

The Evolution of Chromosome Arrangements in *Carex* (Cyperaceae)

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Abstract Sedges (*Carex*: Cyperaceae) exhibit remarkable agmatoploid chromosome series between and within species. This chromosomal diversity is due in large part to the structure of the holocentric chromosomes: fragments that would not be heritable in organisms with monocentric chromosomes have the potential to produce viable gametes in organisms with holocentric chromosomes. The rapid rate of chromosome evolution in the genus and high species diversification rate in the order (Cyperales Hutch., sensu Dahlgren) together suggest that chromosome evolution may play an important role in the evolution of species diversity in *Carex*. Yet the other genera of the Cyperaceae and their sister group, the Juncaceae, do not show the degree of chromosomal variation found in *Carex*, despite the fact that diffuse centromeres are a synapomorphy for the entire clade. Moreover, fission and fusion apparently account for the majority of chromosome number changes in *Carex*, with relatively little duplication of whole chromosomes, whereas polyploidy is relatively important in the other sedge genera. In this paper, we review the cytologic and taxonomic literature on chromosome evolution in *Carex* and identify unanswered questions and directions for future research. In the end, an integration of biosystematic, cytogenetic, and genomic studies across the Cyperaceae will be needed to address the question of what role chromosome evolution plays in species diversification within *Carex* and the Cyperaceae as a whole.

Introduction

Chromosomes evolve more dynamically in sedges than in any other group of flowering plants. The genus *Carex* L. exhibits a particularly exceptional aneuploid series, ranging from $n=6$ to $n=66$ (Tanaka, 1949), with every number from $n=6$ to $n=47$ represented by at least one species (Roalson et al., 2007). Intraspecific variation in *Carex* is equally remarkable: although chromosome number was initially thought to be invariant within species (Heilborn, 1924; Löve et al., 1957), more than 100 *Carex* species are known to comprise numerous cytotypes, with some taxa spanning a range of ten haploid (n) chromosomes from highest to lowest count

(Wahl, 1940; Tanaka, 1948, 1949; Davies, 1956; Cayouette & Morisset, 1986a; Whitkus, 1991; Hoshino, 1992; Rothrock & Reznicek, 1998). Despite an enormous amount of chromosome counting in the Cyperaceae, there are substantial gaps in our knowledge of the pattern and process of chromosome evolution in the genus.

Chromosome evolution has been a driving force in speciation in diverse groups of organisms (Stebbins, 1950; White, 1978; Grant, 1981; Coyne & Orr, 2004; Ayala & Coluzzi, 2005). Given the high rate of diversification in the Cyperales Hutch. (sensu Dahlgren et al., 1985), which is composed predominantly of the sedges (Magallon & Sanderson, 2001), and the high diversity of *Carex* in particular (ca. 2,000 species worldwide; Reznicek, 1990), it is hard to avoid suspecting that chromosome evolution has played an important role in the evolution and diversification of the genus. In this article, we review the cytological and taxonomic literature to address four major issues regarding chromosome evolution in *Carex*: (1) chromosome structure, (2) mechanisms of chromosome evolution, (3) phylogenetic patterns of chromosome evolution, and (4) evidence for chromosomal speciation in the genus. Characterizing the pattern and process of chromosome evolution is a first step toward the ultimate goal of cytological studies in Cyperaceae: understanding how chromosome evolution and genome rearrangements affect recombination, gene expression, reproductive isolation and speciation.

Cytological Characteristics of the Cyperaceae

While not unique cytologically, the Cyperaceae exhibit three distinctive chromosome traits that are highly unusual among angiosperms: (1) production of a single pollen grain (pseudomonad) per pollen mother cell, (2) post-reductional meiosis, and (3) diffuse centromeres. Microsporogenesis in the Cyperaceae mirrors megasporogenesis in most angiosperms: three nuclei of each pollen mother cell (PMC) degenerate after failing to undergo DNA replication (Juel, 1900; Hoshino & Shimizu, 1986; Brown & Lemmon, 2000). With rare exceptions, each PMC consequently produces only one pollen grain in the Cyperaceae, whereas microsporogenesis in most angiosperms results in four pollen grains per PMC. The Cyperaceae-type of pollen development is known from only one other family, the dicot family Epacridaceae [=Ericaceae] (Brown & Lemmon, 2000). Heilborn (cited in Davies, 1956) held that the seeming lack of allopolyploidy in *Carex* might be attributable at least in part to this phenomenon, as the degeneration of all but one nucleus per PMC “prevents the formation of unreduced pollen dyads in any interspecific hybrid” (Davies, 1956: 351). However, it does not appear that the process of microsporogenesis in *Carex* would prevent meiotic nondisjunction (Hoshino & Shimizu, 1986), and consequently it is not clear how it could play a role in limiting polyploidization. Moreover, unreduced pollen dyads are known at least from one species in section *Phacocystis* Dumortier (Cayouette & Morisset, 1986b) and a putative intersectional hybrid (Luceño, 1994), where they result in gametes with twice the parental chromosome number.

Unlike most groups of organisms, the Cyperaceae undergo post-reductional meiosis. In pre-reductional meiosis, which is the more common type, homologous chromosomes segregate in the first round of meiosis, and sister chromatids

segregate in the second. In post-reductional meiosis, this order is reversed (Battaglia & Boyes, 1955). Post-reductional meiosis was first observed in *Carex* by Heilborn (1928) and demonstrated conclusively by Wahl (1940). Battaglia and Boyes (1955) regrettably did not discuss *Carex* at length in their seminal review on the topic, apparently because Tanaka (1937, 1939, 1940a, b, c, 1941a, b) had reported pre-reductional meiosis from the Cyperaceae. The order of meiosis has been confirmed, however, using molecular cytogenetic methods (Hoshino et al., 1999).

Invariably associated with post-reductional meiosis in angiosperms is the absence of localized centromeres (Battaglia & Boyes, 1955). Chromosomes in sedges and other organisms that undergo post-reductional meiosis are holocentric, meaning that centromeric activity is distributed along the entire chromosome (Håkansson, 1954). Holocentric chromosomes are also often described as polycentric or possessing a diffuse centromere. Greilhuber [1995: 397] has pointed out that the distinction between holocentry and polycentry is not particularly important, given that centromeric sequences must alternate with noncentromeric sequences. Heilborn (1928) noted that *Carex* chromosomes lack an obvious constriction, but it took more precise studies (e.g., Sharma & Bal, 1956) to demonstrate that this was due to lack of a localized centromere. Holocentry had previously been identified in the chromosomes of the Juncaceae (in *Luzula* DC.; De Castro, 1950; La Cour, 1952), the family sister to the Cyperaceae. In holocentric chromosomes, fragments that arise by breakages are retained during meiosis and inherited in Mendelian fashion (Faulkner, 1972; Luceño, 1993). Consequently, breakages may result in viable gametes with aneuploid numbers that can become stabilized through backcrossing or selfing. Holocentric chromosomes occur throughout the Cyperaceae and Juncaceae as well as in four other angiosperm genera (*Cuscuta* L. subgenus *Cuscuta* [Pazy & Plitmann, 1994; Guerra & García, 2004], *Drosera* L. [Sheikh et al., 1995], *Chionographis* Maxim. [Tanaka & Tanaka, 1977], and *Myristica fragrans* Houtt. [Flach, 1966]); nematodes, including *Caenorhabditis elegans* Maupas (Buchwitz et al., 1999); and insects of several orders, including Lepidoptera, Heteroptera, and Odonata (Perez et al., 2000; Nokkala et al., 2002; Wang & Porter, 2004). Chromosome structure appears to be uniform across the Cyperaceae, and in fact the diffuse centromere has been described as the “one obvious and convincing chromosomal higher-level synapomorphy in monocotyledons” (Greilhuber, 1995: 380). All reports of localized centromeres in the family appear to be based on misinterpretations of somatic chromosomes, which frequently show constrictions even if the chromosome is holocentric (Greilhuber, 1995: 403).

Faulkner (1972) noted that the biological implications of the distinctive traits of Cyperaceae pollen development and chromosome structure are not fully appreciated or understood. Thirty years later, this observation is still accurate. All three traits clearly play a role in the mechanism and tempo of chromosome evolution in the Cyperales, as we review in the remainder of this paper. It is unclear, however, why they do not lead to a dominance of agmatoploidy in the other genera in the family, which also exhibit the same characters. Chromosome structure also provides no obvious explanation of why chromosome arrangements are more variable in *Carex* than in the other sedge genera.

Mode of Chromosome Evolution in *Carex*: Agmatoploidy, Aneuploidy, Polyploidy

Whereas chromosome number change in most angiosperms proceeds by duplication of chromosomes, chromosome number changes in *Carex* are primarily by fission and fusion (Wahl, 1940; Davies, 1956; Hoshino, 1981). Malheiros-Gardé and Gardé (1950) coined the term *agmatoploidy* to describe chromosome number changes via fission in *Luzula* (Juncaceae). The term has subsequently been used to describe decreases due to fusion as well (Löve et al., 1957; Luceño, 1994). Agmatoploidy contrasts with strict or quantitative *aneuploidy*, which refers to chromosome number changes due to duplication of single chromosomes; *polyploidy*, which refers to duplications of whole sets of chromosomes; and *dysploidy*, which is a general term for chromosome number changes by any mechanism that involve only a subset of an organism's chromosomes (Löve et al., 1957).

Heilborn (1924: 210) held that although chromosome fission probably played a role in the origins of *Carex*, more recent chromosome number increases in the genus must be a consequence of chromosome duplications (also see Tanaka, 1949; Schmid, 1982). Tanaka (1949: 23) reported that in 44 plants from 21 different species, the pattern “ $(n-2) II+IIII+II$ ” is predominant, which he suggested was evidence of quantitative aneuploidy (either one duplication and one deletion or two duplications). However, this pattern provides no more evidence for aneuploidy than for agmatoploidy: it may be evidence of two fissions with desynapsis in one pair (Luceño, 1994). Moreover, genus-wide and even order-wide surveys (including all of Cyperaceae and Juncaceae) demonstrate that chromosome number is inversely related to total chromosome volume and nuclear DNA content (Tanaka, 1948; Hoshino, 1981; Nishikawa et al., 1984; Roalson et al., 2007). In contrast, there is an approximately flat relationship between ploidy level and nuclear DNA content (*C*-value) across most angiosperms (Leitch & Bennett, 2004). The frequency of univalents and heteromorphic trivalents at meiosis across a wide range of species in the genus also suggests that chromosome number changes are due to breakages, fusions, or translocations (Wahl, 1940; Hoshino, 1981; Hoshino, 1992; Hoshino et al., 1994; Hoshino & Okamura, 1994; Hoshino & Onimatsu, 1994; Hoshino & Waterway, 1994; for interpretations of homologies in multivalents of different types, see Faulkner, 1972).

Quantitative aneuploidy has been suggested as an important mode of evolution in the *Carex flava* L. complex (Schmid, 1982) based primarily on the fact that trivalents in the *C. flava* complex as studied in Switzerland are typically isomorphic. *Carex brevior* (Dewey) Mackenzie ex Lunell ($n=24, 26, 28, 30, 32, 34$) presents stronger evidence for quantitative aneuploidy. The presence of only even-numbered haploid counts and tetravalents in most individuals counted suggests that chromosome number change in that species may be associated with chromosome duplication rather than or in addition to agmatoploid changes (Rothrock & Reznicek, 1998).

Polyploidy has been demonstrated in a few species in the genus (Tanaka, 1949), though it plays a much more important role in chromosome evolution in the rest of the family (Luceño et al., 1998; Vanzela et al., 2000; Yano et al., 2004; Yano & Hoshino, 2005). The only seemingly clear-cut case of polyploid speciation with invariant chromosome numbers in *Carex* is in section *Chlorostachyae* Tuck. ex

Meinsh. [= *Capillares* (Asch. & Graebn.) Rouy] (Löve et al., 1957). However, this study, which suggests speciation associated with autopolyploid changes in chromosome number, is based on mitotic counts, which are notoriously difficult to interpret in *Carex* except in species with the lowest numbers. Moreover, sampling in the study appears not to be adequate to justify the claim of invariant chromosome numbers within the species studied.

Autopolyploidy is well documented in just three carices (Tanaka, 1949). The best-characterized of these is *C. siderosticta* Hance. In this species, approximately two-thirds of the chromosomes form tetravalent associations at meiosis, as expected when the genome has doubled in size through doubling of the entire chromosome complement (Tanaka, 1940b; Yan-Cheng & Qiu-Yun, 1989). Yet more chromosome races are found than just the basic diploids ($2n=12$) and tetraploids ($2n=24$), suggesting that agmatoploidy has also played a role in the evolution of this species (Yan-Cheng & Qiu-Yun, 1989). Another example of autopolyploidy can be found in *C. dolichostachya* Hayata subsp. *dolichostachya* [= *C. multifolia* Ohwi] ($2n=30, 60, 64, 65, 66$). This species has a putative tetraploid form, but in the tetraploid all chromosomes form bivalent associations (Tanaka, 1940c), a situation which is expected in allotetraploids rather than autotetraploids. Allotetraploid origin has not been proposed for this species.

As hypothesized by Heilborn (1924) and supported by most subsequent workers, allopolyploidy is rare if present at all in *Carex*. Only two taxa have been proposed to have allotetraploid origins. The first, *C. jackiana* Boott subsp. *parciflora* (Boott) Kük. [= *C. parciflora* Boott], was studied in detail by Tanaka (1949), who crossed two cytotypes of the species that both displayed regular meiosis ($n=19 \times n=22$). The hybrid offspring exhibited highly disrupted meiosis, with numerous univalents and trivalents, and one to three tetravalents in more than half of the plants. Approximately half of the two largest size classes of chromosomes formed what Tanaka termed a “primary pairing affinity” with another chromosome of the same size class, the other half forming associations that suggested structural rearrangements. Tanaka interpreted this as evidence of two distinct sets of chromosomes in the parental gametes and thus of allopolyploid origin for the parents. No ancestral species were proposed. The other putative allotetraploid is *C. roraimensis* Steyerm., a South American species whose chromosome count ($2n=124$) is one of the largest in the genus and roughly the sum of the mean counts of its putative ancestral sections. Molecular phylogenetic data support a hybrid origin for *C. roraimensis*, with the parent species arising from section *Ovales* Kunth (ITS data) and section *Stellulatae* Kunth (ETS data), though the sister species in both sections is inconclusive (Hipp et al., 2006). In numerous PMC counts from the single individual studied, chromosomes were consistently found in bivalent associations, which argues against autopolyploidy. Chromosome evolution thus appears to proceed by a variety of mechanisms in *Carex*, with agmatoploid changes playing a particularly important role.

Systematic Distribution of Chromosome Numbers

Following the seminal work of Heilborn (1924, 1928, 1932), several researchers have undertaken genus-wide studies of chromosome variation (notably Tanaka,

1937, 1939, 1940a, b, c; Wahl, 1940; Tanaka, 1941a, b, 1948, 1949; Davies, 1956; Hoshino, 1981; Löve, 1981), and numerous other researchers have counted chromosome numbers in one or a few sections or geographic areas (e.g., Faulkner, 1972; Whitkus, 1981; Löve, 1982; Cayouette & Morisset, 1986a; Nijalingappa & Bai, 1990; Luceño, 1993; Rothrock & Reznicek, 1996). To date, approximately 16 percent of described *Carex* species have been sampled (Roalson, 2008).

Heilborn observed that closely related carices frequently have similar chromosome numbers (Heilborn 1924: 167). Subsequent studies reveal phylogenetic autocorrelation in chromosome number frequency and size class distribution at and below the sectional level (Wahl, 1940; Tanaka, 1949; Löve et al., 1957; Hipp et al., 2007). Recent evidence in section *Ovales* suggests that phylogenetic patterning of chromosome numbers is a consequence of clade-specific equilibria rather than neutral phylogenetic autocorrelation (Hipp, 2007).

Heilborn also hypothesized that the direction of evolutionary change in the genus has been from lower to higher chromosome numbers (Heilborn, 1924: 188), and subsequent researchers (Davies, 1956; Hoshino, 1981; Schmid, 1982; Luceño & Castroviejo, 1993) have assumed this hypothesis to be true. Heilborn's hypothesis implies that ancestral character state reconstructions should recover lower numbers at the deepest nodes of a phylogeny. At least five studies have endeavored to investigate whether there is a directional trend in chromosome number evolution by mapping chromosome numbers onto explicit phylogenetic hypotheses. Four of these studies (Naczi, 1992; Roalson et al., 2001; Hipp, 2007; Hipp et al., 2007) recover intermediate or higher-end chromosome counts at the root of their respective trees, suggesting that chromosome number in the genus evolves by both increases and decreases. This supports Reznicek's (1990) argument that chromosome number decreases are not uncommon in *Carex*. Only two studies (Crins & Ball, 1988; Crins, 1990) find a linear sequence of chromosome numbers compatible with their phylogenetic hypotheses, with an increasing trend supported by assumed polarity of ecological and morphological characters. However, these latter two studies utilize chromosome number as partial evidence for rooting their phylogenetic trees. Luceño and Castroviejo (1991) also provide compelling evidence for fission-dominated intraspecific pattern of chromosome evolution within *C. laevigata* Sm. which, if true, might apply as well to the interspecific pattern within the section. More recent studies suggest that *C. gibba* Wahlenb., which has the lowest chromosome count known in subgenus *Vignea* ($2n=34, 36$) (Tanaka, 1949), is sister to the remainder of the subgenus (Ford et al., 2006). Moreover, *C. siderosticta* Hance ($n=6, 12$), which has the smallest number of chromosomes known in the genus *Carex* and has been hypothesized to represent an ancestral type within the genus (Löve et al., 1957), has recently been shown to occupy a basal position in the genus (Waterway et al., 2008). These findings suggest that chromosome numbers may trend upward in the evolution of *Carex*, but that this trend is probably a consequence of the fact that the ancestral chromosome count is near the lower bound (i.e., chromosome number is strongly constrained at the lower end (necessarily, $n \geq 1$), but not strongly constrained at the upper end).

Associated with hypotheses about chromosome number of the ancestor to the genus are hypotheses regarding base chromosome numbers. Heilborn (1924) noted chromosome number maxima at $2n=56, 84$, and 112, and from these he inferred the

base haploid chromosome number (X) of *Carex* to be 7. Based on a greater number of counts, Wahl (1940) showed that the optima were probably not multiples of 7, and he inferred a secondary base number of $X=5$. A more recent study finds maxima mostly at multiples of 6 (Roalson et al., 2007). The most detailed analysis (Löve et al., 1957) argues for a base number of $X=5$. Both Heilborn and Löve, however, believed that chromosome numbers were essentially invariant within species, and consequently they considered base numbers within the genus to be readily inferable from counts of individual species. Faulkner (1972), based on his studies in section *Phacocystis*, observed that intraspecific chromosomal variability likely obscures ancestral states. Given the uncertainty that intraspecific variation introduces into ancestral character state reconstructions, the concept of base chromosome numbers may be not be useful in *Carex*.

Intraspecific Chromosomal Variation

Despite Heilborn's (1924) view, intraspecific agmatoploidy or aneuploidy is widespread in *Carex* (e.g., Tanaka, 1940b, c; Wahl, 1940; Tanaka, 1948, 1949; Faulkner, 1972; Whitkus, 1981; Cayouette & Morisset, 1985, 1986a, b; Whitkus, 1988; Luceño & Castroviejo, 1991; Whitkus, 1991; Hoshino et al., 1994; Hoshino & Waterway, 1994; Rothrock & Reznicek, 1996, 1998, 2001; Naczi, 1999). Some species exhibit variation within populations (Luceño & Castroviejo, 1991) or even individual plants (Schmid, 1982; Luceño, 1994). Typically, the different euploid chromosome races within species all show regular meiosis and are indistinguishable from one another morphologically (Schmid, 1982; Cayouette & Morisset, 1986b; Whitkus, 1988; Rothrock & Reznicek, 1996, 1998), though some chromosome races with even-numbered diploid counts in *C. aquatilis* Wahlenb. exhibit disrupted meiosis (Cayouette & Morisset, 1986b). Some regional endemics (e.g., *Carex scoparia* Schkuhr ex Willdenow var. *tessellata* Fernald & Wiegand of Maine, U.S. A.; P. E. Rothrock unpublished) and even some widespread taxa (e.g., *C. macloviana* D'Urv.; Whitkus, 1991) are apparently invariant in chromosome number, despite counts of individuals from numerous populations. In the former cases, invariance is likely a consequence of recent origin from a single population, though this has not been studied using explicit estimates of species age. It is less clear why some widespread taxa may be invariant in chromosome number, if indeed they are. A survey of more distant populations for some of these taxa would be helpful in ascertaining whether the apparent invariance is due to limited sampling or, alternatively, whether there are constraints on chromosomal variation within some taxa in the genus.

A few studies demonstrate apparent correlations between geography and intraspecific chromosomal variation. *Carex laevigata*, for example, exhibits a stepwise chromosome-number relationship between adjacent populations, with chromosome numbers inversely proportional to latitude (Luceño & Castroviejo, 1991). A correlation between latitude and chromosome number has also been found in *C. jackiana* Boott subsp. *parciflora* (Boott) Kük. [= *C. parciflora* Boott] (Tanaka, 1949), while a strong negative correlation between latitude and chromosome number has been demonstrated in *C. oxyandra* (Franch. & Sav.) Kudô (Hoshino, 1992). The

apparent geography-cytotype correlation reported for *C. conica* Boott (Hoshino & Waterway, 1994) is less straightforward: variation in chromosome numbers and morphology in this species show similar patterns of geographic autocorrelation, suggesting that this might be a case of taxonomic divergence rather than biogeographic divergence of chromosome races within a single species. Correlations between geographic distance and chromosome number difference are most likely a stochastic effect of chromosome divergence associated with species range expansion, as there is no evidence for a correlation between environment and interspecific patterns of chromosome number variation in the genus (Haskell, 1952, cited in Davies, 1956). Most species show little or no relationship between chromosome number and geography (Cayouette & Morisset, 1986b; Whitkus, 1991; Luceño, 1994). Thus, while the incidence of polyploidy across angiosperms increases with latitude (Grant 1981: 313–315) there is not a general correlation between latitude and chromosome number in *Carex*.

Chromosomal Variation Associated with Hybridization and Speciation

Chromosome evolution plays an important role in plant speciation (Stebbins, 1950; White, 1978; Grant, 1981; Ayala & Coluzzi, 2005). The variation in chromosome number within *Carex* has been posited as an explanation for the taxonomic diversity of the genus at least since Heilborn, who regarded the mutations giving rise to new chromosome numbers and other chromosomal mutations to be “the most important processes in the evolution of new species” in the genus (Heilborn, 1924: 190). Heilborn was aware of the potential for chromosomal rearrangements to change linkage relationships within a species’ genome (1924: 189) and saw the potential “recombination of the characters” as a source of additional variation within species. This, in Heilborn’s view, suggested that increases in chromosome number would increase the potential for recombination among characters and consequently variation within species through decreased size of linkage groups. Faulkner (1972) also suggested that structural changes play a role in the divergence (or at least evolution) of populations through position effects and rearrangement of linkage groups, a view that was further articulated by Whitkus (1988) in his discussion of species diversification in the *C. pachystachya* Cham. ex Steud. complex of western North America.

Experimental research on the potential role of chromosome number change in species diversification has focused on meiotic chromosome pairing relationships within an individual and fitness of offspring resulting from crosses between individuals with different chromosome numbers (Tanaka, 1949; Faulkner, 1973; Cayouette & Morisset, 1985; Whitkus, 1988). Hybrids between species with widely divergent chromosome counts typically exhibit a greater proportion of univalents, trivalents and other irregular meiotic chromosome associations than hybrids between species with similar numbers of chromosomes (Faulkner, 1973; Schmid, 1982; Cayouette & Morisset, 1985; Whitkus, 1988; Cayouette and Catling 1992; Luceño, 1994). Moreover, while agamaploid individuals are typically fertile (Whitkus, 1988; Hoshino et al., 1993), pollen fertility correlates negatively with the proportion of irregular meiotic associations (Cayouette & Morisset, 1985; Whitkus, 1988).

These data suggest that chromosomal rearrangements may play a role in reproductive isolation between populations, and thus the “hybrid-dysfunction” model of chromosomal speciation (Ayala & Coluzzi, 2005) may apply in *Carex*.

Whitkus (1988) made detailed comparisons of intraspecific and interspecific crosses involving different chromosome races in the *C. pachystachya* complex of Western North America. He found that crosses between chromosome races within *C. pachystachya* suffered a decrease in fertility comparable to crosses between *C. pachystachya* and the related species *C. macloviana* and *C. preslii* Steud. Tanaka (1949) also investigated the effects of intraspecific crosses between chromosome races within *C. oxyandra*, *C. gibba*, and *C. jackiana* subsp. *parciflora*. He found that meiotic associations become increasingly irregular as the difference in chromosome counts between individuals crossed increases. He also found limited or negligible decreases in fertility of F1s in intraspecific crosses. These findings suggest that chromosome races within a species may be incipient or even cryptic species. However, they do not demonstrate whether chromosome number changes precede or follow from population divergence. This distinction is crucial to understanding the role of chromosome evolution in species diversification.

Conclusions and Directions for Future Research

Although many of the Cyperaceae have not yet been studied, all observations indicate that diffuse centromeres are a synapomorphy for the Cyperales (Greilhuber, 1995) and that agmatoploid chromosome evolution predominates in the genus *Carex*. However, we have little understanding of the mechanisms of chromosome evolution in sedges, and still less of the effects of chromosome evolution diversification of the genus. Coordinated research on fundamental questions regarding chromosome evolution in sedges will both illuminate diversification patterns in one of the most diverse angiosperm clades and do much to explain how chromosome evolution operates in other organisms with holocentric chromosomes, few of which have been studied. Three major research questions stand out:

Why does Carex display a wider range of chromosomal variation and a lower rate of polyploidy than the other members of the Cyperales? No obvious variation in chromosome structure accounts for the substantial karyotypic variability in *Carex* relative to other genera of the Cyperaceae and many other organisms with holocentric chromosomes (Dernberg, 2001). However, no one has studied centromere organization in sedges, and a detailed comparative study may be fruitful. A recent study in the Cyperales demonstrates that centromere-specific histone H3 is present at nearly even strengths along heterochromatic regions of the chromosomes in *Luzula nivea* (L.) DC. (Juncaceae, $2n=12$; Nagaki et al., 2005), mirroring findings in *C. elegans* (Maddox et al., 2004). Comparative mapping of centromeric regions in exemplars scattered across the Cyperales would allow us to address the question of whether *Carex* is more variable in centromere distribution and position. Because asymmetric distribution of centromeric activity in structural heterozygotes has the potential to select for chromosomal variants through meiotic drive (Pardo-Manuel de Villena & Sapienza, 2001), increased rate of centromere evolution may lead to higher rates of fixation of chromosomal variants. Moreover, as speciation genes are

associated with centromeres in other organisms (e.g., Turner et al., 2005), mapping centromere positions in *Carex* would provide a framework for evaluating the relationship between speciation and chromosome structure. Another question of interest is whether recombination and fusion hotspots (Luceño, 1994; Hey, 2004; Butlin, 2005) exist within sedges, and whether such hotspots are conserved across the family (Murphy et al., 2005). Recombination hotspots, which are also hotspots for double-strand breakages, have not been found in *C. elegans*, which also possesses holocentric chromosomes (Hey, 2004). Knowing whether recombination hotspots occur in any organisms with holocentric chromosomes and understanding the evolution of such hotspots may help explain differences in chromosome number variance. Until we investigate genome structure in the Cyperaceae, we are unlikely to understand why the *Carex* karyotype is so variable and what implications that variability may have for the evolution of the genus.

What are the macroevolutionary patterns and trends in chromosome evolution?

Phylogenetic comparative methods are suited to addressing hypotheses about directional trends in character evolution (Pagel, 1997, 1999; Lutzoni et al., 2001; Huelsenbeck et al., 2003). However, when intraspecific variance in chromosome number exceeds interspecific variance, as is the case in many sedge clades, character coding for traditional phylogenetic comparative approaches is problematic (Hipp et al., 2007), and methods that do not account for intraspecific variance are error-prone (Harmon and Losos, 2005). The sparse taxon sampling to date in sedges also limits our ability to estimate the large numbers of parameters associated with complicated models of chromosome number evolution (Burnham & Anderson, 2002). Recent work (Hipp, 2007) demonstrates the suitability of Martins and Hansen's (1997) generalized linear model for testing hypotheses regarding chromosome number evolution in the genus. With more complete sampling within species (to estimate intraspecific variance more accurately) and across the genus, employment of such methods should make it possible not just to test whether there is a bias toward fission or fusion in the evolution of the *Carex* karyotype—this is the question that has driven most chromosome number research in *Carex*—but also to understand the role of selection in the evolution of chromosome arrangements.

What role has chromosome evolution played in species diversification? Numerous studies of natural and artificial hybrids demonstrate that chromosome number change often accompanies population divergence and speciation in *Carex*. However, crossing studies alone do not reveal whether chromosome evolution proceeds from or drives speciation. Moreover, the relative rates of fission, fusion, translocation, inversion and duplication in the evolution of chromosome number in *Carex* are unknown. It is consequently difficult to estimate the role that chromosomal rearrangements may play in suppressing recombination and thus potentially driving chromosomal speciation even in the face of ongoing gene flow between populations (Rieseberg, 2001; Butlin, 2005). Comparative linkage mapping and fluorescence in-situ hybridization studies could provide the data needed to address this question by revealing the nature of chromosome rearrangements associated with chromosome number change and the effects of rearrangements on recombination rates in hybrids. Such work would be of broad interest for biologists working on organisms with holocentric chromosomes, as relatively few genomic studies have been undertaken in organisms with holocentric chromosomes (Albertson, 1993; Wang & Porter, 2004).

Teasing apart the roles of ecological divergence and chromosomal divergence in the diversification of sedges will demand a synthetic approach that combines molecular cytogenetics with crossing studies, linkage mapping and QTL analyses (e.g., Lexer et al., 2003). In the end, it will be the integration of biosystematic, cytogenetic and genomic studies across the Cyperaceae that allows us to address the question of what role chromosome evolution plays in species diversification within *Carex* and the Cyperaceae as a whole.

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