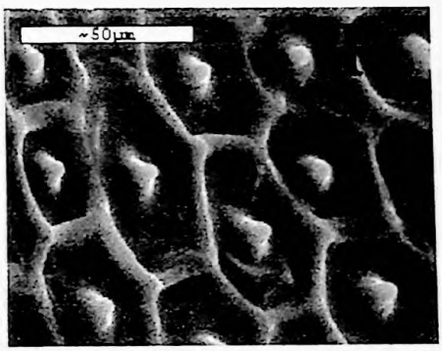




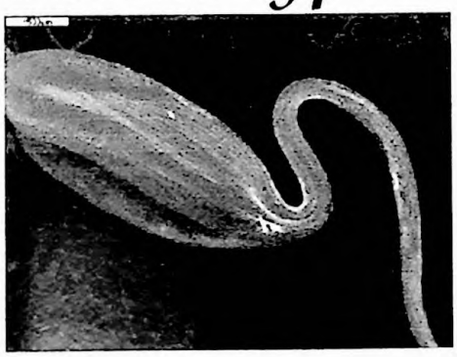
Carex shortiana Dewey

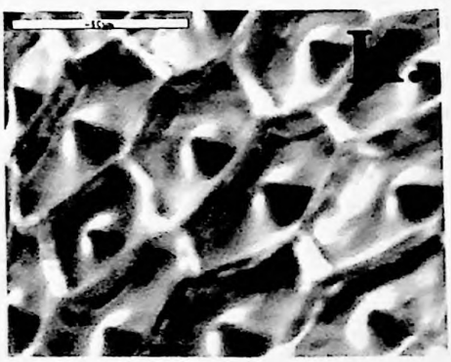






Carex typhina Michx.





Carex squarrosa L.

Table 16. Achene Micromorphology Measurements. All measurements taken from just below the neck of the achene.

TAXA	CW	CC	CA	NS	SB	SBW	SBH
C. x deamii	31.22	86.97	543.83	5.7	Absent	NA	NA
C. shortiana	47.41	118.6	931.05	6	Present(1/cell)	25.35	16.61
C. typhina	43.94	129.54	1207.02	5.5	Present(1/cell)	19.63	12.92
C. squarrosa	55.3	156.02	1730.13	6.17	Present(1/cell)	21.77	13.57

CW = Cell Width(micrometers)
CC = Cell Circumference(micrometers)
CA = Cell Area(square micrometers)
NS = Number of Sides
SB = Silica Body(present or absent)
SBW = Silica Body Width(micrometers)
SBH = Silica Body Height(micrometers)

Perigynium, Pistillate Scale, and Leaf Epidermal Micromorphology

Univariate Analysis

Analysis of Variance (ANOVA)

According to ANOVA procedure of SAS/STAT (1990), five of the twelve characters (SL, PCL, PCW, PCR, and SCPRK) used in multivariate analysis proved to be significantly different among taxa to at least the .01 probability level (Table 17). Best among these were PCL, PCW, and SCPRK which were significantly different among taxa to the .001 probability level.

Duncan's Multiple Range Test

The Duncan's Multiple Range Test was used under the PROC ANOVA procedure of SAS/STAT (1990). According to the Duncan groupings produced by this analysis, very few significant differences were found between the four taxa (Table 18). Significant differences were found between *C.* x *deamii* (Mean = 41.16 μm, Figure 13 A-D)and *C. shortiana* (Mean = 24.63 μm, Figure 13 E-H) for only one character (PCL). *C. typhina* (Figure 13 I-L) and *C. squarrosa* (Figure 13 M-P) were also significantly different for only one character (SCPRK).

The results of the Duncan groupings places *C.* x *deamii* immediately between *C. shortiana* and *C. typhina* for three characters (SW, PCW, and SCPRK) and between *C. shortiana* and *C. squarrosa* for two characters (LCW, and LCR). For the remaining seven characters *C.* x *deamii* demonstrated the largest or smallest value.

17

Table ????. Table of characters used in micromorphological multivariate analysis along with their F and p values according to ANOVA.

Character	F Value	P Value
LCL	3.86	0.0382
LCW	2.52	0.1076
LCR	2.48	0.1112
SL	5.30	0.0148
SW	0.07	0.9745
SR	0.55	0.6563
SD	2.89	0.0793
LFPRK	2.00	0.1678
PCL	16.16	0.0002
PCW	29.00	0.0001
PCR	9.27	0.0019
SCPRK	11.00	0.0009

Table 18 ???. Mean values +/- one standard deviation for micromorphological characters used in the assessment of the hybrid origin of Carex x deamii Herm.. The letter following these values depicts the groupings assigned by Duncan's Multiple Range Test (unlike letters indicate a significant difference). Characters followed by an asterisk were used in multivariate analysis.

Character (Unit)	*C.* x *deamii*	*C. shortiana*	*C. typhina*	*C. squarrosa*
LCL(micrometers)	43.05+/-12.24A,B	36.05+/-6.03B	55.99+/-7.98A	48.90+/-6.97A,B
LCW(micrometers)	16.48+/-1.37B	17.20+/-2.08A,B	20.22+/-2.01A	17.90+/-2.54A,B
LCR	2.60+/-0.65A,B	2.10+/-0.29B	2.77+/-0.23A	2.74+/-0.22A
SL(micrometers)	23.81+/-1.22B	23.94+/-1.30B	26.41+/-0.94A	25.50+/-0.84A,B
SW(micrometers)	19.21+/-1.22A	19.19+/-1.90A	19.53+/-2.38A	18.96+/-1.30A
SR	1.25+/-0.13A	1.26+/-0.18A	1.37+/-0.21A	1.35+/-0.12A
SD(micrometers)	34.11+/-12.41B	42.18+/-21.77A,B	62.10+/-12.76A	40.50+/-4.33A,B
LFPRK	2.00+/-0.00A	1.50+/-0.58A	2.00+/-0.00A	1.50+/-0.58A
PCL(micrometers)	41.16+/-2.05A	24.63+/-4.07B	35.32+/-5.37A	38.71+/-1.72A
PCW(micrometers)	20.85+/-4.99B	17.32+/-6.43B	42.30+/-5.97A	44.55+/-2.91A
PCR	2.10+/-0.71A	1.50+/-0.34A	0.84+/-0.06B	0.87+/-0.07B
SCPRK	1.00+/-0.00C	1.00+/-0.00C	1.50+/-0.58B	2.00+/-0.00A

LCL = Abaxial Leaf Epidermal Cell Length (micrometers)

LCW = Abaxial Leaf Epidermal Cell Width (micrometers)

LCR = Abaxial Leaf Epidermal Cell L/W Ratio

SL = Stomata Length (micrometers)

SW = Stomata Width (micrometers)

SR = Stomata L/W Ratio

SD = Distance between stomata (micrometers)

LFPRK = Leaf Prickle hairs: 1 = no, 2 = yes

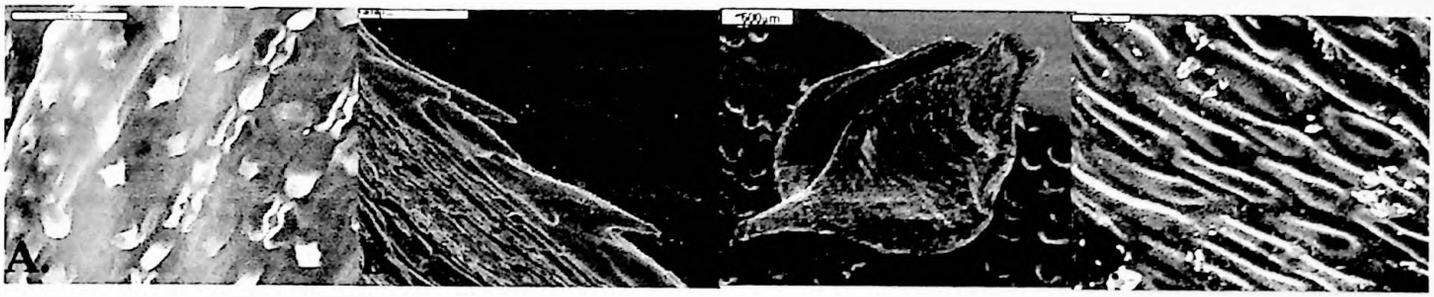
PCL = Perigynium Cell Length (micrometers)

PCW = Perigynium Cell Width (micrometers)

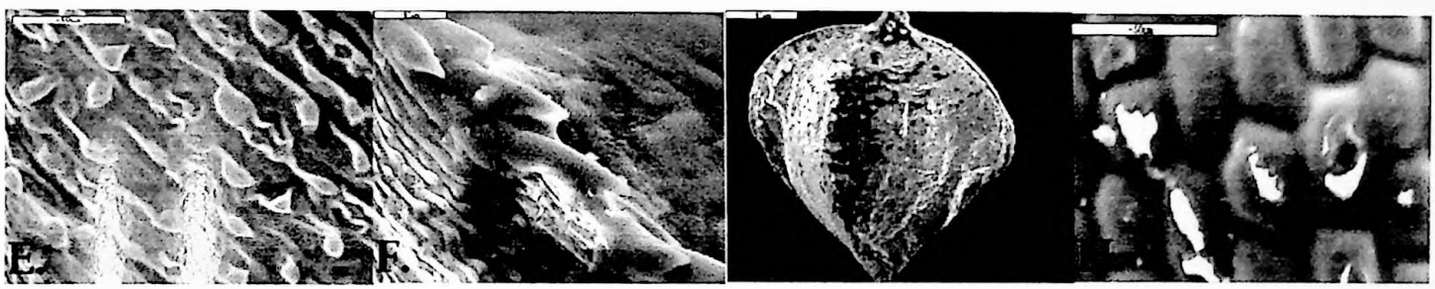
PCR = Perigynium Cell L/W Ratio

SCPRK = Pistillate Scale Prickle hairs: 1 = no, 2 = yes

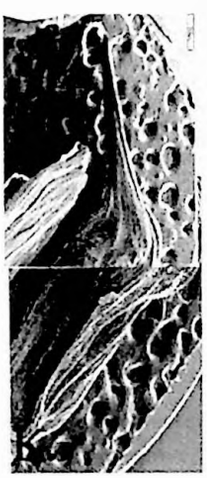
Figure 13. SEM images of *Carex* x *deamii* Herm. and its putative parents for abaxial leaf and perigynium features. A.) *C.* x *deamii* abaxial leaf surface showing stomata and distinct papillae, B.) *C.* x *deamii* abaxial leaf surface showing prickles at the leafs edge, C.) *C.* x *deamii* whole perigynium, D.) *C.* x *deamii* perigynium epidermal cells showing rectangular shape, E.) *C. shortiana* abaxial leaf surface showing stomata and distinct papillae, F.) *C. shortiana* abaxial leaf surface showing prickles at the leafs edge, G.) *C. shortiana* whole perigynium, H.) *C. shortiana* perigynium epidermal cells showing rectangular shape, I.) *C. typhina* abaxial leaf surface showing stomata and indistinct papillae, J.) *C. typhina* abaxial leaf surface showing prickles at the leafs edge, K.) *C. typhina* whole perigynium, L.) *C. typhina* perigynium epidermal cells showing square shape, M.) *C. squarrosa* abaxial leaf surface showing stomata and indistinct papillae, N.) *C. squarrosa* abaxial leaf surface showing prickles at the leafs edge, O.) *C. squarrosa* whole perigynium, P.) *C. squarrosa* perigynium epidermal cells showing square shape.

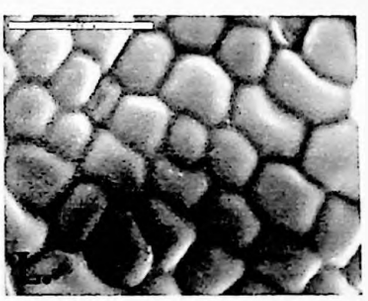


Carex x *deamii* Herm.

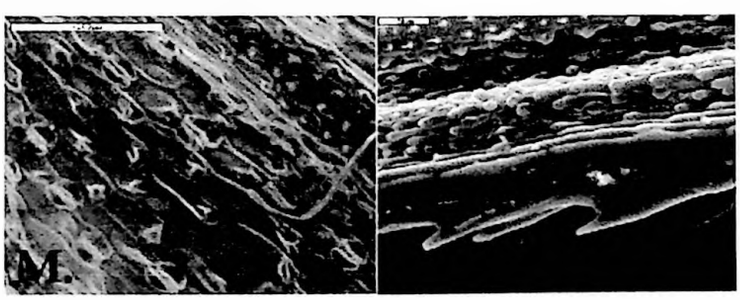


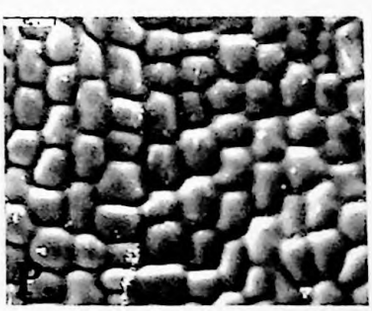
Carex shortiana Dewey





Carex typhina Michx.



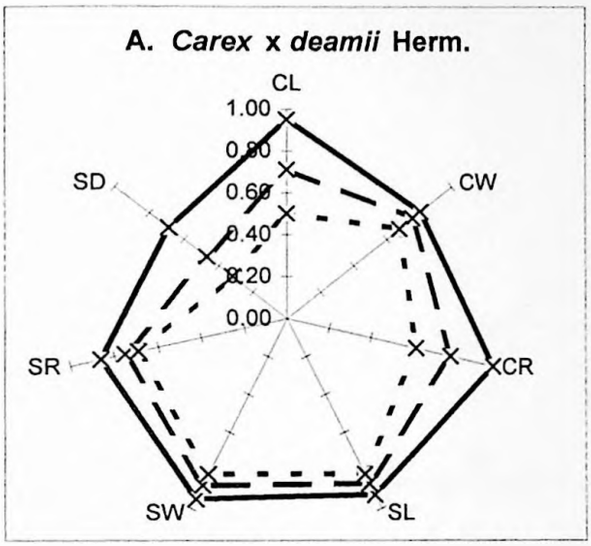


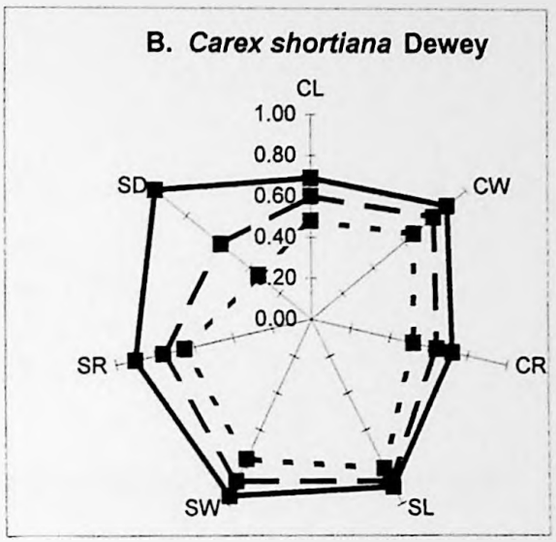
Carex squarrosa L.

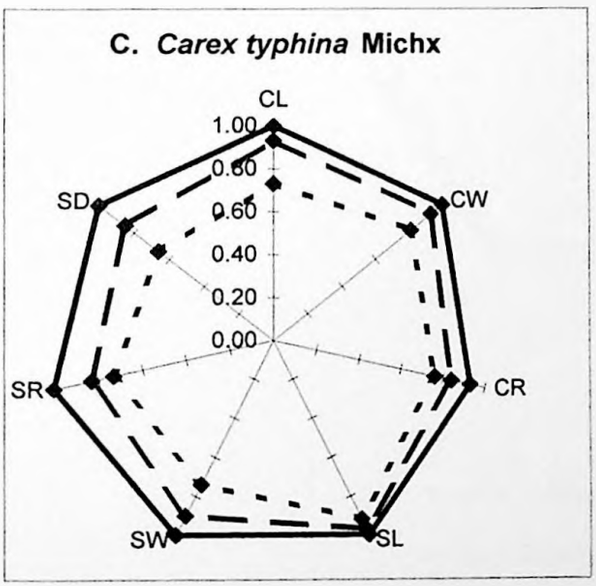
Polygonal Graph Analysis

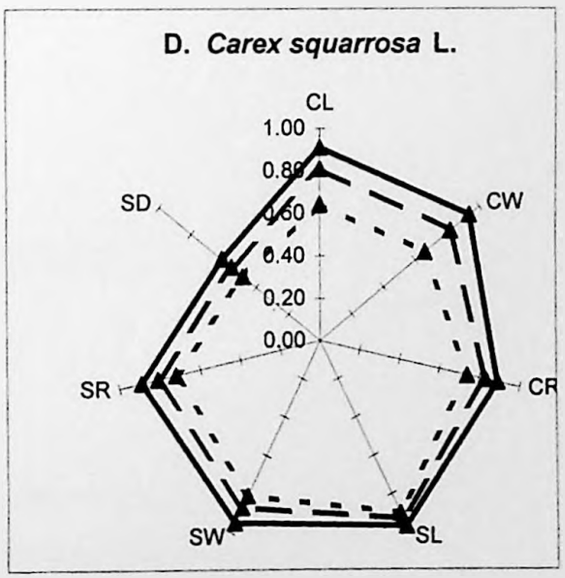
Polygonal graphs of relative values for each character were created to visually compare the micromorphological characters between and among taxa. Two sets of polygonal graphs were constructed. The first set (Figures 14-15) shows each taxa for the first seven characters used in multivariate analysis (LCL, LCW, LCR, SL, SW, SR, and SD). Actual and relative values for these characters are given in Table 19. In this set of polygonal graphs, LCL and SD are the most variable characters for all taxa. SD is especially variable for *C. shortiana* (Mean = 42.18 μm; SD = 21.77), *C.* x *deamii* (Mean = 34.11 μm; SD = 12.41), and *C. typhina* (Mean = 62.10 μm; SD = 12.76). SL is the least variable character in this set (*C.* x *deamii*: Mean = 23.81 μm, SD = 1.22; *C. shortiana*: Mean = 23.94 μm, SD = 1.30; *C. typhina*: Mean = 26.41 μm, SD = 0.94; *C. squarrosa*: Mean = 25.50 μm, SD = 0.84). When mean values are placed on the same polygonal graph (Figure 15), it is clear that *C. typhina* is the most robust for all characters represented in the first set of polygonal graphs. None of the characters reliably separate *C. typhina* from *C. squarrosa* or *C.* x *deamii* from *C. shortiana*.

Figure 14. Polygonal graph of maximum, mean, and minimum values of micromorphological characters (CL, CW, CR, SL, SW, SR, and SD) for A.) *Carex* x *deamii* Herm., B.) *Carex shortiana* Dewey, C.) *Carex typhina* Michx., and D.) *Carex squarrosa* L..


A. Carex x deamii Herm.
CL
1.00
0.80
0.60
0.40
0.20
0.00
CW
CR
SL
SW
SR
SD


B. Carex shortiana Dewey
CL
1.00
0.80
0.60
0.40
0.20
0.00
CW
CR
SL
SW
SR
SD


C. Carex typhina Michx
CL
1.00
0.80
0.60
0.40
0.20
0.00
CW
CR
SL
SW
SR
SD


D. Carex squarrosa L.
CL
1.00
0.80
0.60
0.40
0.20
0.00
CW
CR
SL
SW
SR
SD

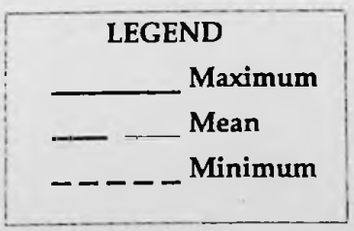

LEGEND
Maximum
Mean
Minimum

Figure 15. Polygonal graph of mean values of micromorphological characters (CL, CW, CR, SL, SW, SR, and SD) for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L..

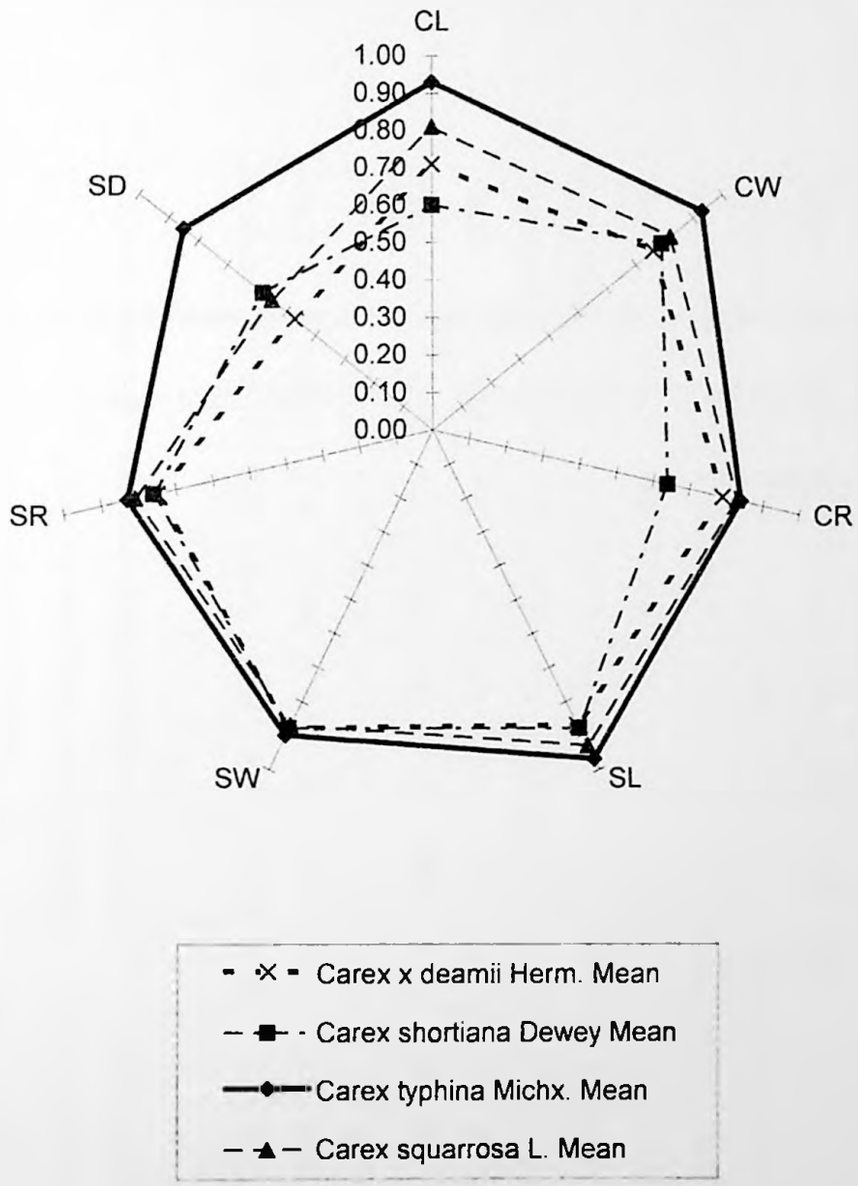

CL
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
CW
CR
SL
SW
SR
SD
Carex x deamii Herm. Mean
Carex shortiana Dewey Mean
Carex typhina Michx. Mean
Carex squarrosa L. Mean

Table 19. Actual and relative values for micromorphological characters (CL,CW, CR, SL, SW, SR, and SD) of *Carex* x *deamii* and its putative parents.

		CL		CW		CR		SL		SW		SR		SD	
		A	R	A	R	A	R	A	R	A	R	A	R	A	R
Carex x *deamii* Herm.	Maximum	57.61	0.95	17.74	0.81	3.30	1.00	25.41	0.93	20.77	0.95	1.43	0.86	50.60	0.69
	Mean	43.05	0.71	16.48	0.76	2.60	0.79	23.81	0.87	19.21	0.88	1.25	0.75	34.11	0.47
	Minimum	30.00	0.50	14.81	0.68	2.03	0.62	22.44	0.82	17.78	0.82	1.14	0.69	23.38	0.32
Carex shortiana Dewey	Maximum	41.81	0.69	19.15	0.88	2.37	0.72	24.89	0.91	20.83	0.96	1.50	0.90	72.88	1.00
	Mean	36.05	0.60	17.20	0.79	2.10	0.64	23.94	0.88	19.19	0.88	1.26	0.76	42.18	0.58
	Minimum	29.01	0.48	14.39	0.66	1.73	0.52	22.04	0.81	16.59	0.76	1.08	0.65	24.72	0.34
Carex typhina Michx.	Maximum	60.50	1.00	21.80	1.00	3.06	0.93	27.33	1.00	21.76	1.00	1.66	1.00	73.14	1.00
	Mean	55.99	0.93	20.22	0.93	2.77	0.84	26.41	0.97	19.53	0.90	1.37	0.83	62.10	0.85
	Minimum	44.03	0.73	17.55	0.81	2.51	0.76	25.13	0.92	16.19	0.74	1.21	0.73	48.16	0.66
Carex squarrosa L.	Maximum	54.80	0.91	20.58	0.94	2.95	0.89	26.38	0.97	20.70	0.95	1.48	0.89	44.94	0.61
	Mean	48.90	0.81	17.90	0.82	2.74	0.83	25.50	0.93	18.96	0.87	1.35	0.81	40.50	0.55
	Minimum	38.83	0.64	14.47	0.66	2.45	0.74	24.70	0.90	17.65	0.81	1.20	0.72	35.18	0.48

LCL = Abaxial Leaf Epidermal Cell Length (micrometers)

LCW = Abaxial Leaf Epidermal Cell Width (micrometers)

LCR = Abaxial Leaf Epidermal Cell L/W Ratio

SL = Stomata Length (micrometers)

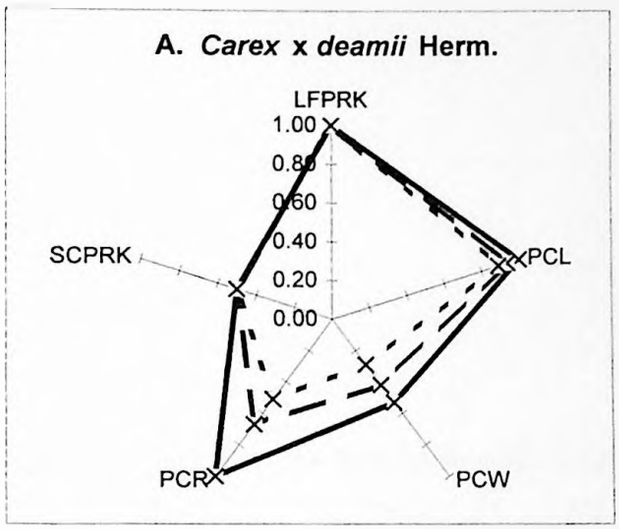
SW = Stomata Width (micrometers)

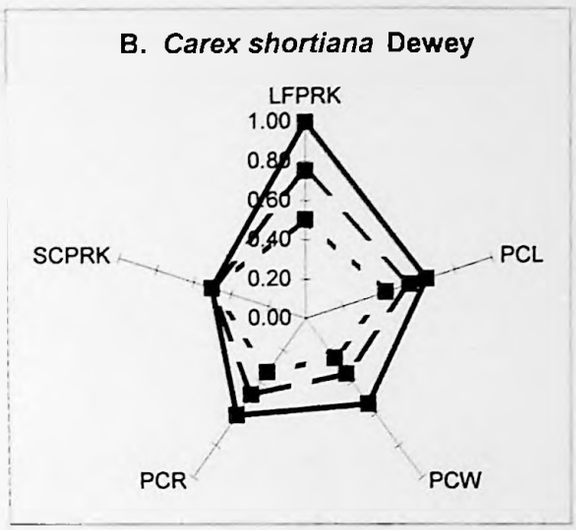
SR = Stomata L/W Ratio

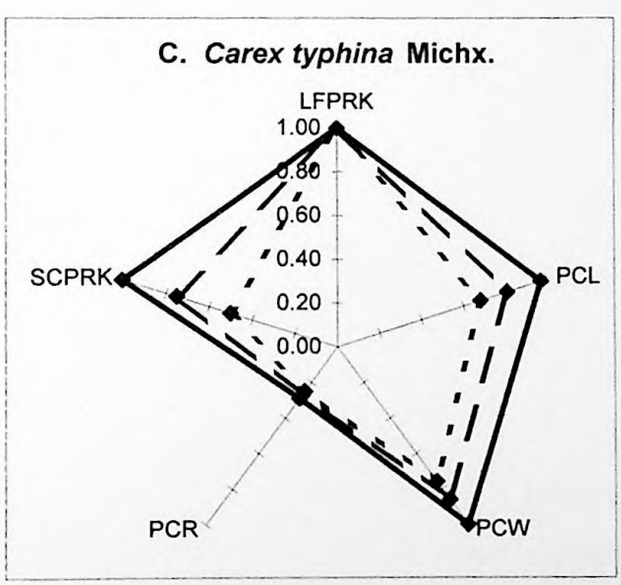
SD = Distance between stomata (micrometers)

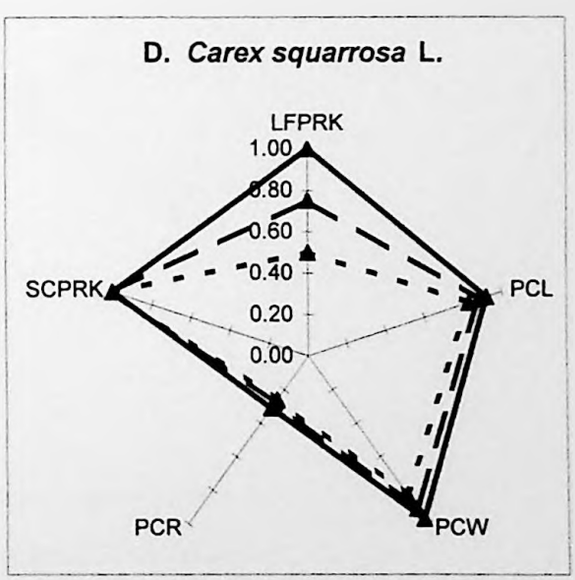
The second set of polygonal graphs (Figures 16-17) show each taxa for the the remaining five characters used in multivariate analysis (LfPrk, PCL, PCW, PCR, and ScPrk). Actual and relative values of these characters are given in Table 20. *C.* x *deamii* and *C. typhina* always had prickle hairs at leaf edges while *C. shortiana* and *C. squarrosa* specimens were found to possess both character states: 1 = no prickle hairs present and 2 = prickle hairs present. When mean values are placed on the same graph (Figure 17) it is clear that *C.* x *deamii* and *C. shortiana* have a distinctly different appearance from that of *C. typhina* and *C. squarrosa*. Perigynium cell measurements were somewhat variable for *C.* x *deamii* and *C. shortiana*, yet cell measurements of both taxa were consistently longer than wide (*C.* x *deamii* PCL = 41.16 +/- 2.05 μm, PCW = 20.85 +/- 4.99 μm; *C. shortiana* PCL = 24.63 +/- 4.07 μm, PCW = 17.32 +/- 6.43 μm). *C. typhina* and *C. squarrosa*; on the other hand, were always near square with the width (*C. typhina* = 42.30 +/- 5.97 μm; *C. squarrosa* = 44.55 +/- 2.91 μm) being only slightly greater than the length (*C. typhina* = 35.32 +/- 5.37 μm; *C. squarrosa* = 38.71 +/- 1.72 μm). Pistillate scale prickles (SCPRK) were present on *C. typhina* and C. squarrosa, yet they could not be found on *C.* x *deamii* or *C. shortiana*.

Figure 16. Polygonal graph of maximum, mean, and minimum values of micromorphological characters (LFPRK, PCL, PCW, PCR, and SCPRK) for A.) *Carex* x *deamii* Herm., B.) *Carex shortiana* Dewey, *Carex typhina* Michx., and D.) *Carex squarrosa* L..


A. Carex x deamii Herm.
LFPRK
1.00
0.80
0.60
0.40
0.20
0.00
SCPRK
PCL
PCR
PCW


B. Carex shortiana Dewey
LFPRK
1.00
0.80
0.60
0.40
0.20
0.00
SCPRK
PCL
PCR
PCW


C. Carex typhina Michx.
LFPRK
1.00
0.80
0.60
0.40
0.20
0.00
SCPRK
PCL
PCR
PCW


D. Carex squarrosa L.
LFPRK
1.00
0.80
0.60
0.40
0.20
0.00
SCPRK
PCL
PCR
PCW

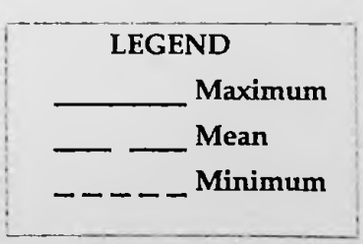

LEGEND
Maximum
Mean
Minimum

Figure 17. Polygonal graph of mean values of micromorphological characters (LFPRK, PCL, PCW, PCR, and SCPRK) for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L..

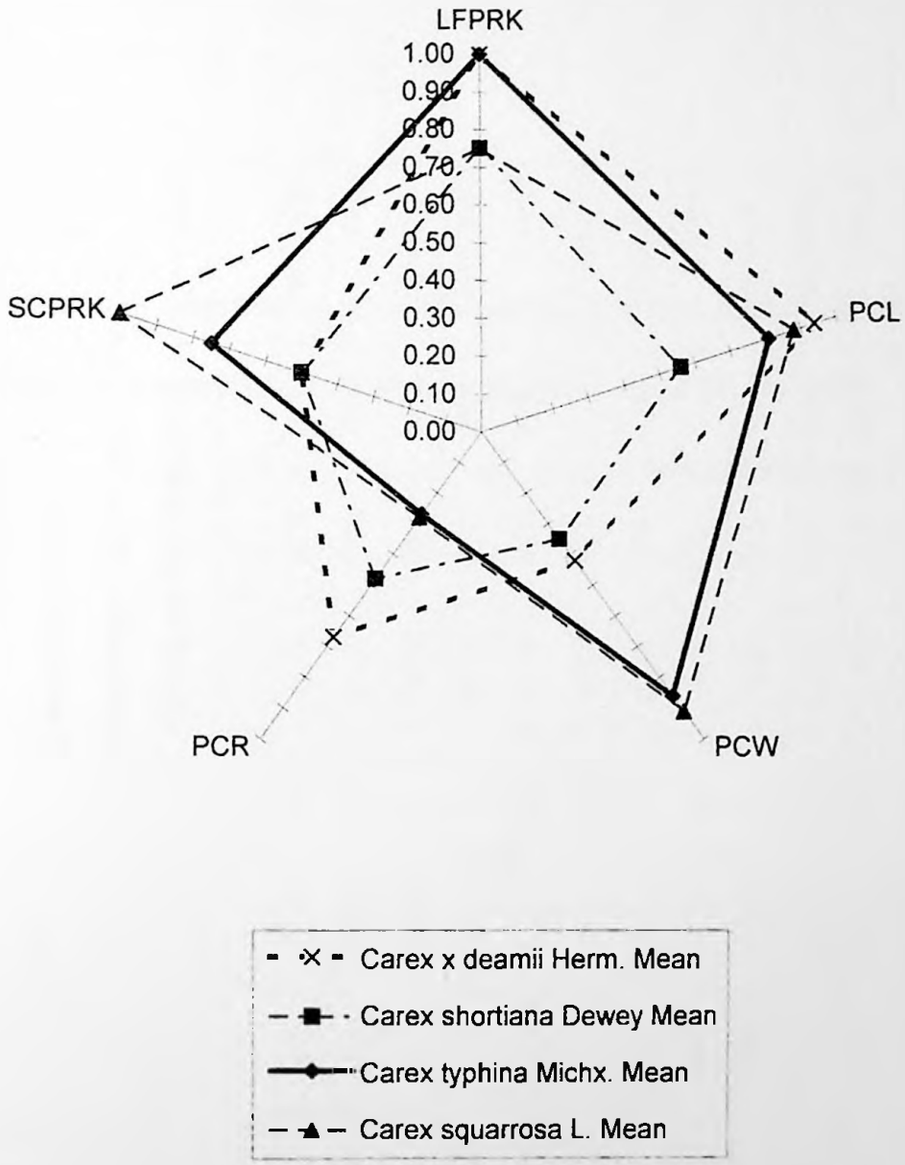

LFPRK
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
PCL
PCW
PCR
SCPRK
Carex x deamii Herm. Mean
Carex shortiana Dewey Mean
Carex typhina Michx. Mean
Carex squarrosa L. Mean

Table 20. Actual and relative values for micromorphological characters (LFPRK, PCL, PCW, PCR, and SCPRK) of Carex x deamii and its putative parents.

		LF	PRK	PC	L	PC	W	PC	R	SC	PRK
		A	R	A	R	A	R	A	R	A	R
Carex x *deamii* Herm.	Maximum	2.00	1.00	43.78	1.00	25.79	0.53	3.12	1.00	1.00	0.50
	Mean	2.00	1.00	41.16	0.94	20.85	0.42	2.10	0.67	1.00	0.50
	Minimum	2.00	1.00	38.79	0.89	14.01	0.29	1.59	0.51	1.00	0.50
Carex shortiana Dewey	Maximum	2.00	1.00	28.31	0.65	26.49	0.54	1.89	0.61	1.00	0.50
	Mean	1.50	0.75	24.63	0.56	17.32	0.35	1.50	0.48	1.00	0.50
	Minimum	1.00	0.50	18.87	0.43	12.47	0.25	1.07	0.34	1.00	0.50
Carex typhina Michx.	Maximum	2.00	1.00	42.54	0.97	49.08	1.00	0.90	0.29	2.00	1.00
	Mean	2.00	1.00	35.32	0.81	42.30	0.86	0.84	0.27	1.50	0.75
	Minimum	2.00	1.00	29.82	0.68	37.18	0.76	0.78	0.25	1.00	0.50
Carex squarrosa L.	Maximum	2.00	1.00	40.33	0.92	47.57	0.97	0.97	0.31	2.00	1.00
	Mean	1.50	0.75	38.71	0.88	44.55	0.91	0.87	0.28	2.00	1.00
	Minimum	1.00	0.50	36.33	0.83	40.61	0.83	0.80	0.26	2.00	1.00
	LFPRK = Leaf Prickle hairs; 1 = no, 2 = yes										
	PCL = Perigynium Cell Length (micrometers)										
	PCW = Perigynium Cell Width (micrometers)										
	PCR = Perigynium Cell L/W Ratio										
	SCPRK = Pistillate Scale Prickle hairs; 1 = no, 2 = yes										

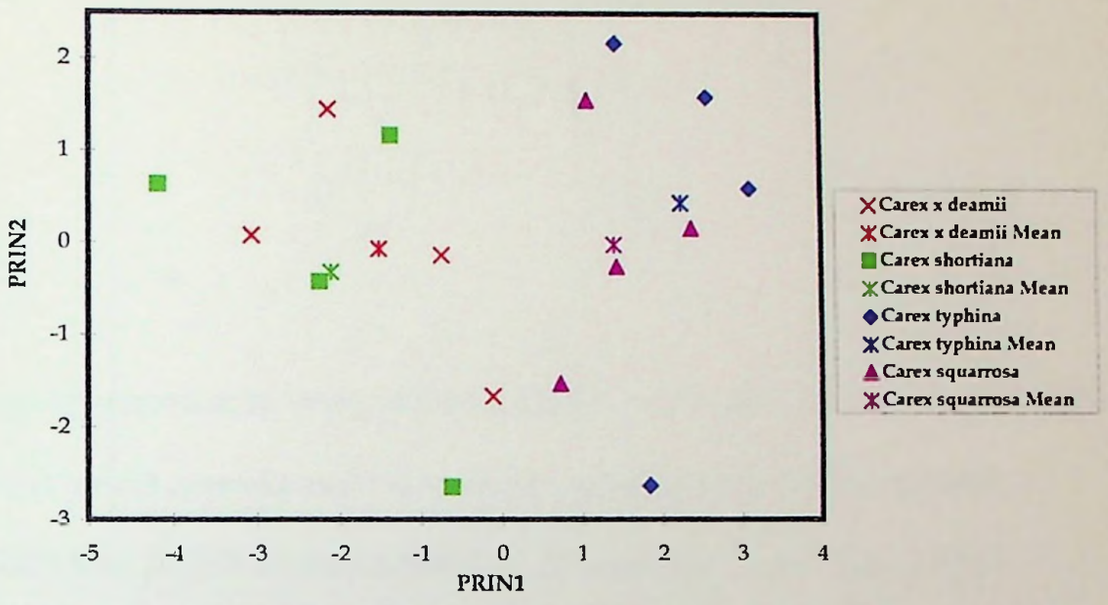
Multivariate Analysis

Principal Component Analysis (PCA)

PCA was used to analyze twelve micromorphological characters. Scatterplots of PRIN2 x PPRIN1 (Figure 36), PRIN3 x PRIN1 (Figure 37), PRIN 4 x PRIN1 (Figure 38), and PRIN5 x PRIN1 (Figure 39) were created to graphically portray any micromorphological relationships among the four taxa.. Percent of total variation displayed by the first five principal component axes ranged from 6.4 % to 38.9 % with PRIN 1 being the most important followed in order by each successive axis (Table 21). All scatterplots showed *C.* x *deamii* and *C. shortiana* with overlapping character ranges or at least closely clustered near one another. Likewise, *C. typhina* and *C. squarrosa* consistently showed overlapping character ranges or were closely clustered. Based on eigenvector values of each character for each principal component axis SL, LCL, and PCW were most important in separating the two groups (1st group = *C.* x *deamii* and *C. shortiana*; 2nd group = *C. typhina* and *C. squarrosa*) on PRIN1. None of the species separated well on PRIN2. On PRIN3 *C.* x *deamii* and *C. shortiana* were separated from one another primarily by PCL and LFPRK while *C. typhina* and *C. squarrosa* were not separated along this axis. On PRIN4 *C. typhina* and *C. squarrosa* were separated by SCPRK and SD while *C.* x *deamii* and *C. shortiana* were not separated. Finally on PRIN5 *C. typhina* and *C. squarrosa* were disassociated by LFPRK, PCR, and PCL. Table 22 shows the eigenvector values of each character for each principal component axis.

Figure 18. Principal Component Analysis (PCA) plots of micromorphological data for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Scatterplot of PRIN1 and PRIN2, B.) Scatterplot of PRIN1 and PRIN3.

A. Principal Component Analysis (PCA) plot of PRIN1 and PRIN2 for *Carex* x *deamii* Herm. and its Putative Parents using Micromorphological Characters.



B. Principal Component Analysis (PCA) plot of PRIN1 and PRIN3 for *Carex* x *deamii* Herm. and its Putative Parents using Micromorphological Characters.

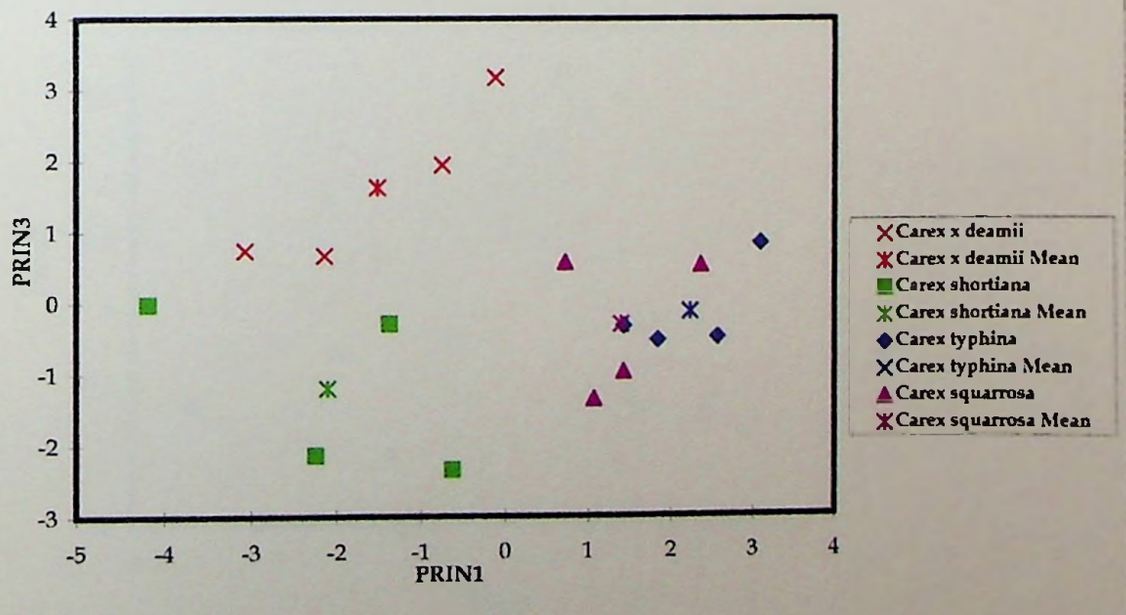
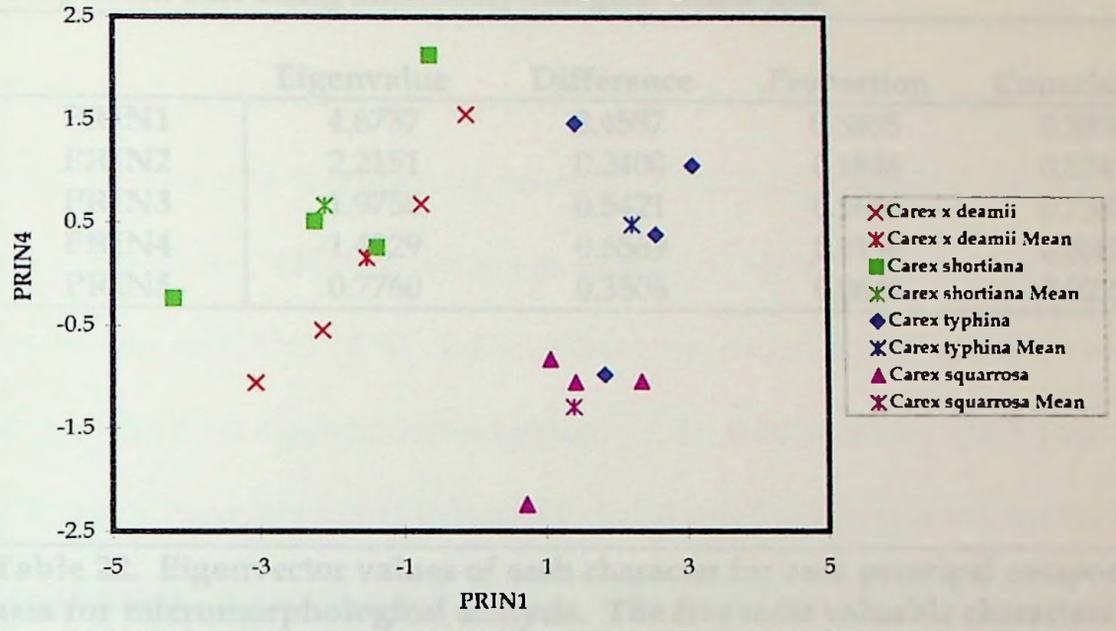


Figure 19. Principal Component Analysis (PCA) plots of micromorphological data for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Scatterplot of PRIN1 and PRIN4, B.) Scatterplot of PRIN1 and PRIN5.

A. Principal Component Analysis (PCA) plot of PRIN1 and PRIN4 for *Carex* x *deamii* Herm. and its Putative Parents using Micromorphological Characters.



B. Principal Component Analysis (PCA) plot of PRIN1 and PRIN5 for *Carex* x *deamii* Herm. and its Putative Parents using Micromorphological Characters.

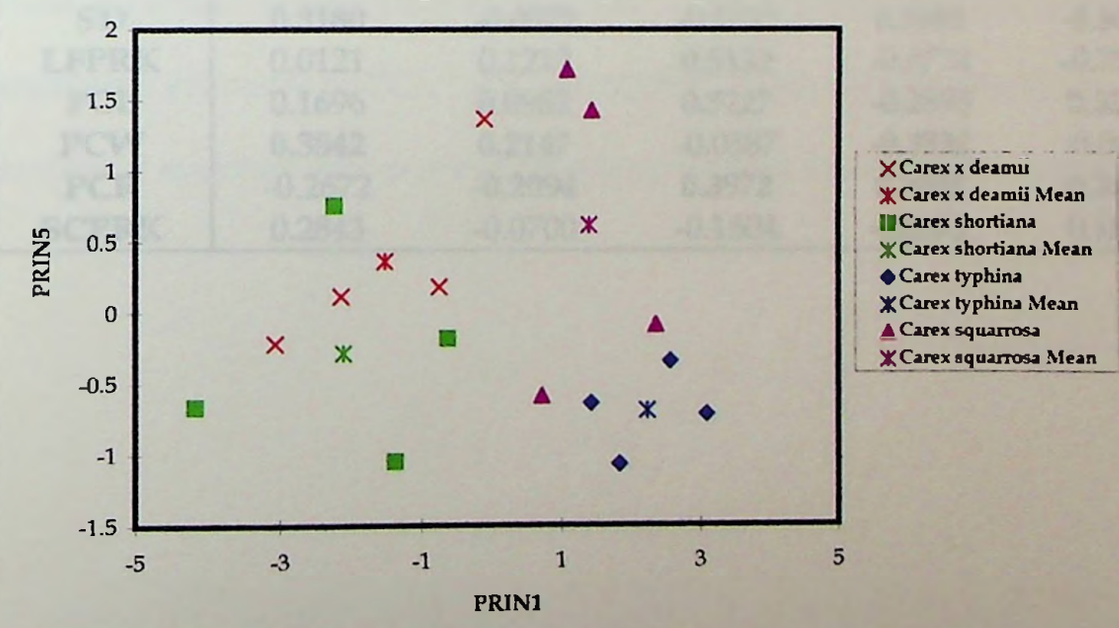


Table 21. Eigenvalues and percent variation represented by each principal component axis using micromorphological characters.

	Eigenvalue	Difference	Proportion	Cumulative
PRIN1	4.6737	2.4587	0.3895	0.3895
PRIN2	2.2151	0.2400	0.1846	0.5741
PRIN3	1.9750	0.5421	0.1646	0.7387
PRIN4	1.4329	0.6569	0.1194	0.8581
PRIN5	0.7760	0.3506	0.0647	0.9227

Table 22. Eigenvector values of each character for each principal component axis for micromorphological analysis. The five most valuable characters in separating the four taxa are highlighted for each axis.

Character	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5
LCL	**0.3965**	0.1315	0.1664	0.2950	0.1908
LCW	0.2873	**0.2800**	**-0.2552**	**0.3500**	**0.2117**
LCR	**0.3309**	-0.0548	**0.4099**	0.1459	0.1527
SL	**0.4052**	-0.1367	-0.0052	0.0891	-0.1523
SW	-0.0506	**0.6594**	0.0223	0.0738	-0.0433
SR	0.2326	**-0.5650**	-0.0275	-0.0231	-0.0896
SD	**0.3180**	-0.0972	-0.1322	**0.3992**	**-0.3035**
LFPRK	0.0121	0.1212	**0.5132**	-0.0774	**-0.7136**
PCL	0.1696	0.0982	**0.5227**	-0.2893	**0.3268**
PCW	**0.3842**	**0.2147**	-0.0587	**-0.3532**	-0.0412
PCR	-0.2672	**-0.2094**	**0.3972**	**0.3038**	**0.3461**
SCPRK	0.2843	-0.0700	-0.1504	**-0.5377**	0.1807

Canonical Discriminant Analysis (CDA)

Canonical Discriminant Analysis (CDA) was used to analyze the twelve characters used in PCA. A scatterplot of CAN2 x CAN1 was created to visually display the relationship between taxa (Figure 20). The plot of CAN2 x CAN1 accounted for 99.8 % of the total variation. CAN1 represented 98.3 % of the variation while CAN2 contributed an additional 1.6 %. The remaining variation was found on CAN3 (.2 %). Table 23 shows each canonical discriminant axis along with their eigenvalues and percent of the total variation which they display. Characters found to be most effective in separating along each canonical discriminant axis were determined by using the values for the total canonical structure. Values closest to positive or negative 1 were most important in separating along each respective axis. *C.* x *deamii* was quite separated from the other three taxa and *C. shortiana* and *C. typhina* were clustered in close proximity while *C. squarrosa* was also somewhat isolated. PCR, SCPRK, and PCW were most important in separating along CAN 1. On the CAN2 axis, taxa were separated primarily by PCL, LCR, and SCPRK. Table 24 shows the total canonical structure values of each character for each canonical discriminant axis.

Figure 20. Canonical Discriminant Analysis (CDA) plot of macro-morphological data for *Carex* x *deamii* Herm., *Carex shortiana* Dewey, *Carex typhina* Michx., and *Carex squarrosa* L.. A.) Scatterplot of CAN1 and CAN2.

Canonical Discriminant Analysis (CDA) plot of CAN1 and CAN2 for *Carex* x *deamii* Herm. and its Putative Parents using Micromorphological Characters.

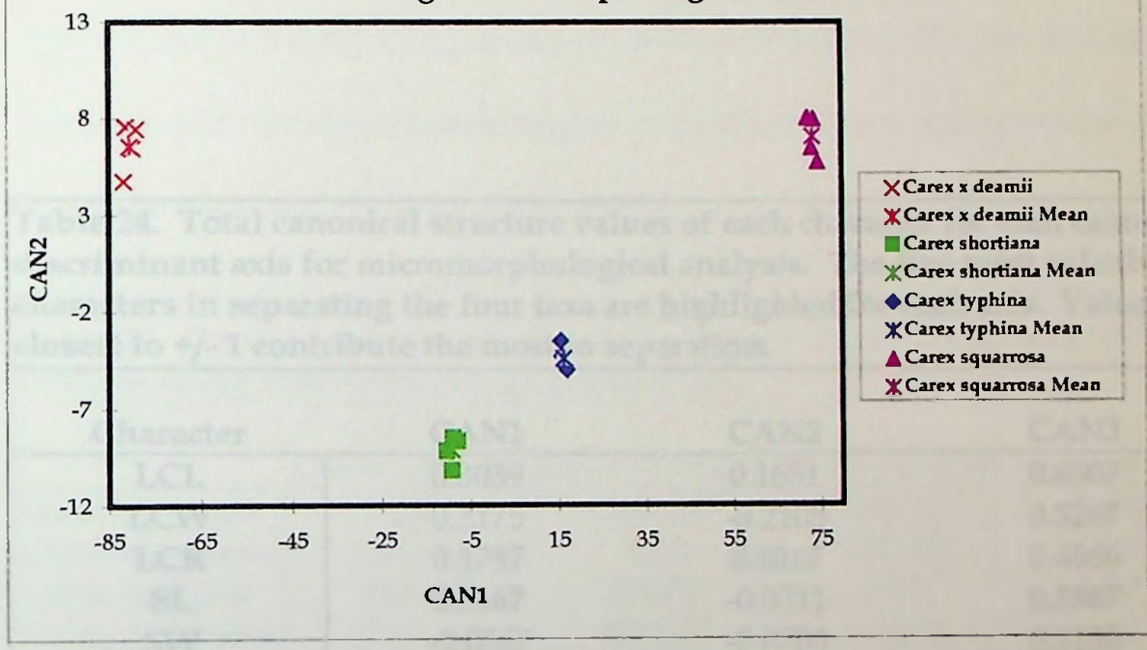


Table 23. Eigenvalues and percent variation represented by each canonical discriminant axis for micromorphological analysis.

	Eigenvalue	Difference	Proportion	Cumulative
CAN1	4071.6670	4006.2670	0.9825	0.9825
CAN2	65.4007	58.1850	0.0158222	0.9983
CAN3	7.2157	NA	0.0017	1.0000

Table 24. Total canonical structure values of each character for each canonical discriminant axis for micromorphological analysis. The five most valuable characters in separating the four taxa are highlighted for each axis. Values closest to +/- 1 contribute the most to separation.

Character	CAN1	CAN2	CAN3
LCL	0.3039	0.1651	**0.6507**
LCW	0.3175	-0.2105	**0.5247**
LCR	0.1797	**0.4017**	0.4666
SL	**0.5167**	-0.0211	**0.5867**
SW	-0.0367	-0.0700	0.1128
SR	0.2705	0.0233	0.2328
SD	0.2382	**-0.3401**	**0.5323**
LFPRK	**-0.3356**	0.0885	0.4925
PCL	-0.0794	**0.8173**	0.3955
PCW	**0.7336**	**0.2868**	**0.5438**
PCR	**-0.7672**	0.1394	-0.3220
SCPRK	**0.7618**	**0.3585**	0.1740

Discriminant Analysis

The discriminant analysis (PROC DISCRIM) procedure of SAS/STAT (1990) produces a generalized squared distance to species (Table 25). This distance to species places *C.* x *deamii* closest to *C. shortiana* (5350). *C. typhina* is next closest at 9506 and *C. squarrosa* is farthest apart with a generalized squared to species of 23,683. *C. shortiana* and *C. typhina* are placed very close together with a value of 708.59. Comparison of predetermined identification of each specimen with the classification suggested by discriminant analysis showed all taxa to be identified correctly (Table 26).

Table 25. Generalized squared distance to species derived from discriminant analysis using micromorphological characters.

	Carex x *deamii*	*Carex shortiana*	*Carex typhina*	*Carex squarrosa*
Carex x *deamii*	0.00	5350.00	9506.00	23683.00
Carex shortiana	5350.00	0.00	708.59	7071.00
Carex typhina	9506.00	708.59	0.00	3412.00
Carex squarrosa	23683.00	7071.00	3412.00	0.00

Table 26. Classification results based upon discriminant analysis (PROC DISCRIM of SAS/STAT 1990) for micromorphological analysis.

	Percent Correct	Number of Specimens Classified into Group			
		C. x *deamii*	*C. shortiana*	*C. typhina*	*C. squarrosa*
C. x deamii	100	4	0	0	0
C. shortiana	100	0	4	0	0
C. typhina	100	0	0	4	0
C. squarrosa	100	0	0	0	4
Total	100	4	4	4	4

HYWIN Analysis (Hypothesizing Hybrids using Weighted Intermediacy)

HYWIN was used to see which species would represent the most probable parents of *C.* x *deamii* based upon micromorphological characters. In the HYWIN analysis using micromorphological data, the top 87 triples out of a possible 1,680 triples were reported to achieve a .95 probability level. Three of the four *C.* x *deamii* specimens showed up as hybrids in the top 87 triples. *C.* x *deamii* was determined to be a hybrid in 14 triples (Table 27). *C. shortiana* was determined to be the closest parent in nine triples. Six of these triples had *C. typhina* as the second closest parent while only 3 triples had *C. squarrosa* as the most likely second parental species. In the remaining five triples with *C.* x *deamii* as a hybrid, other *C.* x *deamii* specimens were determined to be the most likely parent specimens.

Table 27. ***Carex*** **x** ***deamii*** **specimens and most likely parent species as determined by HYWIN for micromorphological data. Rank and scores for the determining criteria are also given.**

Rank	Hybrid	Parent1	Parent2	IN	EQ	PD	NP	HS
8	DEAM1	SHOR1	SQUA4	0.191	-0.007	0.496	0.262	1.6798
9	DEAM1	SHOR1	TYPH3	0.242	-0.218	0.650	0.262	1.6746
22	DEAM1	DEAM2	DEAM4	0.268	0.037	0.321	0.176	1.5522
26	DEAM2	SHOR1	TYPH3	-0.045	-0.061	0.650	0.384	1.5440
32	DEAM1	SHOR1	SQUA2	0.119	-0.122	0.528	0.262	1.5261
47	DEAM1	DEAM2	DEAM3	0.199	0.013	0.304	0.184	1.4900
55	DEAM1	DEAM2	SHOR4	0.178	0.023	0.314	0.180	1.4695
61	DEAM1	DEAM2	SHOR1	0.233	-0.162	0.384	0.189	1.4551
65	DEAM3	SHOR1	SQUA3	0.292	-0.336	0.479	0.165	1.4323
71	DEAM2	SHOR1	TYPH4	-0.067	-0.148	0.633	0.384	1.4185
72	DEAM1	DEAM4	TYPH3	0.256	-0.398	0.560	0.176	1.4171
76	DEAM1	SHOR2	TYPH3	-0.050	-0.135	0.593	0.311	1.4080
78	DEAM1	SHOR1	TYPH4	0.071	-0.299	0.633	0.262	1.4049
84	DEAM1	SHOR1	TYPH1	0.028	-0.290	0.645	0.262	1.3835

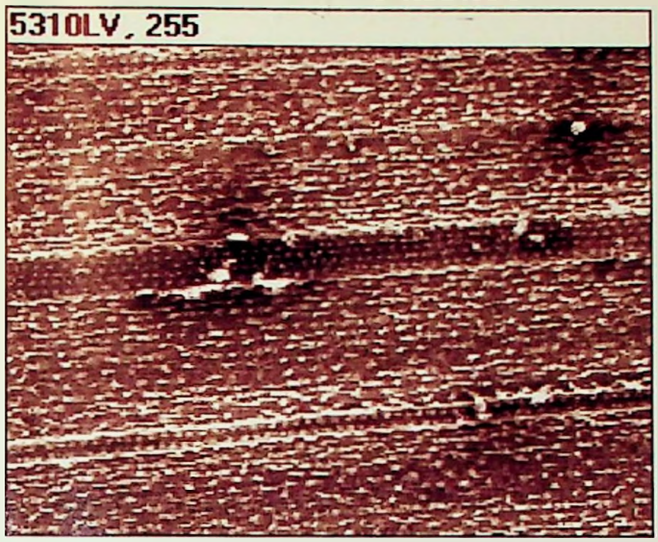
IN = Hybrid Intermediacy Score
EQ = Measure of Equality of the two distances between the possible hybrid and each of the two possible parents.
PD = Distance between possible parents
NP = Nearest Parent
HS = Hybrid Score

X-Ray Analysis

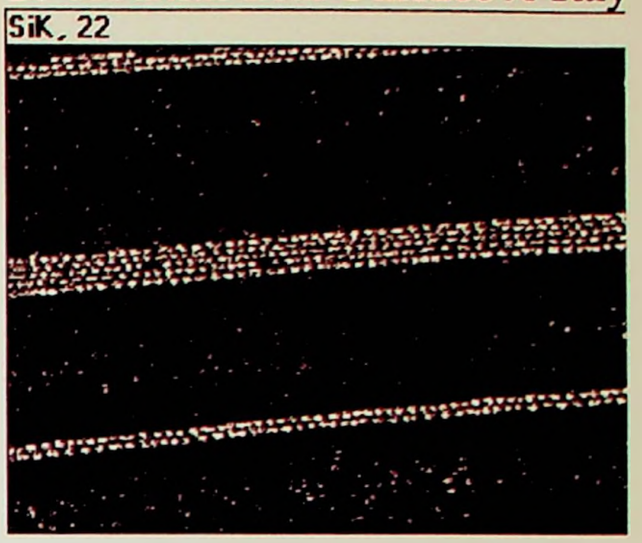
Elemental X-Ray analysis showed silica to be present in all parts examined (Figure 21). Silica was found on the leaves associated with the veins, on papillae and prickles on the leaf surface, throughout the achene, and even on stigma lobes. The occurrence on all parts except the stigma lobes is easy to determine. On the leaves, the silica serves to protect the vascular tissue from damage. On the achene, the silica bodies provide a natural scarification process. A possible reason for silica to be present on the stigma lobes is that the silica is a leftover from the evolutionary past when anthers and stigmas originated from leaf material.

Figure 21. Images of X-Ray analysis showing presence and distribution of silica in various *Carex* parts.

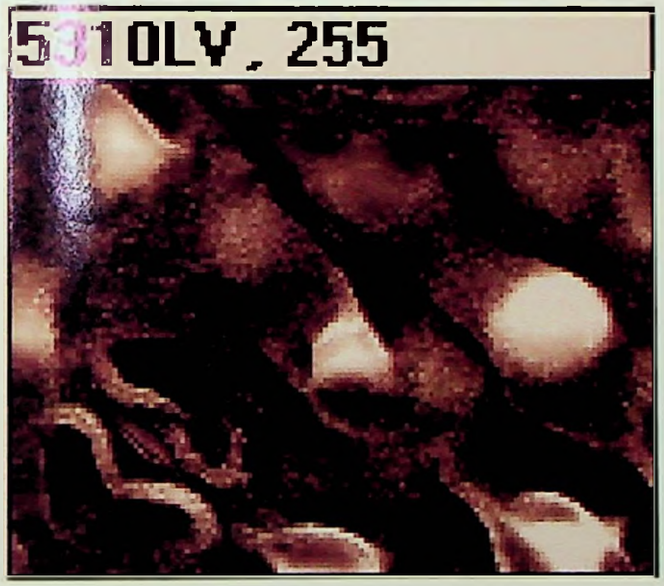
A. Abaxial Leaf Surface



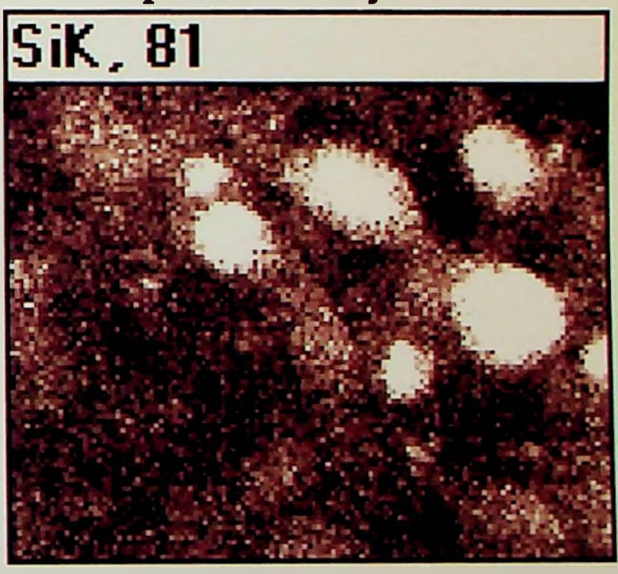
B. Abaxial Leaf Surface X-Ray



C. Papillae

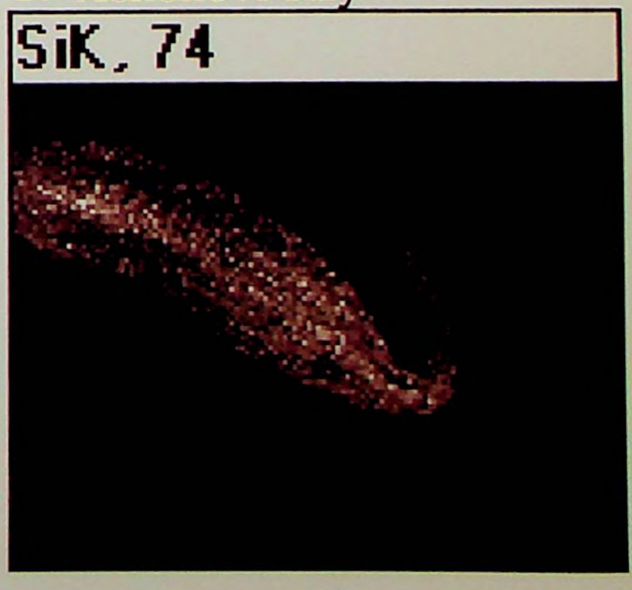


D. Papillae X-Ray

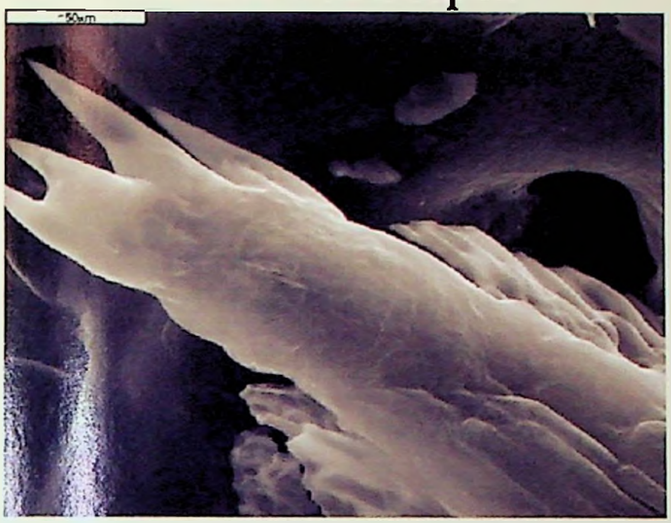


E. Achene

F. Achene X-Ray



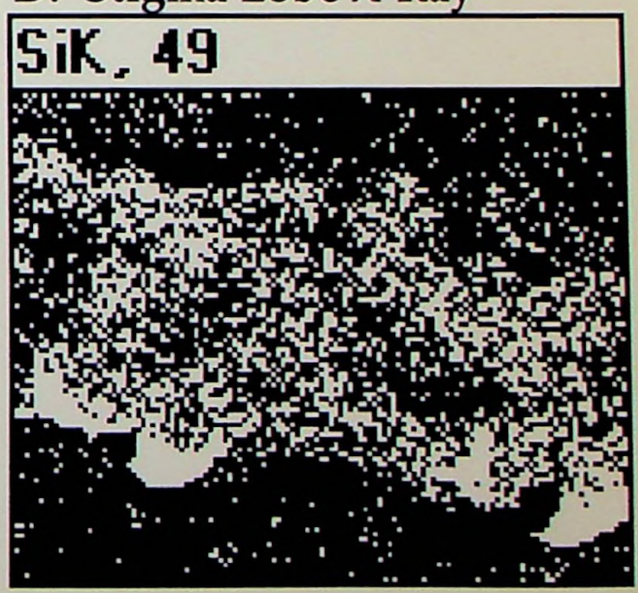
A. Pistillate Scale Apex



B. Pistillate Scale Apex X-Ray

C. Stigma Lobe

D. Stigma Lobe X-Ray



CHAPTER IV.

DISCUSSION

Analysis of Pollen and Achene Viability

The mean percent viability of pollen for the hybrid was found to be only 2.3 % (N=514) while the viability of all three of the other taxa always averaged over 90 %. This low viability is very typical for hybrids between *Carex* species of different sections as is suspected in this case (Cayouette and Catling 1992).

Nevertheless, the fact the hybrid was at least partially fertile is very significant. In past studies, no viable pollen grains were found for C. x *deamii* (Dan K. Evans, pers. comm.). This may be because mature herbarium specimens were used in past studies and the pollen grains that remained were nonviable anyway.

Since both pollen and achenes were found to be almost 100 % unviable, it can be safely concluded that individual plants of the hybrid, C. x *deamii*, are unlikely to spread unless by vegetative means.

Analysis of Macromorphological Characters

Univariate Analysis

All characters used in univariate analysis were found to be significantly different among taxa to at least the .01 probability level. Perigynium and pistillate scale characters had a probability level of .0001 along with very high F values suggesting that these would be the best characters in separating the four taxa (Table 5).

Duncan's Multiple Range Test showed that the taxa could be differentiated for many macromorphological characters (Table 6). *C.* x *deamii* and *C. shortiana* were significantly different for seventeen of the nineteen characters. Pistillate/staminate ratio (PSR) and pistillate scale width were the only characters not significantly different between the two taxa. This is very important since these two taxa are placed in the same subgeneric section. These characters will allow easier determination of the hybrid when it is found in a mixed population with *C. shortiana*.

The two putative paternal parents of the hybrid, *C. typhina* and *C. squarrosa*, are both in subgeneric section Squarrosae. There has been confusion in the past about the taxonomic relationship of these two taxa. Atypical specimens of *C. typhina* often bear a resemblance to *C. squarrosa* and some *C. squarrosa* have an overall morphological appearance similar to that of *C. typhina*. However, this study makes it clear that there are many characters which can be used to differentiate these two species. According to Duncan's Multiple Range Test the

two taxa were significantly different for seventeen characters. Staminate length (STL) and perigynium width (PEW) were the only characters not found to be significantly different.

Bivariate Analysis

Bivariate scatterplots (Figures 15-17) showed *C.* x *deamii*, *C. shortiana*, and *C. typhina* overlapping macromorphologically while *C. squarrosa* specimens were somewhat separated. This suggests that *C. shortiana* and *C. typhina* are the most likely parental species.

Multivariate Analysis

All PCA and CDA scatterplots showed that the four taxa could easily be differentiated based upon macromorphological multivariate analysis. PCA and CDA allow a visual image of the relationship between the taxa and also provide a method for determining the best characters in separating closely related species. The eigenvector values (Table 10) of PCA and the total canonical structure (Table 12) of CDA are the tools used to determine the best characters for separating taxa. All four taxa were separated on PRIN 1 primarily by perigynium length (PEL) and perigynium beak length (PKL). These two characters are especially effective in separating *C.* x *deamii* and *C. shortiana* because there is no overlap in their character value ranges (*C.* x *deamii* PEL=2.90 - 4.01 mm, PKL=0.47 - 1.21 mm; *C. shortiana* PEL=2.02 - 2.60 mm, PKL=0.12 - 0.34 mm). Although, *C. typhina* and *C. squarrosa* were significantly different for both

characters, macromorphological overlap present between the two taxa renders these characters unacceptable for separating the two species.

Analysis by CDA showed more separation and thus helps to clarify the macromorphological relationships present between the taxa. The scatterplot of CAN2 x CAN1 (Figure 20) shows all four taxa to cluster individually. In this plot, the hybrid *C.* x *deamii* occupies an intermediate position between *C. shortiana* and *C. typhina* while *C. squarrosa* is placed at a distance from the other taxa. Here, the most important characters for differentiating taxa are spike number (SN), perigynium beak length (PKL), and perigynium length (PEL).

Putative Parent Identification

Hybrid intermediacy is often used to determine a hybrids origin. In this case, the hybrid *C.* x *deamii* was found to be directly intermediate between *C. shortiana* and *C. typhina* for thirteen characters (SL, SW, SR, PN, SN, PEL, PEW, PER, PBL, PBR, PKL, PBB, and SCR) and directly between *C. shortiana* and *C. squarrosa* for only four characters (LW, PL, SCK, and SCW). This lends support to *C. shortiana* and *C. typhina* as probable parental taxa.

The phenomenon of matrocliny shows that hybrids most often bear a closer resemblance to the maternal contributor. The fact that *C.* x *deamii* was placed closest to *C. shortiana* for fifteen of nineteen characters helps lend some credence to the assumption that *C. shortiana* is the maternal contributor to the hybrid. Likewise, the fact that *C. typhina* was also placed near the hybrid supports its role as a probable paternal contributor.

All PCA plots (Figures 18-19) showed that *C.* x *deamii* and *C. shortiana* were clearly macromorphologically similar. This further solidifies *C. shortiana*'s place as the likely maternal parent. Furthermore, CDA (Figure 20) placed the hybrid directly intermediate to *C. shortiana* and *C. typhina* showing that these taxa are the most likely parental species. The fact that *C. squarrosa* was consistently separated from the other taxa was significant. In previous macromorphological multivariate analysis of these taxa (Puckett and Evans 1994), *C. typhina* and *C. squarrosa* clustered sufficiently close to make it difficult to determine which was most closely related to *C.* x *deamii*.

Discriminant Analysis quantitatively supports *C. shortiana* and *C. typhina* as the likely parental contributors of *C.* x *deamii* (Table 13).

HYWIN Analysis provides further evidence for *C. shortiana* and *C. typhina* as parental contributors to *C.* x *deamii*. *C. shortiana* was consistently placed as *C.* x *deamii*'s closest probable parent while *C. typhina* was found to be the most likely second parent (Table 15).

C. x *deamii* is macromorphologically most similar to *C. shortiana* and is therefore most likely to be confused with this species. *C. typhina* and *C. squarrosa* have also been shown to be very similar in macromorphological characters. To allow easier determination of these taxa using macromorphological characters a key and description of taxa is provided below:

Key to taxa based upon macromorphological data

Perigynium Beak Length less than 1.50 mm.....Section Shortianae

Perigynium Beak ~ 0.91 mm long (0.45-1.21 mm).....(1.) *Carex* x *deamii*

Perigynium Beak ~ 0.21 mm long (0.12-0.34 mm).....(2.) *Carex shortiana*

Perigynium Beak Length greater than 1.50 mm.....Section Squarrosae

Pistillate part of terminal spike 1.8 to 3.3X longer.....(3.) *Carex typhina*
than wide; style straight.

Pistillate part of terminal spike 0.93 to 1.58X longer.....(4.) *Carex squarrosa*
than wide; style with very noticeable curve.

Description of taxa based upon macromorphological data

(1.) *Carex* x *deamii* Herm.

Plants: caespitose, ~ 64 cm high to top of inflorescence (SD = 14.70). **Leaves:** ~ 6-7 per culm, 75.50 cm long (SD = 16.86), 5.57 mm wide (4.58-7.14 mm) with 28 parallel veins on average (SD = 3.57). **Inflorescence:** 61.89 mm long consisting of 3 to 4 spikes. **Terminal spike:** gynecandrous, 36.01 mm long (19.27-46.09 mm) and 8.04 mm wide (5.74-9.66 mm). Pistillate part from 1.75 to 3.75 times longer than wide, 22.05 mm long (12.48-27.50 mm). Staminate part 13.96 mm long (6.79-21.73 mm). **Perigynia:** 3.52 mm total length (2.90-4.01 mm) and 2.06 mm wide (1.44-2.71 mm); perigynia body 1 to 2 times longer than wide, 2.61 mm long (2.18-2.91 mm); perigynia beak noticeably two-toothed, 0.91 mm long (0.47-1.21 mm). **Pistillate Scale:** 3.22 mm long (2.00-4.00 mm) by 1.36 mm wide (0.75-2.81 mm).

(2.) *Carex shortiana* Dewey

Plants: caespitose, ~ 64 cm high to top of inflorescence (SD = 20.58). Leaves: ~ 6 per culm, 69.04 cm long (SD = 20.66), 6.35 mm wide (5.30-7.45 mm) with 27 parallel veins on average (SD = 2.92). **Inflorescence:** **????** mm long consisting of 4 to 5 spikes. **Terminal spike:** gynecandrous, 30.03 mm long (19.49-38.92 mm) and 5.22 mm wide (4.43-5.96 mm). Pistillate part from 2.5 to 5 times longer than wide, 18.68 mm long (12.29-26.08 mm). Staminate part 11.37 mm long (5.46-22.42 mm). **Perigynia:** 2.31 mm total length (2.02-2.60 mm) and 2.03 mm wide (1.66-2.31 mm); perigynia body virtually the same length as width, 2.10 mm long (1.89-2.38 mm); perigynia beak indistinctly two-toothed and very short, 0.21 mm long (0.12-0.34 mm). **Pistillate Scale:** 2.46 mm long (1.75-3.61 mm) by 1.38 mm wide (1.13-1.90 mm).

(3.) *Carex typhina* Michx.

Plants: caespitose, ~ 53 cm high to top of inflorescence (SD = 7.86). **Leaves:** ~ 6-7 per culm, 73.21 cm long (SD = 10.68), 6.45 mm wide (4.47-7.85 mm) with 27 parallel veins on average (SD = 1.34). **Inflorescence:** 45.75 mm long consisting of 1 to 3 spikes. **Terminal spike:** gynecandrous, typically somewhat pointed at the apex, 41.92 mm long (30.50-49.65 mm) and 13.14 mm wide (9.87-16.20 mm). Pistillate part from 1.8 to 3.3 times longer than wide, 32.84 mm long (24.77-39.86 mm). Staminate part 9.09 mm long (5.07-13.10 mm). **Perigynia:** 6.76 mm total length (5.82-8.22 mm) and 2.51 mm wide (1.60-3.58 mm); perigynia body 1.4 to 2.6 times longer than wide, 4.42 mm long (3.57-5.42 mm); perigynia beak

distinctly two-toothed, 2.34 mm long (1.80-2.98 mm). **Pistillate Scale:** 4.79 mm long (4.07-5.80 mm) by 1.45 mm wide (1.10-2.12 mm).

(4.) ***Carex squarrosa*** **L.**

Plants: caespitose, ~ 58 cm high to top of inflorescence (SD = 11.51). **Leaves:** ~ 5-6 per culm, 74.80 cm long (SD = 15.63), 4.12 mm wide (3.32-5.15 mm) with 22 parallel veins on average (SD = 2.72). **Inflorescence:** 34.05 mm long consisting of 1 to 2 spikes. **Terminal spike:** gynecandrous, typically rounded at the apex, 33.83 mm long (20.54-40.92 mm) and 17.54 mm wide (14.10-19.70 mm). Pistillate part from 1 to 1.5 times longer than wide, 23.24 mm long (13.13-29.37 mm). Staminate part 10.97 mm long (7.41-17.99 mm). **Perigynia:** 8.28 mm total length (6.52-9.77 mm) and 2.68 mm wide (2.00-3.47 mm); perigynia body 1.4 to 2.5 times longer than wide, 5.23 mm long (3.88-6.30 mm); perigynia beak distinctly two-toothed, 3.05 mm long (2.52-3.49 mm). **Pistillate Scale:** 4.49 mm long (3.46-5.24 mm) by 1.15 mm wide (0.90-1.60 mm).

Analysis of Micromorphological Characters

ACHENE MICROMORPHOLOGY

C. x *deamii* achenes failed to produce silica bodies characteristic of sedges (Figure 21). This is because the achenes are abortive and fail to reach maturity. Mehra and Sharma (1965) found that epidermal silica bodies were more common in older plant parts. This helps to explain there absence in *C.* x *deamii* achenes. The fact that *C.* x *deamii* achenes are abortive prevented any quantitative analysis of data from achene measurements.

Achene epidermal cells were much smaller in *C.* x *deamii* than in the other taxa (Table 16). Again, this is probably due to their abortive nature. *C. typhina* and *C. squarrosa* consistently had larger cells, yet the silica bodies of *C. shortiana* were larger than *C. typhina* or *C. squarrosa* (Table 16).

PERIGYNIUM, PISTILLATE SCALE, AND LEAF MICROMORPHOLOGY

Univariate Analysis

ANOVA (Table 17) and Duncan's Multiple Range Test (Table 18) showed that all four taxa were relatively similar for all micromorphological characters. Three characters (SW, SR, and LFPRK), in fact, were not significantly different between any of the four taxa. The remaining characters did, however, separate the taxa into two clearly identifiable groups. The first group contained *C.* x *deamii* and *C. shortiana* and the second group was made up of *C. typhina* and *C. squarrosa*. These groupings were to be expected since *C.* x *deamii* and *C. shortiana* are both placed in section Shortianae and *C. typhina* and *C. squarrosa* are both

placed in section Squarrosae. These two groups can easily be separated using perigynium epidermal cell measurements and the presence or absence of prickles on the pistillate scale apex. The perigynium epidermal cells of *C.* x *deamii* and *C. shortiana* were always rectangular in shape being longer than wide. *C. typhina* and *C. squarrosa*, however, were somewhat square in shape with a slightly greater width than length. Papillae were present on the abaxial leaf surface of all four taxa. *C. typhina* and *C. squarrosa*, however, had much fewer and less distinctive papillae when compared to those of *C.* x *deamii* and *C. shortiana*.

Little variation in micromorphology was found within the two groups. Only PCL was useful in separating *C.* x *deamii* from *C. shortiana*. *C.* x *deamii* perigynium epidermal cells (PCL = 41.16 μm) were nearly twice the length of *C. shortiana*'s perigynium epidermal cells (PCL = 24.63 μm). The only character found to be significantly different between *C. typhina* and *C. squarrosa* was SCPRK. This character, however, occurred in both the present and absent state in *C. typhina*, and therefore not useful in reliably separating these two taxa. The following key reflects the micromorphological differences displayed by the four taxa.

Multivariate Analysis

PCA and CDA scatterplots showed that the four taxa could be separated based upon micromorphological data. All scatterplots separated the taxa into at least two groups representing the distinct subgeneric sections (Shortianae and Squarrosae). This separation was based primarily on stomatal length (SL), leaf

epidermal cell length (LCL), and perigynium epidermal cell width (PCW). However, size ranges of SL and LCL for the four taxa overlap. PCW, although quite variable, is distinctive for the two subgeneric sections, and therefore the best character in separating the two sections. The shape of the perigynium epidermal cells (PCR) is also distinctive for the two sections. Perigynium epidermal cells of *C.* x *deamii* and *C. shortiana* (Section Shortianae) were consistently rectangular with a greater length (PCL) than width (PCW). *C. typhina* and *C. squarrosa* (Section Squarrosae) PCR were squarish with slightly greater widths (PCW) than lengths (PCL).

Multivariate scatterplots of micromorphological characters produced by PCA and CDA achieved minor success in separating taxa within each of the two sections. *C.* x *deamii* and *C. shortiana* were separated in both Principal Component Analysis and Canonical Discriminant Analysis by perigynium epidermal cell length (PCL). Perigynium epidermal cells were nearly twice as long for *C.* x *deamii* (Mean = 41.2 m, Range = 38.8 - 43.8 m) as compared to *C. shortiana* (Mean = 24.6 m, Range = 18.9 - 28.3 m) with no overlap between the two taxa. *C. typhina* and *C. squarrosa* were separated in Principal Component Analysis by SCPRK, LFPRK, PCR, PCL, and SD. However, SCPRK and LFPRK were not very reliable because both taxa were found to possess the present state for these characters. Also, PCR and PCL are not entirely reliable due to a great deal of micromorphological overlap between *C. typhina* and *C. squarrosa* for these

characters. Distance between stomata (SD) is the best micromorphological character for separating *C. typhina* and *C. squarrosa*.

Putative Parent Identification

Often times, through matrocliny, hybrids bear a closer resemblance to their maternal parent. *C. shortiana* clustered nearest to *C.* x *deamii* in all PCA scatterplots supporting the assumption that *C. shortiana* is the maternal contributor to the hybrid. HYWIN also reported *C. shortiana* to be the closest parental species each time *C.* x *deamii* was reported to be a hybrid.

PCA and CDA of micromorphological characters did not, however, help in determining the correct paternal parent species. In each PCA scatterplot the hybrid was placed between *C. shortiana* and both *C. typhina* and *C. squarrosa*. PCA alone was not able to demonstrate the most likely paternal contributor to the hybrid because there wasn't enough separation obtained between *C. typhina* and *C. squarrosa*.

The generalized squared distance to species produced by Discriminant Analysis showed *C. shortiana* and *C. typhina* to be much closer micromorphologically to the hybrid than *C. squarrosa* (Table 25). This is perhaps the best evidence for *C. shortiana* and *C. typhina* as parental species to *C.* x *deamii*. This distance shows a quantitative relationship betwen taxa and therefore takes some of the subjectivity of graph interpretation out of the equation and provides a numerical basis for conclusions.

HYWIN Analysis of micromorphological data also supports *C. shortiana* and *C. typhina* as the likely paternal species to *C.* x *deamii* (Table 27). As mentioned above, *C. shortiana* was determined to be the closest parental species for all *C.* x *deamii* specimens reported as hybrid and *C. typhina* was determined to be the next most likely parental species. This supports *C. shortiana* as the maternal contributor to the hybrid and *C. typhina* as the paternal contributor.

C. x *deamii* has been shown to be very similar micromorphologically to its proposed maternal contributor (*C. shortiana)* and *C. typhina* is also very similar in appearrance to its close relative, *C. squarrosa*. To allow easier determination of these taxa using micromorphological characters a key and description of taxa are provided below:

Key to taxa based upon micromorphological data

Perigynium Epidermal Cells Rectangular* (approximately 1.5 to two times longer than wide); Prickle hairs never present on the apex of pistillate scales. ..... Section Shortianae

Perigynium Epidermal Cells* 38.8 to 43.8 μm long; Achenes without silica bodies in epidermal cells (achenes abortive). ..... (1.) *Carex* x *deamii*

Perigynium Epidermal Cells* 18.9 to 28.3 μm long; Achenes with silica bodies in the epidermal cells (achenes not abortive). ..... (2.) *Carex shortiana*

Perigynium Epidermal Cells Squarish* (cells slightly wider than long; Prickle hairs present or absent on the apex of pistillate scales. ..... Section Squarrosae

Distance between stamata greater than 45 μm; Prickle hairs present or absent on the apex of pistillate scales. ..... (3.) *Carex typhina*

Distance between stomata less than 45 μm; Prickle hairs always present on the apex of pistillate scales. ..... (4.) *Carex squarrosa*

*Perigynium epidermal cells measured just below the neck.

Description of taxa based upon micromorphological data

(1.) *Carex* x *deamii* Herm.

Leaf: Inversely W-shaped; Abaxial epidermal cells rectangular (2.6 times longer than wide (2.0 - 3.3)), 43.0 μm long (30.0 - 57.6 μm) and 16.5 m wide (14.8 - 17.7 μm); Papillae abaxial, distinctive; Prickle hairs present, marginal; stomata abaxial, in rows parallel to long axis of leaf, 23.8 μm long (22.4 - 25.4 μm) and 19.2 μm wide (17.8 - 20.8 μm), 34.1 μm between stomata (23.4 - 50.6 μm).

Pistillate Scale: Three-nerved, prickle hairs never present on apex.

Perigynium: Epidermal cells rectangular (approximately twice as long as wide), 41.2 μm long (38.8 - 43.8 μm) and 20.9 μm wide (14.0 - 25.8 μm).

Achene: Abortive, no silica bodies present in epidermal cells, cells with 5 to 6 sides, 31.22 μm at widest point (26.49-39.88 μm), 543.83 μm^2 in area (390.94-812.75 μm^2).

(2.) ***Carex shortiana*** **Dewey**

Leaf: Inversely W-shaped; Abaxial epidermal cells rectangular (2.1 times longer than wide (1.7 - 2.4 μm)), 36.0 μm long (29.0 - 41.8 μm) and 17.2 μm wide (14.4 - 19.2 μm); Papillae abaxial, distinctive; Prickle hairs present, marginal; stomata abaxial, in rows parallel to long axis of leaf, 23.9 μm long (22.0 - 24.9 μm) and 19.2 μm wide (16.6 - 20.8 μm), 42.2 μm between stomata (24.7 - 72.9 μm).

Pistillate Scale: Three-nerved, prickle hairs never present on apex.

Perigynium: Epidermal cells rectangular (1.1 to 1.9 times longer than wide), 24.6 μm long (18.8 - 28.3 μm) and 17.3 μm wide (12.5 - 26.5 μm).

Achene: Viable, silica bodies present, 25.35 μm wide (18.68-32.88 μm) by 16.61 μm tall (15.06-18.93 μm); epidermal cells with 5 to 7 sides, 47.41 μm at widest point (35.23-55.34 μm), 931.05 μm^2 in area (645.91-1178.48 μm^2).

(3.) ***Carex typhina*** **Michx.**

Leaf: Inversely W-shaped; Abaxial epidermal cells rectangular (2.8 times longer than wide (2.5 - 3.1)), 56.0 μm long (44.0 - 60.5 μm) and 20.2 μm wide (17.6 - 21.8 μm); Papillae abaxial, small and indistinctive; Prickle hairs present, marginal;

Stomata abaxial, in rows parallel to long axis of leaf, 26.4 μm long (25.1 - 27.3 μm) and 19.5 μm wide (16.2 - 21.8 μm), 62.1 μm between stomata (48.2 - 73.1 μm).

Pistillate Scale: Three-nerved, prickle hairs present or absent on apex.

Perigynium: Epidermal cells squarish (slightly wider than long), 35.3 μm long (29.8 - 42.5 μm) and 42.3 μm wide (37.2 - 49.1 μm).

Achene: Viable, silica bodies present, 19.63 μm wide (13.55-23.75 μm) by 12.92 μm tall (10.63-15.72 μm); epidermal cells with 4 to 7 sides, 43.94 μm at widest point (29.80-64.20 μm), 1207.02 μm^2 in area (594.31-232.32 μm^2).

(4.) ***Carex squarrosa*** **L.**

Leaf: Inversely W-shaped; Abaxial epidermal cells rectangular (2.7 times longer than wide (2.5 - 3.0)), 48.9 μm long (38.8 - 54.8 μm) and 17.9 μm wide (14.5 - 20.6 μm); Papillae abaxial, small and indistinctive; Prickle hairs present, marginal; Stomata abaxial, in rows parallel to long axis of leaf, 25.5 μm long (24.7 - 26.4 μm) and 19.0 μm wide (17.7 - 20.7 μm), 40.5 μm between stomata (35.2 - 44.9 μm).

Pistillate Scale: Three-nerved, prickle hairs always present on apex.

Perigynium: Epidermal cells squarish (slightly wider than long), 38.7 μm long (36.3 - 40.3 μm) and 44.5 μm wide (40.6 - 47.6 μm).

Achene: Viable, silica bodies present, 21.77 μm wide (16.53-27.29 μm) by 13.57 μm tall (11.48-15.49 μm); epidermal cells with 6 to 7 sides, 55.30 μm at widest point (40.29-71.04 μm), 1730.13 μm^2 in area (990.20-2509.40 μm^2).

CHAPTER V.

SUMMARY AND CONCLUSIONS

One objective of this study was to assess the viability of *C.* x *deamii* and the three putative parent species. The hybrid, *C.* x *deamii*, and all probable parental species (*C. shortiana*, *C. typhina* and *C. squarrosa*) were analyzed to determine pollen viability. All three possible parental species had a high degree (90.8 % - 94.9 %) of pollen viability while the hybrid was found to be nearly sterile (2.3 % viable). Further, the hybrid did not produce fertile achenes. All achenes observed were noticeably abortive being smaller than normal and shriveled. It is clear that *C.* x *deamii* is of hybrid origin.

The primary objective of this study was to determine the correct parents of the hybrid, *C.* x *deamii*. Both macromorphological and micromorphological data were analyzed to achieve this goal.

All macromorphological analysis supports *C. shortiana* and *C. typhina* as the parental contributors of the hybrid, *C.* x *deamii*. Bivariate analysis repeatedly showed *C. shortiana* and *C. typhina* clustered with the hybrid while *C. squarrosa* was clearly more distant from the hybrid. The multivariate statistical analyses (PCA, CDA, Discriminant Analysis, and HYWIN Analysis) of macromorphological data further verified *C. shortiana* and *C. typhina* as the correct parents. Only PCA was unable to show *C. typhina* as the most likely

second parent species because not enough separation was achieved between *C. typhina* and *C. squarrosa*.

Using micromorphological data, *C. shortiana* and *C. typhina* were also found to be the most likely parents. This conclusion is based on the results of Discriminant Analysis which clearly showed *C. shortiana* and *C. typhina* as the closest species to *C.* x *deamii*. Further, HYWIN Analysis showed *C. shortiana* as the most likely parent species and *C. typhina* as the most likely second parent. PCA and CDA using micromorphological data were unable to determine if *C. typhina* or *C. squarrosa* was the more likely parent. The two taxa were quite similar in appearance and little separation was obtained between them.

Key Characters

Determination of key characters which could be used in identifying the four taxa was another objective of this study. Through the analysis of this data set, many key characters have been found which can help to determine the identity of these quite similar species. Among the most effective macromorphological characters in separating these taxa were perigynium and pistillate scale characters. Section Shortianae, having relatively small perigynia and pistillate scales, can easily be separated from Section Squarrosae having much larger perigynia and pistillate scales.

C. x *deamii* is very similar to *C. shortiana* for both macromorphological and micromorphological characters. It can, however, be differentiated from its maternal contributor for many features. For example, the perigynium beak of *C.*

x *deamii* (~0.91mm) is alway much longer than that of *C. shortiana* (~0.20mm). Micromorphologically the perigynium epidermal cells just below the neck are always much longer for the hybrid than they are for *C. shortiana*.

C. typhina and *C. squarrosa* are also very similar both macromorphologically and micromorphologically. These species can be differentiated easily if typical forms of each are viewed. However, if atypical specimens are obtained the differentiation of these two species becomes much more cloudy. Many macromorphological characters were found to be significantly different between these two taxa, but there ranges all overlap, at least at there extremes. Perhaps the best and easiest character for separating these two species is whether or not the style has a distinct bend (*C. typhina* doesn't have a bend, *C. squarrosa* does have a bend). Micromorphological data of these two species found no real significant differences for any characters.

A number of other questions arise as a result of this study. Could there possibly be two separate hybrids represented within the *C.* x *deamii* collections?

At first glance, it might appear that two separate hybrids could be involved in this study, one hybrid between *C. shortiana* and *C. typhina* and the other between *C. shortiana* and *C. squarrosa*. This would explain the absence of *C. typhina* at *C.* x *deamii* collection sites as well as the results of HYWIN which showed a few C. x deamii specimens to have *C. shortiana* and *C. squarrosa* as there most likely parental species. However, upon closer inspection, evidence is not available to support this claim. In macromorphological HYWIN analysis, C. x

deamii specimens 10 and 15 were found to have *C. shortiana* and *C. squarrosa* as their most likely parental species. Specimen 10 was collected by Deam in 1926 from Pike County, Indiana. *C. shortiana, C. typhina,* and *C. squarrosa* were all present at this site (Hermann 1938). The remaining four *C.* x *deamii* specimens collected at this site were all determined to have *C. shortiana* and *C. typhina* as their most likely parental species. Specimen 15 collected in Illinois by Shildneck and Hess shows similar findings. This specimen was determined by HYWIN to have *C. shortiana* and *C. squarrrosa* as its most likely parental species, yet the other hybrid specimens collected at this site suggested *C. shortiana* and *C. typhina* as the most likely parent species.

C. x *deamii* is only found at sites in four midwestern states (Missouri, Indiana, Illinois, and Kentucky). This raises another question: Why is the hybrid so limited in its range? The answer to this question is relatively simple and straightforward. The hybrid is most likely limited from occurring in other states because of the distribution of its parental species. *C. shortiana*'s range extends only slightly beyond where the hybrid is found. It might be expected, however, for the hybrid to occur in a few more spread areas (namely Ohio, West Virginia and Tennessee) at the periphery of *C. shortiana*'s range. The reason hybrids are not found in this area may be because of the limited abundance of *C. typhina. C. typhina* has a broad geographic range but is not abundant in many parts of its range. In fact, in West Virginia *C. typhina* is listed as a S1 species with five or fewer occurrences known in the state (West Virginia DNR 1995). There are,

however, additional potential sites of the hybrid, *C.* x *deamii* in West Virginia. Guyan Creek Oxbow off Rt. 2 at the border of Cabell and Mason Counties and Crab Creek Lagoon off Route 2 near the Robert C. Byrd Locks and Dam in Mason County are potential hybrid sites since *C. shortiana* and *C. typhina* are both found at these sites. *C. typhina*, however, is very limited at both sites reducing the possibility that a hybrid might occur there.

CHAPTER VI.

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APPENDIX 1

SPECIMENS USED FOR ASSESSMENT OF FERTILITY/STERILITY OF POLLEN

Species	Location	Habitat	Collector
Carex x *deamii* *PBM 250*	Illinois	NA	A. Reznicek
Carex shortiana *PBM 251*	Guyan Creek, Mason Co., WV	Margin of oxbow	P. Marcum
PBM 252	Crab Creek, Mason Co., WV	Edge of lagoon	P. Marcum
Carex typhina *PBM 253*	Guyan Creek, Mason Co., WV	Margin of oxbow	P. Marcum
PBM 254	Crab Creek, Mason Co., WV	Edge of lagoon	P. Marcum
Carex squarrosa *PBM 255*	Guyan Creek, Mason Co., WV	Margin of oxbow	P. Marcum
PBM 256	Crab Creek, Mason Co., WV	Edge of lagoon	P. Marcum
PBM 257	Morris Lane-Blue Run Rd., Lucasville, Scioto Co., OH	Along pond bank.	P. Marcum

APPENDIX 2.

SPECIMENS USED FOR MACROMORPHOLOGICAL ANALYSIS.

Carex x *deamii* F. J. Hermann

ILLINOIS. Macon County: 4 July 1979, *Shildneck C-11204* (SIU); 14 June 1979, *Shildneck C-11117* (SIU). Shelby County: 19 July 1982, *Shildneck C-13193 S-12248* (SIU); 19 July 1981, *Shildneck C-12931* (SIU); 14 June 1981, *Shildneck C-12897* (SIU). Unknown County: *Evans 3841* (SIU).

INDIANA. Pike County: 5 June 1934, *Hermann 6147* (MICH); 5 June 1934, *Hermann 6147* (MICH); 5 June 1934, *Hermann 6147* (MICH); 5 June 1934, *Hermann 6147* (MICH)5 June 1934, *Deam 55011* (IND); 5 June 1934????, *Deam 43090* (MICH).

MISSOURI. Adair County: 19 September 1955, *Steyermark 79705* (F). Howell County: 25 June 1955, *Steyermark 78724* (F). St. Louis County: 30 July 1887, *Letterman s.n.* (F) (annotated by Steyermark ????).

Carex shortiana Dewey.

KENTUCKY. Fayette County: 26 May 1894, Terrill s.n. (IND). Trigg County: 15 May 1976, *Athey 3371* (IND).

ILLINOIS. Jackson County: 19 May 1952, *Mohlenbrock 1683* (SIU). Macon County: 6 June 1972, *Shildneck C-3784* (SIU).

INDIANA. Morgan County: 22 May 1906, *Deam 822* (IND). Rush County: 1 June 1957, *Stares 1596* (IND).

MISSOURI. Greene County: 20 May 1889, *Weller s.n.* (MO) (Annotated by J.A. Steyermark 1954). Jackson County: 8 May 1895, *Mackenzie 560* (MO). Jefferson County: 25 May 1974, *Christ 20* (MO). Lincoln County: 27 May 1916, *Davis 1208* (MO). Monroe County: 5 June 1974, *Hudson 267* (MO). Ralls County: 9 June 1917, *Davis 2479* (MO). Reynolds County: 2 June 1992, *Straughn S100* (MO). St. Louis County: 30 May 1901, *Swonton s.n.* (MO); July 1884, *Kellogg s.n.* (MO); May 1833, *Engelmann 53* (MO).

OHIO. Athens County: 1 July 1961, *LeBlanc s.n.* (MUHW).

WEST VIRGINIA. Mason County: 3 June 1980, *Evans 3720* (MUHW).

UNKNOWN LOCATION. 6 May 1938, *Muller 10078* (MUHW); 26 May 1875, *Chickering s.n.* (US).

Carex typhina Michx.

KENTUCKY. Carlisle County: 18 July 1962, *O'Dell and Windler s.n.* (SIU). Hickman County: 30 June 1962, *O'Dell and Windler 731* (SIU).

ILLINOIS. Williamson County: 21 June 1992, *Basinger 2876* (SIU).

INDIANA. Bartholomew County: 5 June 1921, *Deam 34271* (IND). Cass County: 6 August 1938, *Ek s.n.* (IND). 9 June 1936, *Ek s.n.* (IND). Clark County: 18 June 1935, *Deam 56218* (IND). Daviess County: 3 July 1918, *Deam 25626* (IND). Dubois County: 3 June 1930, *Deam 48687* (IND). Jackson County: 7 June 1913, *Deam 13277* (IND). Pike County: 3 June 1930, *Deam 48721* (IND). Wabash County: 8 July 1932, *Deam 52241* (IND). Warrick County: 11 June 1918, *Deam 25294* (IND). Wells County: 23 July 1923, *Deam 39173* (IND).

MISSOURI. Adair County: 2 July 1933, *Palmer and Steyermark 41149* (MO). Barton County: 5 July 1952, *Palmer 54375* (SIU). Butler County: 11 June 1893, *Eggert s.n.* (MO). Cooper County: 25 July 1991, *Currier 91-023* (MO). Dunklin County: 22 May 1932, *Kellogg s.n.* (MO). Wayne County: 24 May 1994, *Brant 2796* (MO).

Carex squarrosa L.

KENTUCKY. Lyon County: 21 June 1991, *Latortue s.n.* (SIU).

ILLINOIS. Macon County: 16 July 1915, *Clokey 2456* (MO). Union County: 23 May 1992, *Basinger, Middleton and Schott B-2533* (SIU).

INDIANA. Knox County: 7 June 1912, *Deam 11058* (IND). Monroe County: 3 July 1929, *Haas 6352* (IND); 25 June 1982,

Johnson and Ewing 8299 (IND). Posey County: 25 May 1985, *McCrary and Yatskievych 83-62* (IND). Shelby County: 14 July 1912, *Deam 11667* (IND). Spencer County: 1 July 1915, *Deam 16681* (IND). Sullivan County: 6 June 1923, *Deam 38783* (IND). Vanderburgh County: 4 June 1941, *Zeiner s.n.* (IND).

MISSOURI. Iron County: 31 May 1974, *Christ s.n.* (MO). Monroe County: 18 June 1974, *Hudson 521* (MO). Randolph County: 28 May 1981, *Conrad and Dimit 9259* (MO). Stoddard County: 7 May 1981, *Christ 20* (MO); 17 May 1992, *Hudson s.n.* (MO). Vernon County: 20 May 1989, *Chang 482* (MO). Warren County: 26 May 1985, *Brant and Gereau 590* (MO).

WEST VIRGINIA. Mason County: 3 June 1980?????, *Evans 3734* (MUHW). Upshur County: 8 June 1972, *Rossbach 8728* (MUHW)

APPENDIX 3.

SPECIMENS USED FOR MICROMORPHOLOGICAL ANALYSIS.

Carex x *deamii* F. J. Hermann

ILLINOIS. Macon County: 4 July 1979, *P. Shildneck C-11204*(MICH). Shelby County: 19 July 1981, *P. Shildneck C-12931*(MICH); 19 July 1982, *P. Shildneck C-13193*(MICH).

INDIANA. Pike County: 5 June 1934, *F. J. Hermann 6147*(MICH).

Carex shortiana Dewey.

MISSOURI. Jefferson County: 24 May 1974, *A. Christ s.n.*(MO). Ralls County: 6 September 1917, *Rev. J. Davis 3479*(MO). Reynolds County: 2 June 1992, *S. E. Straughn S100*(MO)

WEST VIRGINIA. Mason County: 3 June 1980, *D. K. Evans 3720*(MUHW).

Carex typhina Michx.

MISSOURI. Cooper County: 25 July 1991, *M. Currier 91-023*(MO). Wayne County: 25 May 1994, *A. E. Brant 2796*(MO).

WEST VIRGINIA. Putnam County: 15 May 1980, *B. Brumfield 13*(MUHW). Wayne County: 11 June 1980, *D. K. Evans 3727*(MUHW).

Carex squarrosa L.

MISSOURI. Iron County: 31 May 1974, *A. Christ s.n.*(MO). Stoddard County: 7 May 1981, *A. Christ s.n.*(MO); 17 May 1992, *S. Hudson s.n.*(MO). Warren County: 26 May 1985, *A. E. Brant & R. E. Gereau 590*(MO).