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CHROMOSOME EVOLUTION IN CYPERALES

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ABSTRACT

Karyotypic evolution is a prominent feature in the diversification of many plants and animals, yet the role that chromosomal changes play in the process of diversification is still debated. At the diploid level, chromosome fission and/or fusion are necessary components of chromosomal structural change associated with diversification. Yet the genomic features required for these events remain unknown. Here we present an overview of what is known about genomic structure in Cyperales, with particular focus on the current level of understanding of chromosome number and genome size and their impact in a phylogenetic context. We outline ongoing projects exploring genomic structure in the order using modern genomics techniques coupled with traditional data sets. Additionally, we explore the questions to which this approach might be best applied, and in particular, detail a project exploring the nature of genomic structural change at the diploid level in genus *Carex,* a group in which chromosome fission/fusion events are common and associated with diversification of many of its 2000 species. A hypothesized mechanism for chromosome number change in this genus is agmatoploidy, denoting changes in chromosome number without change in DNA amount through fission/fusion of holocentric chromosomes (chromosomes without localized centromeres). This project includes the creation of bacterial artificial chromosome (BAC) and expressed sequence tagged (EST) libraries to be used in physical and genetic linkage mapping studies in order to reveal the patterns of genome structural variation associated with agmatoploidy in *Carex,* and to explore the sequence and genic characteristics of chromosomal break points in the genome.

Key words: agmatoploidy, chromosome evolution, chromosome numbers, Cyperaceae, Cyperales, genome size, genomics, Juncaceae, phylogenetic relationships.

INTRODUCTION

A prominent feature in plant and animal diversification at the diploid level is karyotypic evolution (White 1978; Grant 1981; Baker and Bickham 1986; King 1993; Rieseberg 2001; Levin 2003). For example, Robertsonian rearrangements are common in a number of mammalian taxa, such as bats (Baker et al. 1985) and mice (Capanna et al. 1977; Nachman and Searle 1995). Inversion polymorphisms are common in *Drosophila* (Dobzhansky 1970), and many plant groups contain an array of karyotypic polymorphisms, both within and among species (for overviews see Stebbins 1950; Grant 1981; Levin 2003).

Although a common correlate in the diversity of many lineages, the role that chromosomal changes play in evolutionary diversification—ranging from race formation through speciation—remains debated. The classic view holds that chromosome structural changes are directly responsible for reproductive isolation and thus the primary agent for speciation. This forms the basis of the stasipatric (White 1968) and saltational speciation (Lewis 1966) models.

More recently, a different view has emerged that posits chromosome structural changes are not directly responsible for reproductive isolation (Coyne and Orr 1998; Noor et al. 2001; Rieseberg 2001). Instead of providing the basis for isolation, karyotypic changes reduce gene flow to allow adaptive differences or genic incompatibilities to become fixed within populations or among neighboring populations (Noor et al. 2001; Rieseberg 2001; Navarro and Barton 2003). This emergent view is based on two general observations. (1) A number of homosequential species/subspecies are reproductively isolated at a genetic level. Empirical evidence comes from the genetics of sexual isolation among races of *D. melanogaster* (Ting et al. 2001), and sterility alleles in a number of domesticated plants such as rice (Li et al. 1997) and tomato (Rick 1971). (2) Populations or species that differ in chromosomal arrangement can show little reduction in interfertility. Examples include paracentric inversions in races of *D. melanogaster* (Coyne et al. 1993) and Robertsonian fusions and pericentric inversions in races of *Sceloporus grammicus* Wiegmann (Reed et al. 1995).

Both views of the role of chromosomal changes in diversification have robust support from empirical studies. Given the wide range of taxa investigated, the variance in our understanding may be a simple reflection of diversity in biological processes. If not, progress in resolving these different views will require studies into the nature of chromosomal changes and their effect on population structure and divergence (Nachman and Searle 1995; Rieseberg 2001).

Numerous types of chromosomal changes contribute to karyotypic evolution at the diploid level. Regardless of the type of rearrangement (aneuploidy, inversions, reciprocal translocations, Robertsonian rearrangements, segmental duplications, or transpositions), a common feature is the fission/fusion of chromosome arms. These changes highlight how two common features of chromosomes, centromeres and telomeres, are malleable. With respect to telomeres, telo-

meric-like sequences have been identified in interstitial regions of vertebrate (Meyne et al. 1990) and plant (The Arabidopsis Genome Initiative 2000) chromosomes. However, probe experiments produce clear telomere signal only at the ends of chromosomes, including those that have undergone rearrangement (Schubert et al. 1992; Wang et al. 1992; Werner et al. 1992; Cox et al. 1993). These observations suggest that telomeric sequences ''degenerate'' when placed in interstitial regions and, thus are generated on new ends of chromosomes. Centromere structure and function varies widely among organisms (Karpen and Allshire 1997; Sullivan et al. 2001). Additionally, centromeres may become deactivated (Page et al. 1995) or arise as neocentromeres (Brown and Tyler-Smith 1995; Warburton et al. 2000). These data have contributed to the view that centromere function (i.e., the locus for kinetochore formation) is primarily determined by epigenetics rather than by centromere sequence (Karpen and Allshire 1997). These observations on the dynamic nature of chromosome components suggest that understanding how karyotypic evolution plays a role in diversification/speciation will require a genomics approach.

The tools of genomics research have expanded our insight into the nature of changes associated with the origin and diversification of taxa, with results ranging from the gene to the whole genome. These insights include the number and location of genes involved in the differences between species (Bernacchi and Tanksley 1997; Bradshaw et al. 1998; Westerbergh and Doebley 2002), the number and interactions of genes involved in reproductive isolation (Rieseberg et al. 1996; Wu and Hollocher 1998), and synteny between diverse genera that reveals a surprising amount of structural conservation (Tanksley et al. 1988, 1992; Paterson et al. 1996; Gale and Devos 1998; Ku et al. 2000). Extending this research to chromosomal structural changes represents one aspect of genomics that can be considered in its infancy (Nadeau and Snakoff 1998; Paterson et al. 2000; Song et al. 2001), but is providing tantalizing insights into the link between genome structural evolution and genome function (The Rice Chromosome 10 Sequencing Consortium 2003; Thomas et al. 2003). Applying a genomics approach to chromosome changes will provide an avenue to test new models for reproductive isolation arising from genic factors associated with karyotypic alterations that Noor et al. (2001) and Rieseberg (2001) have formulated. Results from such exploration would contribute toward understanding a mechanistic view of how diversification and speciation are initiated at the genomic level.

Application of a genomics approach to the above stated goal requires an appropriate study system. Our criteria included which fissions/fusions occur on a regular basis. To insure that comparisons are made with taxa that are recently diverged or in the process of divergence, polymorphisms should be available between sister species or within species. An ideal system to study this process—both at a larger scale and at the finer level of chromosome fission/fusion—is provided by the genus *Carex* in Cyperaceae. The genus has the most extensively developed aneuploid series of any angiosperm genus and is one of the largest genera in the world, with approximately 2000 species (Reznicek 1990). Chromosome numbers range from $n = 6$ to 68 (Davies 1956; Nishikawa et al. 1984), with nearly every haploid number between the two extremes present in the genus (Davies 1956). The origin of the extensive aneuploid series in *Carex* is attributed to the presence of a specialized condition: holocentric chromosomes.

Holocentric chromosomes are defined as chromosomes that contain diffuse or non-localized centromeres. They occur in a limited, but disparate array of life's diversity, with examples from Arthropoda (Hughes-Schrader 1948; Brown et al. 1992), algae (Godward 1954; King 1960), and angiosperms. Within the flowering plants, holocentric chromosomes are found in Cyperaceae and Juncaceae (Malheiros and de Castro 1947; Håkansson 1958), *Cuscuta* L. subgen. *Cuscuta* (Cuscutaceae; Pazy and Plitmann 1991, 1994), *Chionographis* Maxim. (Melanthiaceae; Tanaka and Tanaka 1977, 1980), and *Myristica fragrans* Houtt. (Myristicaceae; Flach 1966). The diffuse centromere condition is associated by far with the greatest species diversity in Cyperaceae and Juncaceae, the main families of the order Cyperales, with many of the approximately 4780 species (Mabberley 1997) possessing this condition (Greilhuber 1995). The presence of holocentric chromosomes has been proposed as the means by which members of the Cyperales (especially *Carex*), have been able to survive and thrive despite extreme levels of aneuploidy (Davies 1956; Grant 1981).

The presence of holocentric chromosomes is not associated with extreme aneuploid change in other groups of organisms. Only Cyperales combine species diversification, holocentric chromosomes, and aneuploid chromosome change. The pattern of change is called agmatoploidy (Malherios-Garde and Garde 1950; Grant 1981) and although the process underlying this change is not clear, several lines of evidence have provided insight. Nuclear irradiation studies (de Castro et al. 1949; LaCour 1953; Håkansson 1954; Davies 1956) indicate that chromosome fragments maintain centromeric activity and are not lost during cell division (Lima-de-Faria 1949). Furthermore, hybrids between plants with different numbers of chromosomes reveal one large chromosome pairing with two small chromosomes (Wahl 1940; Tanaka 1949; Håkansson 1954; Davies 1955; Faulkner 1972; Schmid 1982; Cayouette and Morisset 1985, 1986; Hoshino et al. 1993, 1994; Hoshino and Okamura 1994). Complicating the picture in hybrids from natural populations is the presence of various univalent and multivalent formations suggesting chromosomal structural rearrangements are involved (Tanaka 1949; Faulkner 1973; Schmid 1982; Cayouette and Morissett 1986; Hoshino and Waterway 1994).

Additional information is provided by C-value measurements among chromosome races in *Carex* (Nishikawa et al. 1984) showing that increasing chromosome numbers are not associated with an increase in DNA amounts. Finally, electron microscopy of kinetochore binding sites (Braselton 1971) in *Luzula* and *Cyperus* shows mitotic spindle microtubule attachment to be localized on multiple regions on individual chromosomes. Although the agmatoploid chromosome evolution hypothesis has been used to explain chromosome variation in Cyperales for more than 45 years, it has yet to be explored with modern genomic tools such as mapping studies and sequence analysis.

QUESTIONS

Carex is the largest and most diverse group of organisms exhibiting holocentric chromosomes and agmatoploid evo-

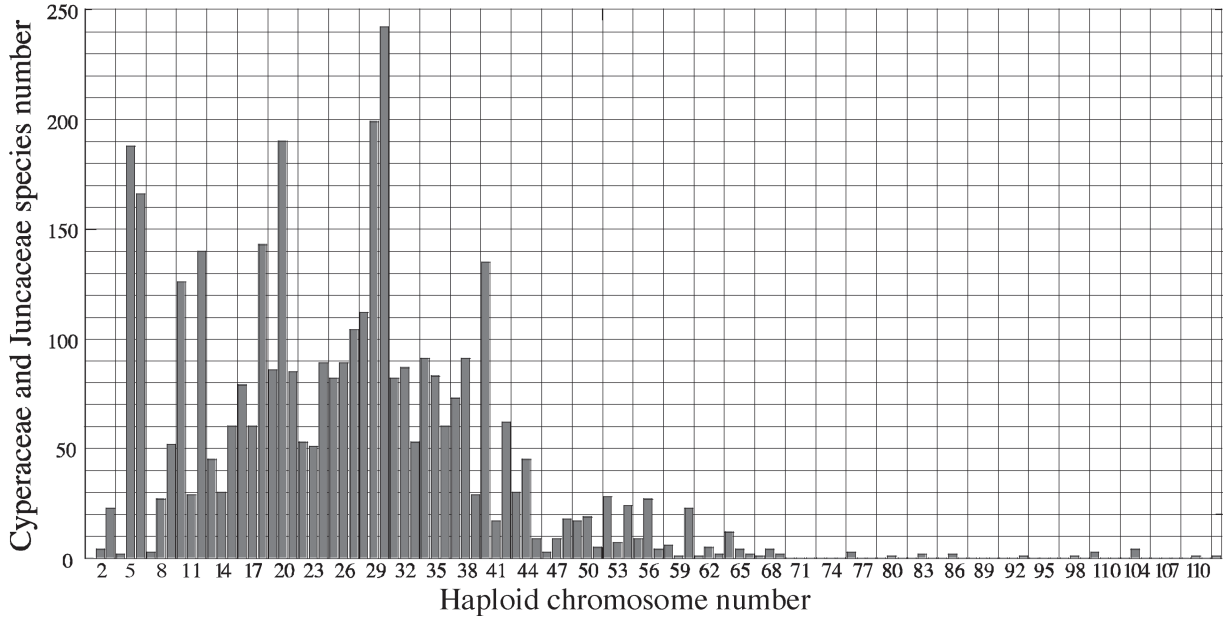

Fig. 1.—Cyperales haploid chromosome number distribution.

lution (Reznicek 1990) with polymorphisms between closely related species, as well as within species (Faulkner 1972; Schmid 1982; Whitkus 1991; Hoshino and Okamura 1994; Hoshino and Onimatsu 1994). Monoecy in the genus allows for artificial crosses to be made with relative ease, and plants are easily cloned. These characteristics make *Carex* a good system to address several questions related to chromosome change and diversification. In particular,

- In *Carex,* the single largest genus of organisms with holocentric chromosomes, what are the patterns of genome structural variation associated with agmatoploidy among chromosome races within and among closely related species?
- What are the sequence characteristics of chromosomal breakage points in the genome? Are there particular sequence motifs where chromosomal breakage always occurs? Associated with this is the question of capping of sequences after chromosome fission. Where fission events have occurred, are these breakpoint sequence regions telomere-like?
- Are there discernable genic changes that map to fission/fusion events? If so, are they related to adaptive difference, assortative mating, or incompatibilities as suggested by Noor et al. (2001) and Rieseberg (2001)?

Additional questions in basic and applied plant genome biology can be addressed with genomics studies in Cyperales including:

- What is the structure of the centromeric and telomeric repeats in Cyperaceae and Juncaceae? How are centromeric elements distributed on holocentric chromosomes in Cyperales? If the hypotheses of holocentric and agmatoploid chromosome number variation are accurate, what is the fate of telomere sequences during chromosome fusion events and how are the chromosome ends capped during chromosome fission events?
- Efforts are underway to characterize centromeric regions of plant chromosomes, including two research projects currently funded by the National Science Foundation's (NSF) Plant Genome Research Program (Copenhaver et al. 1998; NSF Awards 9872481, 9975827). The study of holocentric chromosomes enabled by the construction of the bacterial artificial chromosome (BAC) libraries proposed here will be a valuable resource in furthering the study of chromosome structure/function and inheritance. Such studies have considerable application to agriculture, bringing us closer to constructing plant artificial chromosomes.
- The creation of genomic resources in Cyperales provides an opportunity to make deep node comparisons between Gramineae in Poales and Cyperales. Cyperales and Poales have elevated diversification rates and are likely sister groups, suggesting the mechanism of diversification in the two clades may be a shared characteristic (Magallón and Sanderson 2001). How the differences in genome structure between these groups influence this hypothesis could be explored. Of particular interest are the following: Is the same pattern of co-linear genomes found in grasses (Gale and Devos 1998) also found in Cyperaceae and Juncaceae? What are the structural similarities and differences in gene family organization between the two clades? Finally, what are the similarities and differences in DNA repeat structure (e.g., centromeric repeats) between the two clades?
- *Cyperus rotundus* (L.) Benth. is known as the world's worst weed (Holm et al. 1977). A genome library for this taxon would be effective as a tool to explore the biological basis of the weedy habit and methods of biological control.
- Both *Eleocharis dulcis* Trinius ex Hensch. (Chinese water chestnut) and *C. rotundus* bear starch storage structures (González-Elizondo and Peterson 1997; Umerie and Ezeuzo 2000). *Eleocharis dulcis* is currently used as a human food source and it has been suggested that *C.*

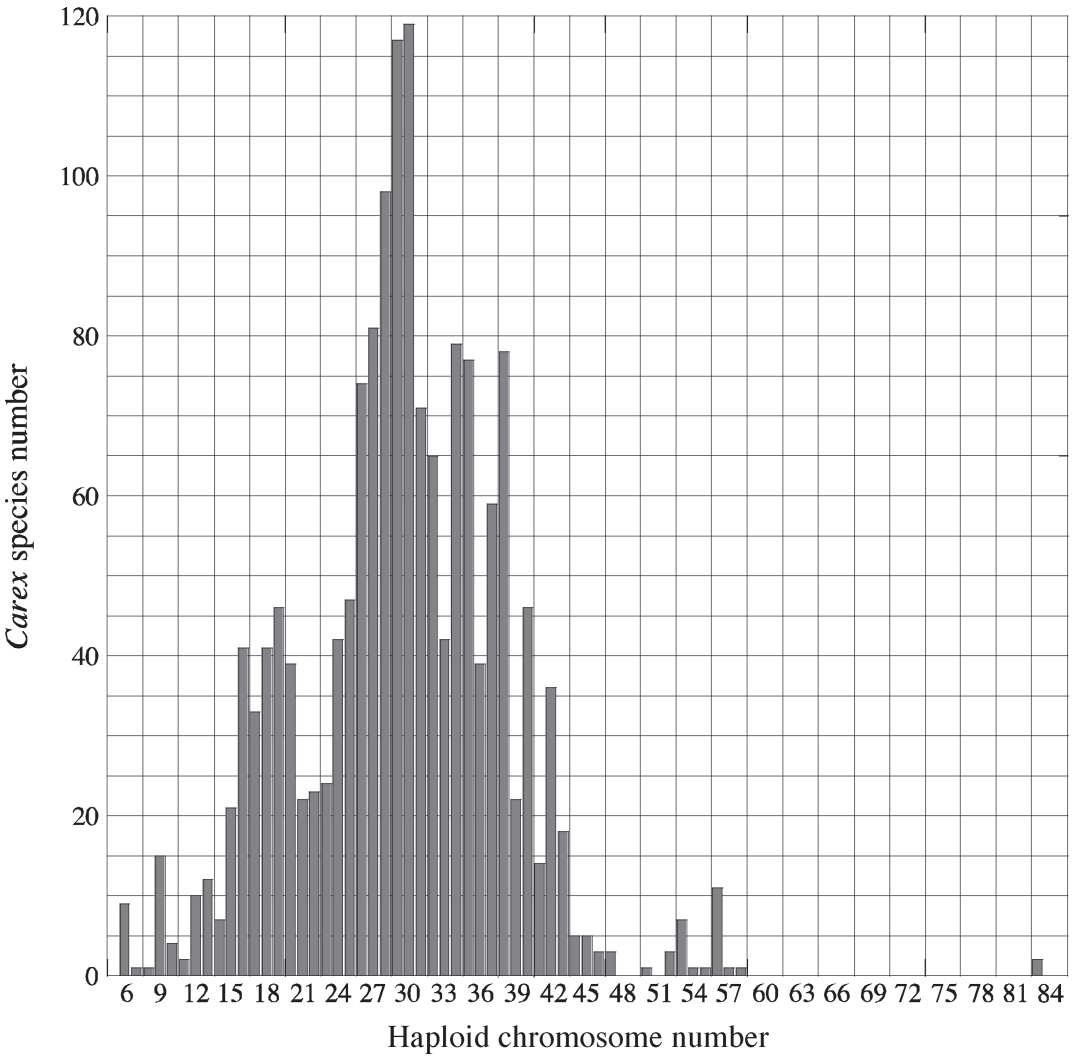

Fig. 2.—*Carex* haploid chromosome number distribution.

rotundus can be used as a starch source for industry and as a food source (Umerie and Ezeuzo 2000). Genomic resources for these species would be useful to explore the evolution of starch storage structures, the genetics of starch reservoir formation, and molecular breeding for increasing starch yield in cultivated varieties of these species.

- Members of Cyperaceae and Juncaceae have been used to clean waste water and to remove toxic compounds from soils and water sources (Chandra et al. 1997; de Souza et al. 1999; Pilon-Smits et al. 1999; Siciliano et al. 2001). Genome libraries could be very helpful in studying the biological pathways whereby wastes and toxic compounds are taken up and stored.

Here we provide background information to begin genome structure interpretation in Cyperales, including an overview of what is known regarding chromosome number, genome size, and phylogenetic relationships, and we outline what needs to be done to address the unresolved issues of genome evolution and mechanisms of diversification in the order.

CHROMOSOME NUMBERS AND GENOME SIZE

Chromosome numbers have been a useful systematic tool for over 100 years. This is true in Cyperales, as with other plant groups, with the first chromosome count in the order published by Juel in 1900, later followed by Heilborn (1918, 1922, 1924, 1928, 1932, 1934, 1936, 1937, 1939), Tanaka (1937*a, b*, 1938, 1939*a, b, c, d*), Wahl (1940), and Davies (1956). To date, more than 4200 chromosome counts have been published in Cyperales (Roalson unpubl. compiled data). Many of these chromosome counts are duplicates of species previously counted, leaving approximately 1500 Cyperales species (ca. 1/3 of the total number) with published chromosome counts. These data provide an opportunity to explore patterns of variation and can be used as a general tool for testing hypotheses of mechanisms of chromosome number change.

Figure 1 presents the distribution of haploid chromosome numbers in Cyperales. The distribution roughly follows a normal distribution, with two deviations from this general pattern. First, the distribution tails off with a reasonably large number of counts in the $n = 50$ to 100+ range, outside

Table 1. Cyperales genome size ranges. For reference, the genome of *Arabidopsis* is approximately 172 megabasepairs (Mbp). Under the Mbp range column there are two numbers listed for Mpb average for some genera. The first number includes all samples of that genus while the second number (in brackets, the number of samples excluded following the average) excludes large outliers. For instance, when the average genome size of *Eleocharis* is considered, inclusion of all samples suggests that the average genome size is 770 Mbp. One sample has a genome size measurement of 5415 Mbp, which is more than 10 times the size of the nearest other measurement. When this species is excluded, the average is considerably smaller at 306 Mbp.

Genus	Mbp range (Mbp average)	Number of counts (number of counts not previously published)
Carex L.	115–1152 (304 [290, −1])	63 (27)
Cladium P. Browne	281	1 (1)
Cyperus L.	146–1348 (572 [443, −1])	7 (2)
Dulichium Pers.	123	1 (1)
Eleocharis R. Br.	245–5415 (770 [306, −1])	11 (9)
Eriophorum L.	368–637 (503)	2
Fuirena Rottb.	149	1 (1)
Juncus L.	221–1789 (626 [534, −1])	8 (3)
Luzula DC.	270–4190 (1224 [995, −1])	14
Rhynchospora Vahl	108–287 (174)	3 (3)
Rostkovia Desv.	441	1
Schoenoplectus (Rchb.) Palla	282	1 (1)
Scirpus L.	215–490 (346)	6 (3)
Scleria P. J. Bergius	284	1 (1)
Uncinia Pers.	1323	1
Total	108–5415 (498)	121 (52)

of the described normal distribution (Fig. 1). Additionally, there are definite peaks associated with the haploid numbers of 5, 6, 10, 12, 18, 20, 30, and 40, suggesting that polyploidization of genomes based on haploid complements of 5, 6, and/or 10 might underlie the more obvious aneuploid pattern. This, at a very coarse level, suggests that multiple types of genomic reorganization, namely agmatoploidy and polyploidy, may be equally involved in the patterning of genome structure in Cyperales.

When chromosome distribution patterns are explored at a finer scale, such as within the genus *Carex* (Fig. 2), we see some similarities and differences to the broader pattern. First, the normal distribution pattern is still evident in the distribution of *Carex* chromosome numbers, but there are fewer very large chromosome numbers, and the inferred polyploid peaks seen in Fig. 1 are not obvious within *Carex.* The differences in patterns seen at the two scales suggest that in different lineages of Cyperales, different mechanisms of chromosome number change might be operating.

Measurement of genome size is a relatively new technique used to estimate the overall size of a genome by means of a variety of techniques from light microscope densitometry measurements (Nishikawa et al. 1984) to fluorescent stains in flow cytometry (Galbraith et al. 1983). The 69 published genome size measurements for Cyperales (Bennett and Leitch 2001) fall far short of the number of chromosome counts available for this order. Ongoing studies are expanding these numbers, including recent flow cytometric measurement of 30 additional species in 11 genera, five of which have not been previously measured (*Cladium, Dulichium, Fuirena, Rhynchospora,* and *Scleria*; Roalson et al. unpubl. data). The current patterns seen in the distributions of genome size suggest that many species of Cyperales have very small genomes (Table 1), although there are a few species with genomes considerably larger than those of the majority of species measured to date.

Table 2. Chromosome number/genome size pairwise comparisons.

Species	2*n*	Mpb
a. *Carex ciliato-marginata* Nakai	12	564
C. siderosticta Hance	24	1152
b. *Carex brownii* Tuckerm.	72	221
C. kobomugi Ohwi	88	221
c. *Luzula elegans* Lowe	6	1446
L. campestris (L.) DC.	12	1568
d. *Luzula elegans* Lowe	6	1446
L. forsteri (Sm.) DC.	24	686

While chromosome numbers or genome sizes can individually provide one view of genome structure, considerably more is seen if we study genome sizes and chromosome numbers together. There are currently 53 species for which both chromosome number and genome size have been measured. Here we will briefly explore two different ways of examining these data: (1) graphical mapping of genome size vs. chromosome number; and (2) tabular pairwise comparisons that might support different mechanisms of genome evolution.

At a very basic level, graphing genome size against chromosome number can test predictions of mechanisms of genome change at a broad scale. If agmatoploidy is the primary mechanism of genome repatterning, we might expect that the scatter of points in a 2D graph (y axis = genome size, x axis = chromosome number) would be equal to or approach a slope of 0. This result would suggest that chromosome number changes without concurrent change in genome size are taking place. Alternatively, if polyploidy or some type of quantitative aneuploidy is occurring, we would expect the points to form or scatter around a positively sloped line. Figure 3 represents genome sizes and chromosome numbers plotted in species for which both measurements have been made. As is easily apparent, the regression line found in this plot does not follow the predictions made for either agmatoploidy or polyploidy. The data available actually suggest that as chromosome numbers increase, genome sizes get smaller. This is a similar result to that presented in an earlier study of 26 Japanese species of *Carex* by Nishikawa et al. (1984). The primary difference between the previous results and ours is that the two ''islands'' of genome size found by Nishikawa et al. (1984) are not present with the increased sampling used in this study (Fig. 3).

While it is not immediately obvious, the pattern seen here can be reconciled with the agmatoploid chromosome number change expectation by invoking one additional mechanism. It might be expected that as fission events occur, there could be some loss of breakpoint end DNA before new telomeres

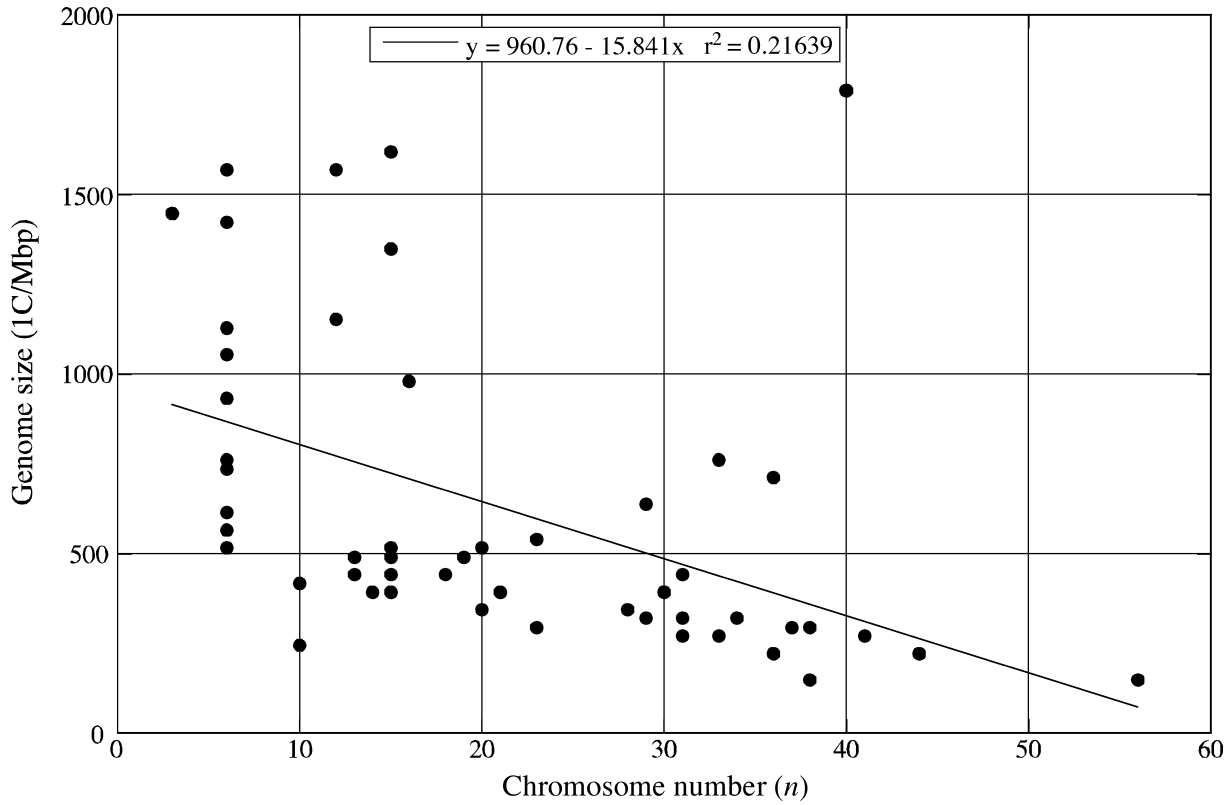

Fig. 3.—Genome size plotted against chromosome number in the Cyperales where genome size and chromosome number are both known for a species. A simple linear r^2 regression is plotted through the data points. Two extremely large genome sizes were excluded.

cap off the new chromosome ends. If this does occur, we might expect that, as the genome becomes more fragmented (continually increasing the number of chromosomes), genome sizes might continually decrease as more breakpoint locations occur, each potentially losing some DNA. These are hypotheses that need to be tested, both by gathering a larger data set of chromosome numbers and genome sizes, and by exploring the genomic organization of chromosomes in regions where breakages (fission events) have occurred.

In making pairwise comparisons within genera or among closely related species, we would expect there to be particular profiles associated with agmatoploid vs. polyploid mechanisms. For instance, if polyploidy is occurring, we would expect to find pairwise comparisons where one species has half of the chromosomes of the second and additionally has half of the genome size as the second. It is important to be aware that if polyploid or aneuploid events are ancient, these patterns would likely be more difficult to detect, as we expect that other genomic events (DNA gains, losses, rearrangements, etc.) will obscure the pattern. Nonetheless, these comparisons allow us to directly test expected patterns of genome structural change. Table 2 represents four pairwise comparisons of genome size and chromosome number that have potential implications for mechanisms of chromosome evolution. The first pairwise comparison (Table 2a) follows the pattern we would expect to see if polyploidy is occurring—*Carex siderosticta* has twice as many chromosomes and a genome that is twice the size of *C. ciliatomarginata.* Alternatively, the second comparison (Table 2b) follows the expectations of agmatoploid chromosome number change, in which *Carex brownii* and *C. kobomugi* both have genome sizes of approximately 221 megabasepairs (Mbp), but differ in their chromosome complement of $2n = 72$ and $2n = 88$, respectively.

The third comparison (Table 2c), *Luzula elegans* vs. *L. campestris,* closely reflects agmatoploid change (fission/fusion) of all chromosomes at the same time with little change in genome size (complete agmatoploidy or complete symploidy sensu Luceño and Guerra [1996]; Table 2). This has been previously described in *Luzula,* particularly associated with the X-ray irradiation studies of chromosome breakage (de Castro et al. 1949; LaCour 1953; Håkansson 1954). It is important to note that if experimental results (such as the irradiation studies) and genome size measurements were not available, this condition might easily be confused with polyploidy.

The fourth comparison (Table 2d) clearly falls outside of what we are currently able to explain cleanly (Table 2). In comparing *L. elegans* with *L. forsteri,* we have a condition where a $2n = 6$ species has a genome that is twice the size of a $2n = 24$ species. Explanations of this pattern can get very complex, but are likely best described by some combination of polyploidy and agmatoploidy.

One problem with the types of comparisons made in this section is that they have been made outside of a phylogenetic framework. While this is necessary when there is no current phylogenetic framework available for these comparisons, it limits our ability to place a directionality to the changes discussed, and limits our ability to differentiate between equally plausible events, such as a fission vs. a fusion event.

In the next section we will discuss the current state of phylogenetic studies in Cyperales.

PHYLOGENETIC RELATIONSHIPS

Given the diversity found in Cyperales, we have only begun to scratch the surface in understanding phylogenetic relationships and patterns of diversification in this large clade. Most of the currently published phylogenetic studies fall into one of two categories: Cyperales/Cyperaceae-level studies (Plunkett et al. 1995; Muasya et al. 1998, 2000*a*) or studies within the Cariceae tribe (Starr et al. 1999, 2003; Yen and Olmstead 2000*a, b*; Roalson et al. 2001; Roalson and Friar 2004). Other studies in Cyperales include those exploring relationships in *Cyperus* s.l. (Muasya et al. 2001*a*, 2002), *Isolepis* R. Br. (Muasya et al. 2001*b*), Mapanioideae (Simpson et al. 2003), *Carpha* Banks & Sol. ex R. Br. (Zhang et al. 2004), *Eleocharis* (Roalson and Friar 2000), and *Scirpus* s.l. (Muasya et al. 2000*b*). Other ongoing studies include, among others, work in Abildgaardieae (Ghamkhar et al. unpubl. data), *Carex* (Dragon et al. unpubl. data; Roalson et al. unpubl. data; Starr et al. unpubl. data; Waterway et al. unpubl. data), *Eleocharis* (Roalson et al. unpubl. data), *Juncus* and Juncaceae (Drábková et al. unpubl. data; Jones et al. unpubl. data; Roalson unpubl. data), *Schoenoplectus* (Young et al. unpubl. data), and *Scirpus* s.l. (Dhooge and Goetghebeur unpubl. data; Roalson et al. unpubl. data).

While we have begun to understand the general patterns of relationships of the order as a whole and major clades of some lineages, very little is know about fine-scale relationships within genera and we are particularly ignorant of patterns of character evolution in the order, morphological, cytological, or other. Most phylogenetic studies have focused on understanding, and in some cases, redefining the classification of the group under study rather than any emphasis on character evolution. The few studies that have explored character evolution have tended to do only basic mapping of characters onto a phylogeny without much focus on interpretation of the patterns seen. The only published Cyperales study to even map chromosome numbers or any characteristic of genome structure was a study of relationships in Cariceae (Roalson et al. 2001). In order to comprehend the patterns and directionality of genome restructuring in Cyperales, it is necessary that more emphasis be placed on exploring chromosome numbers and genome size measurements in a phylogenetic context in order to formulate good hypotheses of patterns and processes of genome structure change.

GENOMICS IN CYPERALES

Currently, several projects are underway or in the planning stages to explore the genome structure of various lineages in Cyperales. A small BAC library has been completed in *Carex lupulina* Muhl. ex Willd. (McCubbin et al. unpubl. data), and further work is continuing to increase the coverage, which is currently approximately 2×.

In addition to basic characterization of this library, we are sequencing the ends of a number of the clones to explore the types of DNA regions currently included in the library, look for potential markers, and locate genic regions of interest (Roalson et al. unpubl. data). Preliminary end-sequencing (11 clones) in the developed library as well as genomic and EST BLAST searches suggest that the clones include a number of gene regions, with partial homology to sequences of lacZ, ubiquitin-conjugating enzyme, ATP/GTP-binding protein, and delta-24 sterol C-methyltransferase gene families. Regions of similarity were found to unknown open reading frames (ORF) or genomic regions of similarity to BAC clones in the plant genera *Arabidopsis* Heynh., *Brassica* L., *Capsicum* L., *Cycas* L., *Glycine* Willd., *Gossypium* L., *Helianthus* L., *Hordeum* L., *Lycopersicon* Mill., *Medicago* L., *Mesembryanthemum* L., *Oryza* L., *Phaseolus* L., *Pinus* L., *Rosa* L., *Solanum* L., *Tamarix* L., *Triticum* L., and *Vitis* L. Additionally, we found one large microsatellite (17 AG repeats) and no sequence homologies to chloroplast or mitochondrial sequences (Roalson et al. unpubl. data).

We will proceed with our studies in genome evolution in Cyperales through a number of avenues. We feel that only through the integration of chromosome number counts, genome size measurements, genomic and cDNA library construction (BACs, ESTs, etc.), physical mapping of genomes, linkage mapping of genomes, large-scale genomic sequencing, and phylogenetic studies will the difficult questions of genome structure and evolution in Cyperales be adequately answered. For this reason, the newly formed Cyperales Genomics Initiative at Washington State University and Sonoma State University will continue to add BAC and EST libraries from across Cyperales to its databanks. While this initiative currently only includes one BAC library (*C. lupulina*), additional libraries from *Carex, Cyperus, Eleocharis, Eriophorum, Juncus,* and *Rhynchospora* are planned. Additionally, physical mapping of the *C. lupulina* BAC library is scheduled for the near future. Crossing studies in *Carex* sect. *Lupulinae* Tuck. ex J. Carey are underway to create progeny arrays for linkage mapping studies, an EST library in *C. lupulina* is in progress, and BAC end sequencing of the *C. lupulina* BAC library continues.

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