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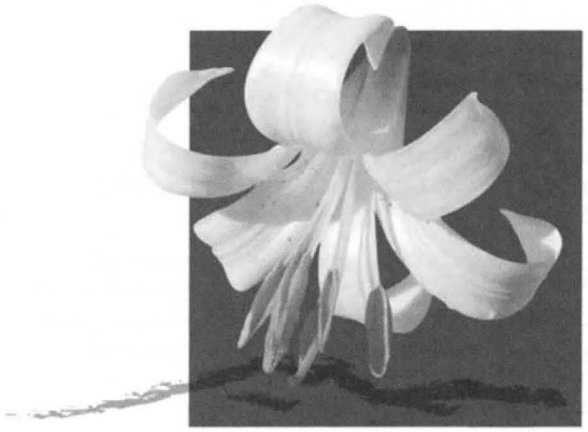

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Excluding Poales

Asparagales

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PHYLOGENY, GENOME SIZE, AND CHROMOSOME EVOLUTION OF ASPARAGALES

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ABSTRACT

Asparagales are a diverse monophyletic order that has numerous species (ca. 50% of monocots) including important crop plants such as *Allium, Asparagus,* and *Vanilla,* and a host of ornamentals such as irises, hyacinths, and orchids. Historically, Asparagales have been of interest partly because of their fascinating chromosomal evolution. We examine the evolutionary dynamics of Asparagales genomes in an updated phylogenetic framework that combines analyses of seven gene regions (*atp*1, *atp*B, *mat*K, *ndh*F, *rbc*L, *trn*L intron, and *trn*L–F intergenic spacer) for 79 taxa of Asparagales and outgroups. Asparagales genomes are evolutionarily labile for many characters, including chromosome number and genome size. The history and causes of variation in chromosome number and genome size remain unclear, primarily because of the lack of data in small clades in the phylogenetic tree and the lack of comparative genetic maps, apart from *Allium* and *Asparagus.* Genomic tools such as bacterial artificial chromosome (BAC) libraries should be developed, as both molecular cytogenetic markers and a source of nuclear genes that can be widely used by evolutionary biologists and plant breeders alike to decipher mechanisms of chromosomal evolution.

Key words: Asparagaceae, bacterial artificial chromosomes (BACs), bimodal karyotype, genomics, *Hesperocallis,* molecular cytogenetics, phylogenomics, polyploidy.

INTRODUCTION

Phylogenomics combines phylogeny and genomics to understand evolutionary patterns and processes. Defined in a narrow sense, phylogenomics originally referred to the use of phylogenetic analyses to determine gene function (Eisen 1998), but this definition has now broadened to include several approaches that combine evolutionary and genomic analyses (Charlesworth et al. 2001; Eisen and Wu 2002; Eisen and Fraser 2003; Feder and Mitchell-Olds 2003). In plants, phylogenomic comparisons have been made or are in progress at several taxonomic levels and often involve the use of genomic information from model taxa (see Soltis and Soltis 2000*a*, 2003; Cronk 2001; Daly et al. 2001; Citerne et al. 2003; Reeves and Olmstead 2003). For example, information from the *Arabidopsis* Heynh. genome has been used in phylogenomic studies to determine genomic evolutionary events across taxa in Brassicaceae (Mitchell-Olds 2001; Hall et al. 2002) and across the monocot/magnoliid-eudicot divide (Bowers et al. 2003). Plant phylogenomic comparisons are also in progress at deeper levels to determine the evolution of genome sizes across the angiosperms (Leitch et al. 1998), the origin of the flower (Soltis et al. 2002), and the evolution of genomes across all green plants (Mandoli and Olmstead 2000; Pryer et al. 2002).

In monocots, phylogenomics is being practiced primarily in the economically important family Poaceae, in which numerous genomic resources are available, such as bacterial artificial chromosome (BAC) libraries, expressed sequence tag (EST) libraries, and the completely sequenced genome of *Oryza* L. (Rudd 2003). Such investigations are applicable at several phylogenetic levels, including comparisons within and between closely related grass genera (e.g., *Oryza*; Ge et al. 1999; Sang 2002; Haas et al. 2003), across Poaceae (Kellogg 2000, 2001; Gaut 2002; Levy and Feldman 2002; Choffnes Inada et al. 2003; Guo and Moose 2003), among commelinids (e.g., Givnish et al. 2000), and finally, comparisons of gene and genome duplication events across the monocot/magnoliid-eudicot divide (e.g., Dias et al. 2003; Vanderpoele et al. 2003). This last form of comparison has revealed the significance of polyploidy in that many plants that behave like diploids are actually paleopolyploids. Researchers in phylogenetics and genomics are now appreciating that the "tree of life" is complicated by ancient and recent cycles of genome doubling and diploidization (Vision et al. 2000; Blanc et al. 2003).

The importance of polyploidy in plant evolution has long been known (Stebbins 1950, 1971; Grant 1971, 1981), and studies of polyploidy continue to experience a renaissance (reviewed in Soltis and Soltis 2000*b*; Wendel 2000; Liu and Wendel 2002, 2003; Ramsey and Schemske 2002; Osborn et al. 2003). Polyploidy and genome size have been associ-

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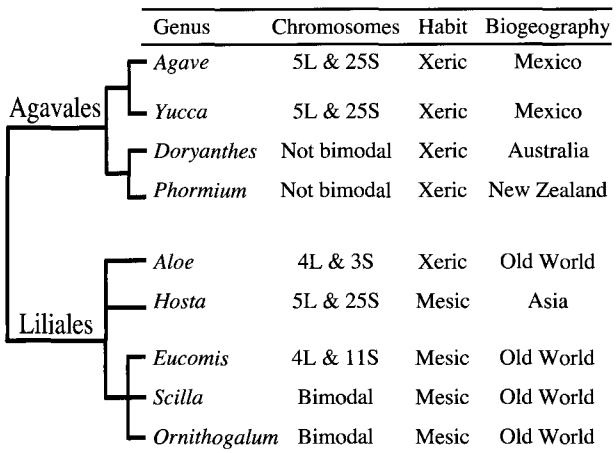


Fig. 1.—Darlington (1963, 1973) proposed multiple origins of the bimodal karyotype. *Agave* and *Yucca* were placed in Agavales whereas *Hosta* and *Eucomis* were placed in a broadly circumscribed Liliales following Hutchinson's classification system (1934, 1959).

ated with a number of life-history traits, including vegetative form, flowering time, and adaptations to particular ecological niches (Levin 1983; Bennett 1987; Gregory 2002). One possible type of polyploidy seen in monocots is the bimodal karyotype. Bimodal karyotypes have chromosomes showing two clearly different sizes, typically with small chromosomes and large acrocentric chromosomes. In the monocots, bimodal karyotypes are found in Alismatales (*Butomus* L., *Hydrilla* Rich., *Najas* L., and *Ruppia* L.) and Poales (e.g., *Milium* L.), but the most spectacular bimodal karyotypes occur in Asparagales, especially but not exclusively in the "desert-loving genera" discussed by Darlington (1963, 1973).

Historically, Darlington and other botanists were fascinated with chromosomal biology and its relationship to plant form and biogeography. One particular puzzle was the bimodal karyotypes of several genera of petaloid monocots. For example, *Agave* L., *Hosta* Tratt., and *Yucca* L., all have 5 pairs of large chromosomes and 25 pairs of small chromosomes, whereas *Aloe* L. has 4 large and 3 small pairs, and *Eucomis* L'Hér. has 4 large and 11 small pairs of chromosomes. If one were to infer phylogenetic relationships from chromosome morphology alone, one would group *Agave, Yucca,* and *Hosta* together. However, *Agave* and *Yucca* are xerophytes native to North America and *Hosta* is a mesophytic plant from Asia. If plant form and biogeography are given an inordinate role in assigning evolutionary relationships, then *Hosta* may be thought to be more closely related to *Eucomis* or other Old World taxa. Indeed, Darlington (1963, 1973) followed Hutchinson's classification system (1934, 1959) and proposed multiple origins of the bimodal karyotype: *Agave* and *Yucca* were placed in Agavales with other xerophytes such as *Doryanthes* Correa and *Phormium* J. R. Forst. & G. Forst., whereas *Hosta* and *Eucomis* were placed in a broadly circumscribed Liliales (Fig. 1).

Since Darlington's time, plant systematics has undergone a dramatic revolution due to molecular data and phylogenetic methodology. With respect to Darlington's scenario for the evolution of the bimodal karyotype, a number of studies has given us new hypotheses about the relationship of Agavaceae to *Hosta* and "New World Hyacinthaceae" such as *Camassia* Lindl., *Chlorogalum* Kunth, and other genera (Bogler and Simpson 1995, 1996; Chase et al. 1995; Pfosser and Speta 1999; Pires et al. 2004; Bogler et al. 2006). Currently, Asparagales are recognized as a large and diverse monophyletic order of monocots that includes ca. 25,000–42,000 species (ca. 50% of monocots or 10–15% of flowering plants, depending on the number of orchid species recognized, which varies from 18,000–35,000 species; Dressler 1993). Asparagales include important crop plants such as *Allium* L., *Asparagus* L., and *Vanilla* Plum. ex Mill., as well as lesser-known crops (*Agave* and *Aloe*) and a host of ornamentals including irises, hyacinths, and orchids. A number of studies have sampled all of the families of Asparagales sensu Angiosperm Phylogeny Group (APG 1998). Following early *rbc*L studies of the monocots (Duvall et al. 1993; Chase et al. 1995), Fay et al. (2000) conducted a four-locus phylogenetic analysis of Asparagales that encompassed most major taxonomic groupings within the order. The primary findings of these analyses were that previous circumscription of Asparagales (Huber 1969; Dahlgren 1980; Dahlgren et al. 1985) needed to be expanded to include Iridaceae and Orchidaceae (formerly placed in Liliales and Orchidales, respectively). In addition, some of the families in Dahlgren's system (e.g., Anthericaceae and Alliaceae) were polyphyletic (Chase et al. 1996; Fay and Chase 1996). As a result, Asparagales were recircumscribed (APG 1998) to include a number of small new families such as Anemarrhenaceae, Boryaceae, Behniaceae, Themidaceae, and Xeronemataceae (reviewed in Fay et al. 2000; Reveal and Pires 2002). Since the combined molecular and morphological phylogenetic analysis of Asparagales presented at the previous monocot conference (Fay et al. 2000; Meerow et al. 2000), continued progress has been made in clarifying Asparagales phylogeny with additional markers (McPherson et al. 2004, submitted; see Graham et al. 2006).

Molecular data have demonstrated that Asparagales can be divided into two major groups: a paraphyletic "lower" asparagoid grade, mostly characterized by simultaneous successive microsporogenesis (except for Hypoxidaceae, Xanthorrhoeaceae, some Orchidaceae, and some Iridaceae), and a "higher" asparagoid clade with successive microsporogenesis (Rudall et al. 1997; Fay et al. 2000; Nadot et al. 2006). A recent morphological analysis of Asparagales also revealed numerous potentially synapomorphic features (Rudall 2002*b*), two of which apparently define Asparagales: the presence of a hypodermal outer layer in roots (Kauff et al. 2000), as well as an inferior ovary (Rudall 2002*a*). However, the "higher" asparagoid clade has a reversal to superior ovaries that is in some instances associated with the presence of septal nectaries. In fact, there is evidence for multiple origins of superior ovaries, zygomorphy, and septal nectaries in Asparagales (Kocyan and Endress 2001; Rudall 2002*a*, *b*; Rudall and Bateman 2002, 2004).

Collectively, these molecular and morphological phylogenetic studies have been recently used to reclassify Asparagales (APG II 2003). In contrast to APG (1998), which recognized 29 families in Asparagales, the APG II (2003) classification had a "bracketed system" that allows for the option of recognizing 25 smaller bracketed families (similar

to APG 1998 with an expanded Agavaceae) or the option of recognizing only 14 families. Specifically, APG II (2003) simplified the "higher" Asparagales by using expanded circumscriptions of two families, Asparagaceae s.l. and Alliaceae s.l., which can be identified by the racemes of Asparagaceae s.l. (with the exception of Themidaceae, as discussed below) and the umbellate inflorescence of Alliaceae s.l. In this sense, Asparagaceae s.l. includes Agavaceae (with *Hesperocallis* A. Gray), Aphyllanthaceae, Asparagaceae s.s., Hyacinthaceae, Laxmanniaceae, Ruscaceae, and Themidaceae. Similarly, Alliaceae s.l. includes Agapanthaceae, Alliaceae s.s., and Amaryllidaceae. Finally, APG II (2003) recognized Xanthorrhoeaceae s.l., which includes Asphodelaceae, Hemerocallidaceae, and Xanthorrhoeaceae s.s. In this study, we extended the four-gene analysis of Fay et al. (2000) to include three additional genes to further clarify the higher-level relationships among these families in Asparagales. We use bracketed families (narrow circumscription in APG II 2003) to facilitate phylogenetic comparisons to the APG 1998 system used by Fay et al. (2000) and others (e.g., Asparagaceae s.s.), but later we referred to the alternative broader circumscriptions (e.g., Asparagaceae s.l.) when making comparisons among more inclusive clades.

Asparagales, like Poales, have experienced dynamic genome and chromosomal evolution. Asparagales have a wide range of chromosome numbers ($2n$ = 4–228, a 57-fold difference) and genome sizes (0.48–75.9 pg, a 158-fold difference) and include both ancient and recent gene duplication and polyploid events (Tamura 1995; Bennett and Leitch 2000). Research into the composition of Asparagales genomes has progressed in four areas. First, surveys of chromosome number and genome sizes now include representatives from all Asparagales except two families (Boryaceae and Lanariaceae; Hanson et al. 2003). More detailed studies of genome sizes within genera have included *Allium* (Ohri 1998) and the slipper orchids (Cox et al. 1998). Second, studies that showed the lack of *Arabidopsis*-like telomeres in *Allium* (Pich et al. 1996*a*, *b*; Pich and Schubert 1998) have been followed up to demonstrate their absence in *Aloe* (Adams et al. 2000*a*, *b*; Weiss and Scherthan 2002) and the large clade that includes Alliaceae s.l., Asparagaceae s.l., and several other families in Asparagales (Adams et al. 2001; Puizina et al. 2003; Sykorova et al. 2003; Weiss-Schneeweiss et al. 2004). Third, in a survey of mitochondrial ribosomal proteins and *sdh* genes, several genes were found to be lost in Asparagales (Adams et al. 2002). In particular, the mitochondrial *sdh*3 gene has been lost in the whole order, the mitochondrial *rpl*2 gene was lost in a clade that includes Alliaceae s.l., Asparagaceae s.l., and Asphodelaceae s.l., and mitochondrial *rps*11 was lost in Alliaceae s.l. (Adams et al. 2002). A parallel loss of a slowly evolving intron, *rps*12, has been observed in two closely related families in Asparagales (Asphodelaceae s.s. and Hemerocallidaceae; McPherson et al. 2004), and the loss of mitochondrial *cox*2 intron 1 has occurred in Ruscaceae (Kudla et al. 2002). Finally, progress has been made in the genomics of ornamental and crop plants such as *Allium, Asparagus,* and *Iris* L., including the construction of genetic maps, cDNA/EST libraries, microarrays, and the application of molecular cytogenetics (reviewed in Havey 2002; see also van Doorn et al. 2003 and Havey et al. 2006).

Given these recent advances in the phylogenetics and genomics of Asparagales, the time is ripe to revisit the evolutionary dynamics of Asparagales genomes. Here we discuss four topics. First, we present a new analysis of the phylogenetic relationships of Asparagales by adding three genes (*ndh*F, *mat*K, *atp*1) to the published four-locus matrix of Fay et al. (2000). We include the enigmatic *Hesperocallis* that was assigned to its own family, Hesperocallidaceae (unplaced in APG 1998). Second, we discuss chromosome evolution and genome size in a phylogenetic context, and we revisit Darlington's hypotheses on bimodal karyotypes. Third, we review mechanisms that can change chromosome number and genome size and outline the genomic tools needed to infer those mechanisms. Finally, we argue for the development of model taxa in Asparagales. Creating genomic resources for model Asparagales taxa will not only help us understand the evolution of Asparagales, but can bridge phylogenomic studies of Poales with emerging studies at the base of the monocots and angiosperms.

MATERIALS AND METHODS

Plant Material

Representatives of all families of Asparagales (29 families sensu APG 1998; 14 or 25 families sensu APG II 2003) were included in this analysis. The genera sampled are the same as in Fay et al. (2000) with the addition of *Hesperocallis.* As in Fay et al. (2000), representatives of Commelinales, Liliales, Pandanales, and Zingiberales were included as outgroups. For the most part, voucher information for the taxa used in these analyses was previously published (Table 1, Fay et al. 2000; Davis et al. 2004) or will be published in forthcoming articles (see also Chase et al. 2006; Givnish et al. 2006; Petersen et al. 2006).

DNA Extraction, Gene Amplification, Sequencing, and Alignment

Because the sequences analyzed here are from several different gene regions and were produced in different laboratories, we will present here only references to more detailed empirical papers. Generally, DNA extraction procedures were from silica-gel-dried leaves (Chase and Hills 1991) or herbarium sheets as summarized by Fay et al. (2000). A description of the amplification procedures for the seven loci can be found in the following references: *rbc*L (Fay and Chase 1996), *atp*B (Hoot et al. 1995), *trn*L–F (primers "c" and "f" of Taberlet et al. 1991, which include two regions, *trn*L intron and *trn*L–F intergenic spacer), *mat*K (Johnson and Soltis 1994), *ndh*F (Pires and Sytsma 2002; McPherson et al. submitted), and *atp*1 (Davis et al. 1998, 2004; Stevenson et al. 2000). GenBank numbers for *rbc*L, *atp*B, and *trn*L–F have been previously published (Fay et al. 2000; Pires et al. 2004). GenBank numbers for *mat*K, *ndh*F, and *atp*1 are in press or will be presented in forthcoming publications and will not be provided here (see Davis et al. 2004; Chase et al. 2006; Givnish et al. 2006; Petersen et al. 2006). This analysis focuses on only 79 taxa of Asparagales and outgroups, but we are preparing a future analysis of ca. 120 taxa that will include all vouchers and GenBank numbers (Pires et al. in prep.).

Data Analysis

Using the parsimony algorithm of the software package PAUP* vers. 4.0b10 for Macintosh (Swofford 2002), tree searches were conducted using the Fitch (equal weights) criterion (Fitch 1971) with 1000 random sequence additions and tree-bisection-reconnection (TBR) branch swapping, but permitting only five trees to be held at each step. All shortest trees collected in the 1000 replicates were swapped on to completion with no tree limit. Internal support was evaluated using 1000 replicates of the bootstrap (Felsenstein 1985), with random sequence addition (100 replicates) and TBR swapping, but permitting only five trees to be held at each step.

Species Diversity, Chromosome Number, and Genome Size in Asparagales

The species diversity for each family of Asparagales was taken from the Angiosperm Phylogeny Website (Stevens 2001 onwards) and updated with other references (e.g., A. W. Meerow pers. comm.; P. Goldblatt pers. comm.). Chromosome numbers were collated from the online Index to Plant Chromosome Numbers (IPCN 1984 onwards) and Angiosperm C-values databases (Bennett and Leitch 2003) and karyological reviews (e.g., Meerow 1984; Tamura 1995; Chase et al. 2000*a*). Presence or absence of bimodal karyotypes was derived from a variety of sources (e.g., Greilhuber 1995; Chase et al. 2000*a*; A. W. Meerow pers. comm.). All genome size estimates were taken from the Angiosperm C-values Database and updated with recent references that pertain to Asparagales (e.g., Hanson et al. 2001, 2003; Stajner et al. 2002). Genome size is reported as the range of haploid DNA content (1 C-value in picograms, followed by number of taxa sampled).

RESULTS

The total aligned matrix consisted of 9414 characters for the six plastid regions: *atp*B accounted for 1518 base pairs (bp), *rbc*L 1428 bp, the *trn*L–F regions 1911 bp (including the *trn*L intron and the *trn*L–F intergenic spacer), *mat*K 1860 bp, and *ndh*F 2697 bp. The total aligned matrix was 10,676 characters for the combined plastid-mitochondrial matrix (9414 characters from plastid regions and 1262 bp for the mitochondrial gene *atp*1). A total of 1720 and 1734 base positions were excluded from the plastid matrix and the combined plastid-mitochondrial matrix, respectively, either at the beginning or end of sequences or where alignment of the *trn*L–F sequences proved ambiguous (Fay et al. 2000). Of the included characters (7694 for plastid and 8942 for combined matrixes), 2490 (32%) and 2680 (30%) were potentially parsimony informative, respectively. The aligned data matrices for *rbc*L, *atp*B, and *trn*L–F were unchanged from the original Fay et al. (2000) matrix, in which it was noted that *rbc*L and *atp*B were length-conserved whereas *trn*L–F required a number of insertions/deletions (indels), which were excluded characters. The alignment of *ndh*F, *mat*K, and *atp*1 was relatively straightforward and required few indels.

Preliminary analyses of individual plastid DNA regions gave similar topologies as expected because these regions are inherited on the same linkage group. Preliminary analyses of the individual mitochondrial region (*atp*1) gave a highly unresolved phylogenetic tree. Thus, further tree searches were conducted only on the plastid data (*rbc*L/*trn*L–F/*atp*B/*mat*K/*ndh*F) and the combined plastid-mitochondrial data (*rbc*L/*trn*L–F/*atp*B/*mat*K/*ndh*F/*atp*1). Both the plastid and combined plastid-mitochondrial analyses were conducted with all 79 taxa. However, we also performed analyses that excluded *Aphyllanthes* L. (not shown). The tree with *Aphyllanthes* excluded was almost identical in topology to that found when *Aphyllanthes* was included, except that relationships among Asparagaceae s.l. were more stabilized (e.g., fewer polytomies and higher bootstrap percentages as found by Fay et al. [2000] and McPherson et al. [submitted]). For the remainder of this article, we present the results found when *Aphyllanthes* was included (Fig. 2).

The combined Fitch analysis produced one most-parsimonious tree with both matrices (plastid and combined plastid-mitochondrial matrix). The plastid matrix gave a tree length (TL) of 13,301, with consistency index (CI) = 0.42, and retention index (RI) = 0.54 (excluding uninformative characters). For the combined matrix, the TL was 14,482, CI = 0.45, and RI = 0.55. There was only one minor area of discordance between the plastid and combined plastid-mitochondrial trees with respect to the relationships of three genera within Agavaceae (*Anthericum* L., *Echeandia* Orteg., and *Leucocrinum* Nutt. ex A. Gray). Given their overwhelming similarity, we show the single tree found in the combined plastid-mitochondrial analysis in Fig. 2. Fitch branch lengths (ACCTRAN optimization) and bootstrap percentages (BP) for the combined matrix are shown above the branches with bootstrap percentages for the plastid matrix below the branches. We report these bootstrap percentages below (combined plastid-mitochondrial BP/plastid BP) for the relationships among the families and major clades of Asparagales.

Asparagales sensu APG (1998) and APG II (2003) are monophyletic (89/86 BP). The "higher" asparagoids (hereafter called the Alliaceae–Asparagaceae clade) form a strongly supported monophyletic group (100/100 BP) that contains two well-resolved clades, Alliaceae s.l. (98/96 BP) and Asparagaceae s.l. (90/89 BP). Alliaceae s.l. (sensu APG II 2003) includes three monophyletic groups (families of APG 1998) with Agapanthaceae sister to Alliaceae s.s. and Amaryllidaceae (92/92 BP). Asparagaceae s.l. (sensu APG II 2003) includes a number of families, but in both the analyses that included and excluded *Aphyllanthes* we found three main clades. The first clade (92/89 BP) is the expanded Agavaceae (sensu APG II 2003), which includes several families recognized in APG (1998): Agavaceae s.s., Anemarrhenaceae, Anthericaceae, Behniaceae, Hesperocallidaceae, and Herreriaceae. The second clade (63/55 BP) in Asparagaceae s.l. consists of Hyacinthaceae and Themidaceae; and the third clade (53/<50 BP) has Aphyllanthaceae and Laxmanniaceae sister to a monophyletic group (90/78 BP) that consists of Asparagaceae s.s. and Ruscaceae. In the analysis that excluded *Aphyllanthes*, these three main clades had higher support (100/100 BP, 98/89 BP, and 98/98 BP, respectively).

The Alliaceae–Asparagaceae clade is sister (100/100 BP) to a strongly supported (100/100 BP) Xanthorrhoeaceae s.l. (sensu APG II 2003). Xanthorrhoeaceae s.l. consist of three

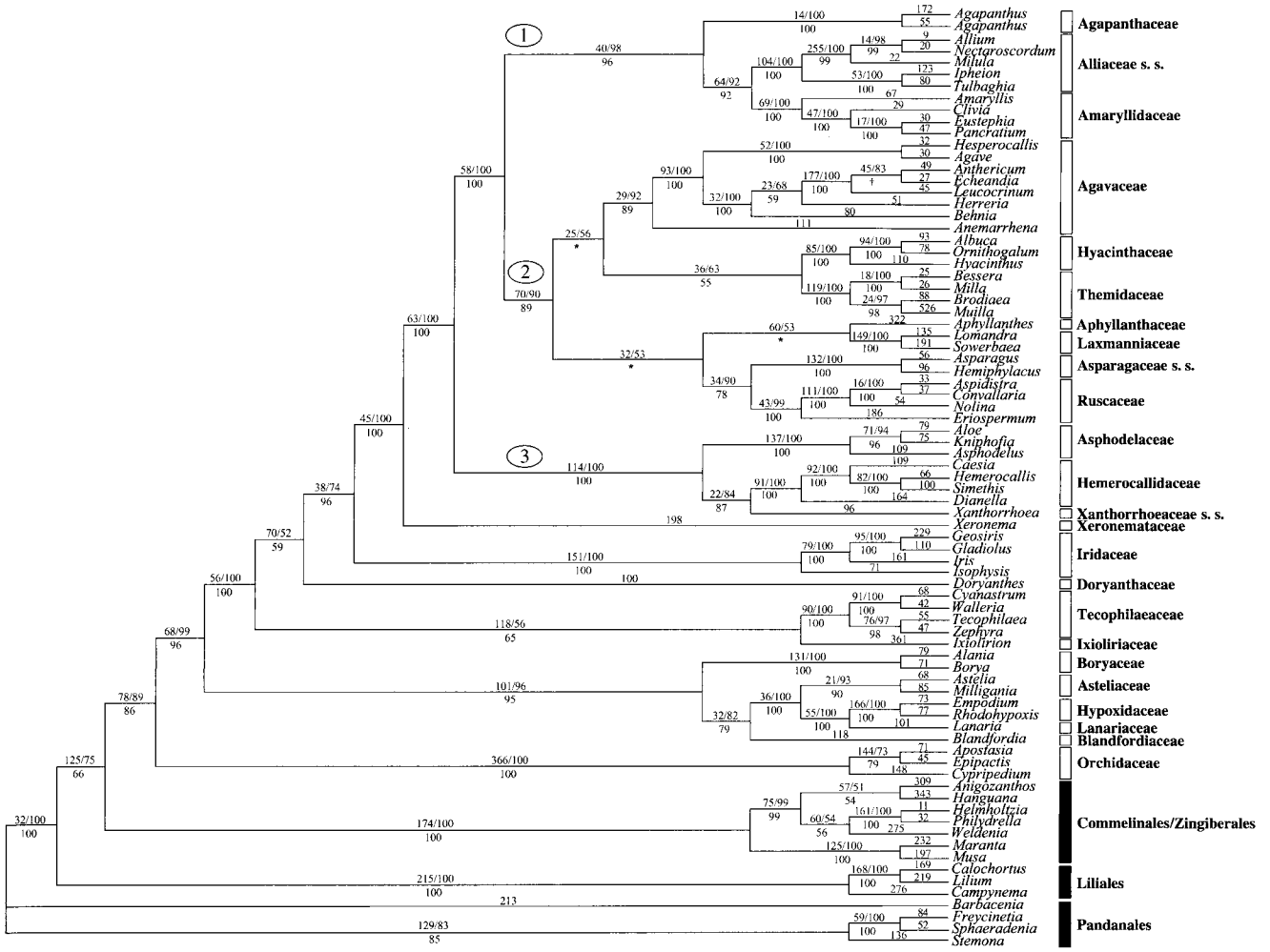


Fig. 2.—Single most-parsimonious tree of Asparagales obtained under the Fitch criterion (14,478 steps) using plastid (*atp*B, *rbc*L, *trn*L intron and *trn*L–F intergenic spacer, *ndh*F, *mat*K) and mitochondrial (*atp*1) sequence data. Branch lengths (ACCTRAN) and bootstrap percentages (branch/bootstrap) are shown above the branches for the combined plastid and mitochondrial dataset. Bootstrap percentages [BP] including only plastid data are shown under the branches. Branches that lack support are represented by an asterisk [*] (asterisks indicate <50% BP). Discordance between the plastid-mitochondrial tree and plastid tree topologies is represented by †. Solid bars to right of the phylogenetic tree indicate outgroup orders. Open bars indicate 23 of 24 narrow bracketed families of Asparagales (APG II 2003; but we sink Hesperocallidaceae into Agavaceae [Pires et al. 2004]). Circled numbers above three branches indicate a broader familial circumscription that recognizes 14 families of Asparagales: 1 corresponds to Alliaceae s.l., 2 corresponds to Asparagaceae s.l., and 3 corresponds to Xanthorrhoeaceae s.l. (sensu APG II 2003).

clades that correspond to families recognized earlier (APG 1998), with Asphodelaceae sister to a clade (84/87 BP) that includes Hemerocallidaceae and Xanthorrhoeaceae s.s. (Devey et al. 2006). The Alliaceae–Asparagaceae clade and Xanthorrhoeaceae s.l. are sister (100/100 BP) to Xeronemataceae alone. Collectively, this large clade is then sister (74/96 BP) to Iridaceae.

We find weak support for Doryanthaceae as sister to all of the above families (52/59 BP). The sister relationship of Ixioliriaceae and Tecophilaeaceae is also weakly supported (56/65 BP). However, the clade that includes Doryanthaceae, Ixioliriaceae, Tecophilaeaceae, and the above previously mentioned families is strongly supported (100/100 BP). In turn, this clade is sister (99/96 BP) to the "astelioids," a clade that includes five families (Rudall et al. 1998*a*; Fay et al. 2000). The monophyly of the "astelioids" is well supported here (96/95 BP). This group has Boryaceae sister to a clade of four families where Hypoxidaceae are sister to Lanariaceae (100/100 BP), and Asteliaceae (100/100 BP) and Blandfordiaceae (82/79 BP) are successive sisters to this pair of families. Orchidaceae are monophyletic (100/100 BP) and are well supported as sister to rest of Asparagales (86/89 BP).

DISCUSSION

Given the enormous resources that have been invested in understanding the genomes of the grass family (e.g., the sequencing of the *Oryza* genome and the construction of numerous genomic libraries), one must wonder whether or not the genomic tools developed for Poaceae will be applicable to the other major lineages of the monocots. Recent studies (Kuhl et al. 2004; Havey et al. 2006) have found that nuclear sequences of expressed genes of *Asparagus* are more similar

to those of the eudicot *Arabidopsis* than to *Oryza,* indicating that the grass genomes may not necessarily model the evolution of other monocot genomes. Here we discuss four areas of the phylogenetics and genomics of Asparagales: (1) phylogenetic relationships and morphological evolution of Asparagales in light of a new phylogenetic analysis based on seven loci (*rbc*L, *atp*B, *trn*L intron, *trn*L–F intergenic spacer, *ndh*F, *mat*K, *atp*1); (2) chromosome evolution and genome size in Asparagales with an emphasis on Darlington's hypotheses on bimodal karyotypes; (3) genomic tools needed to infer mechanisms that can cause chromosomal evolution or change genome size; and (4) criteria for developing model taxa in Asparagales.

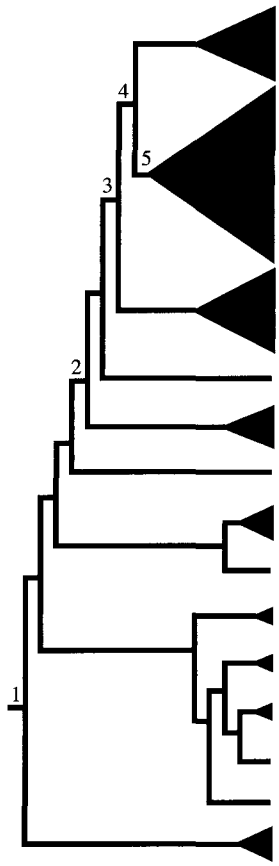
Phylogeny and Morphological Evolution of Asparagales

Evolutionary relationships in Asparagales.—At the previous monocot conference, Fay et al. (2000) presented a combined analysis of morphological characters and sequences for four plastid DNA regions, (*rbc*L, *atp*B, *trn*L intron, and *trn*L–F intergenic spacer) for Asparagales and related groups. The higher-level phylogenetic relationships found here (Fig. 2) are particularly congruent with Fay et al. (2000) and other previous studies (Chase et al. 1995, 2000*b*; Rudall et al. 1997). However, the addition of three additional genes (*mat*K, *ndh*F, and *atp*1) to those used by Fay et al. (2000) generally gave higher bootstrap support to many clades. Three notable areas of increased phylogenetic support are: (1) Orchidaceae (100/100 BP) are sister to the rest of the Asparagales (89/86 BP); (2) monophyly of the "astelioids" (96/95 BP); and (3) Alliaceae s.s. sister to Amaryllidaceae (92/92 BP). These results are consistent with recently published analyses that used 17 plastid genes but fewer taxa (Graham et al. 2006; McPherson et al. submitted) .

However, uncertainties remain in two parts of the Asparagales phylogenetic tree. First, the exact relationships of Doryanthaceae, Ixioliriaceae, and Tecophilaeaceae remain unresolved. Like Fay et al. (2000), we found weak support for Ixioliriaceae and Tecophilaeaceae (56/65 BP), with that clade in a polytomy or weakly sister to Doryanthaceae and the remainder of Asparagales (see Fig. 2). McPherson et al. (submitted) found strong bootstrap support for a sister relationship of Ixioliriaceae and Tecophilaeaceae with molecular data; however, they did not sample Doryanthaceae (but see Graham et al. 2006). Analyses of morphological data and base chromosome number support the sister relationship of Ixioliriaceae and Tecophilaeaceae (Stevenson and Loconte 1995) and place *Doryanthes* as sister to Iridaceae (Rudall 2002*b*). The second problematic area in the phylogenetic tree is the effect that the monotypic Aphyllanthaceae have in destabilizing relationships within Asparagaceae s.l. in the Alliaceae–Asparagaceae clade. McPherson et al. (submitted) and Fay et al. (2000) explored this issue in phylogenetic analyses of Asparagales that both included and excluded *Aphyllanthes.* McPherson et al. (submitted) found Aphyllanthaceae sister to Agavaceae (sensu APG II 2003), but with weak support. In our analysis we found Aphyllanthaceae sister to Laxmanniaceae (53/<50 BP) with weak support and much lower bootstrap percentages within Asparagaceae s.l. (APG II 2003). However, in our analyses that exclude Aphyllanthaceae (not shown), we found strong support for a clade consisting of Asparagaceae s.s., Laxmanniaceae, and Ruscaceae (98/98 BP), as well as Hyacinthaceae sister to Themidaceae (98/89 BP) as found in McPherson et al. (submitted).

In addition to the progress made in clarifying higher-level relationships among Asparagales, new hypotheses exist for relationships among taxa specifically discussed by Darlington (1963, 1973) when he was hypothesizing about chromosomal evolution and bimodal karyotype (Fig. 1). Specifically, we now have a clearer idea about the relationships between *Agave, Aloe, Hosta,* and the "New World hyacinths" such as *Camassia* and *Chlorogalum* (Bogler and Simpson 1995, 1996; Chase et al. 1995, 2000*b*; Pfosser and Speta 1999; Fay et al. 2000). One ambiguity was the phylogenetic placement of the enigmatic *Hesperocallis* (monotypic Hesperocallidaceae), which was unplaced in APG (1998) and mentioned by Fay et al. (2000) as a critical taxon to sample in future studies. In this study, we place *Hesperocallis* within Agavaceae, as opposed to Alliaceae or Hemerocallidaceae as previously thought by Traub (1982) and Hutchinson (1959), and we recommend the submergence of this family in Agavaceae (APG 1998) or Asparagaceae s.l. (APG II 2003). The exact relationship of *Hesperocallis* to other genera of Agavaceae is discussed in detail elsewhere (Pires et al. 2004; Bogler et al. 2006).

Morphological synapomorphies and parallelisms in Asparagales.—Morphological synapomorphies and reversals among major clades in Asparagales have been recently documented by Rudall et al. (1997), Rudall (2002*a*, *b*), Stevens (2001 onward) and others. We briefly review these here. Simultaneous microsporogenesis is a synapomorphy for Asparagales, but this is reversed to successive microsporogenesis in the Alliaceae–Asparagaceae clade, Hypoxidaceae, and Xanthorrhoeaceae s.s. The presence of a hypodermal layer in the roots (Kauff et al. 2000) and an inferior ovary also define Asparagales; however, there are reversals to a superior ovary in five families (Amaryllidaceae s.s., some Agavaceae, some Hemerocallidaceae, Tecophilaeaceae, and one genus of Iridaceae [Kocyan and Endress 2001; Rudall 2002*a*, *b*; Rudall and Bateman 2002, 2004]). Historically, this was an important character in classification because the presence of an inferior ovary separated Amaryllidaceae from Liliaceae (e.g., Cronquist 1988). The seeds of Asparagales have a phytomelan crust, but phytomelan is secondarily lost in some taxa within Ruscaceae (e.g., *Eriospermum* Jacq.) and is not present in Orchidaceae. Whereas no morphological synapomorphies unite the "astelioids," three of the five families in that clade (Asteliaceae, Hypoxidaceae, and Lanariaceae) possess branched hairs and mucilage canals (Rudall et al. 1998*a*). Umbellate inflorescences characterize Alliaceae s.l. (including Agapanthaceae and Amaryllidaceae), and a similar character (but with bracts subtending each pedicel, which is found only in *Agapanthus* L'Hér. and *Tulbaghia* L. in Alliaceae) is found in Themidaceae, which includes genera such as *Brodiaea* Sm. once thought related to Alliaceae (Fay and Chase 1996; Fay et al. 2000; Pires et al. 2001; Pires and Sytsma 2002; APG II 2003). Flowers with bilateral symmetry are found in at least four clades: Orchidaceae (cf. Rudall and Bateman 2002, 2004), some Iridaceae (e.g., *Diplarrhena* Labill.; Rudall and Goldblatt 2001), some



Family	# Species	2*n*	Bimodal	Genome Size	Exemplars
Alliaceae s.l.	1600-1675	10-138	Yes	6.5-74.5 (170)	*Allium, Amaryllis*
Asparagaceae s.l.	2290-2650	4-190	Yes	0.65-75.9 (122)	*Asparagus, Agave*
Xanthorrhoeaceae s.l.	805-890	9-84	Yes	0.76-34.15 (195)	*Aloe, Hemerocallis*
Xeronemataceae	2	32	No	3.38 (1)	*Xeronema*
Iridaceae	1900	6-228	Yes	0.48-31.38 (90)	*Iris, Gladiolus, Freesia*
Doryanthaceae	2	48	No	3.31 (1)	*Doryanthes*
Tecophilaeaceae	23	16-48	No	2.56 (1)	*Tecophilaea*
Ixioliriaceae	3	24-72	No	1.02 (1)	*Ixiolirion*
Boryaceae	12	28-56	No	unknown	*Borya*
Asteliaceae	36	16-210	No	1.27 (1)	*Astelia*
Hypoxidaceae	100-220	18-200	No	1.42 (1)	*Hypoxis*
Lanariaceae	1	unknown	?	unknown	*Lanaria*
Blandfordiaceae	4	34, 68	No	8.13 (1)	*Blandfordia*
Orchidaceae	18,000-35,000	10-164	Yes	1.10-38.83 (193)	*Vanilla*

Fig. 3.—A phylogenetic tree of all 14 families of Asparagales with broader familial circumscriptions (sensu APG II 2003). The numbers on the branches of the tree correspond to synapomorphies based on mitochondrial or telomeric sequences described in the Discussion. The number of species per family is taken primarily from Stevens (2001 onward). The width of the black triangles at the tips of the phylogenetic tree indicates the relative level of taxon sampling of the respective families in this study and not the actual diversity of the families. Range of 2*n* chromosome numbers taken from an Angiosperm C-values Database and other sources (e.g., Tamura 1995). Presence or absence of bimodal karyotypes is from a variety of sources (e.g., Greilhuber 1995). Genome size is reported as the range of haploid DNA content (1 C-value in picograms, followed by number of taxa sampled). DNA content data were collated from the Angiosperm C-values Database (website) and updated with recent references (e.g., Hanson et al. 2003).

Tecophilaeaceae (e.g., *Cyanella* L., *Zephyra* D. Don.; Simpson and Rudall 1998), and some Alliaceae s.l. (*Agapanthus* and many amaryllids are zygomorphic, at least in some whorls [e.g., *Gilliesia* Lindl.; Rudall et al. 2002]). Chemical characters also serve as synapomorphies for large clades, with anthraquinones uniting Xanthorrhoeaceae s.l. (Kite et al. 1995), and alkaloids and alliaceous chemistry defining Amaryllidaceae and Alliaceae s.s., respectively. Parallel evolution also occurs in Asparagales with respect to underground parts. For example, corms are found in Ixioliriaceae, Tecophilaeaceae, Themidaceae, and some Iridaceae (Dahlgren et al. 1985). Perhaps the most spectacular morphological parallelisms are in overall plant habit, with leaf succulence (adaptations to xeric environments) occurring in Agavaceae and Asphodelaceae, and to a lesser degree in Ruscaceae (e.g., *Dracaena* Vand. ex L., *Nolina* Michx.) and even some Iridaceae (Rudall et al. 2000). Similarly, Behniaceae, Blandfordiaceae, Ruscaceae, Tecophilaeaceae, and some Agavaceae (e.g., *Hosta*) possess net-veined leaves that are associated with mesic, forest-understory environments (see Givnish et al. 2006 on concerted evolution of net-veined leaves and fleshy fruits in monocots). Given these morphological parallelisms, it can often be difficult to assign macromorphological characters to separate two families for taxonomic keys, as illustrated by African taxa of Anthericaceae and Asphodelaceae (Stedje and Nordal 1994). Undoubtedly, numerous micromorphological characters may give additional evidence for synapomorphies and convergent evolution in Asparagales. Collectively, these studies highlight the importance of reciprocal illumination between molecular and morphological data when evaluating character evolution.

Genome and Chromosome Evolution in the Asparagales

Studying the dynamics of genome and chromosomal evolution requires a clear phylogenetic framework to infer the directionality of genome size changes over time (Bennetzen and Kellogg 1997; Bennetzen 2002; Gaut 2002; Wendel et al. 2002*a*, *b*). To facilitate comparisons among clades recognized as families, we used our phylogenetic analysis (Fig. 2) to make a summary phylogram of relationships among families (Fig. 3, with alternative broad circumscription of

families sensu APG II 2003). We discuss three phenomena in a phylogenetic perspective: (1) genomic evolution of mitochondrial and telomeric sequences; (2) species diversity among families; and, (3) chromosome and genome size evolution, including the presence of bimodal karyotypes.

Mitochondrial evolution and telomere composition in Asparagales.—Most land plant mitochondrial genomes contain genes for 30–40 proteins, but in angiosperms, and particularly the monocots, there has been frequent gene loss and gene transfer to the nucleus (Adams et al. 2002). Most angiosperms have similar "typical" *Arabidopsis*-type telomeres that cap the ends of chromosomes, but within Asparagales these typical telomeres have been replaced with different kinds of telomeres that are human-type (Adams et al. 2001; Weiss and Scherthan 2002; Puizina et al. 2003; Sykorova et al. 2003; Weiss-Schneeweiss et al. 2004). The evolution of Asparagales genomes in the form of mitochondrial DNA losses and telomere composition serve as synapomorphies for major clades in Asparagales (Fig. 3). The numbers above some of the branches of the phylogenetic tree in Fig. 3 correspond to the following genomic events. (1) The mitochondrial succinate dehydrogenase gene *sdh3* is absent in all Asparagales, although whether it is a synapomorphy for the order is confounded by its frequent absence in Poales and Liliales (Adams et al. 2002). (2) The replacement of the "typical" *Arabidopsis*-type telomere with human-type telomeres marks a clade that includes Iridaceae, Xanthorrhoeaceae s.l., and the Alliaceae–Asparagaceae clade (Adams et al. 2001; Puizina et al. 2003; Sykorova et al. 2003; Weiss-Schneeweiss et al. 2004). (3) The mitochondrial ribosomal protein gene *rpl2* is absent in Xanthorrhoeaceae s.l. and the Alliaceae–Asparagaceae clade (Adams et al. 2002). However, because Xeronemataceae were not sampled for *rpl2*, it is not certain whether the loss of *rpl2* is a synapomorphy for this node of the phylogenetic tree or the clade that includes Xeronemataceae. (4) The mitochondrial ribosomal protein *rps11* was lost in Alliaceae s.l. (Adams et al. 2002), although because Agapanthaceae were not sampled this may be a synapomorphy for Alliaceae s.s. and Amaryllidaceae. In addition to serving as genomic markers for major clades in Asparagales, the losses of mitochondrial and plastid genes also serve as synapomorphies for individual families such as Alliaceae, Asparagaceae, Iridaceae, Ruscaceae, and Xanthorrhoeaceae (Adams et al. 2002; Kudla et al. 2002; McPherson et al. 2004). The most striking mitochondrial DNA loss or transfer in Asparagales has occurred in *Allium,* which has rapidly lost so many genes that its mitochondrial genome has suddenly become animal- or fungal-like (Adams et al. 2002). Davis et al. (1998, 2004) and Petersen et al. (2006) describe the implications of mitochondrial DNA evolution for inferring monocot phylogenetic trees. How these genomic events correspond to the evolutionary diversification of these particular clades within Asparagales is unknown.

Species diversity in the families of Asparagales.—The clades that we recognize as families in Asparagales vary widely with respect to their number of species. Figure 3 illustrates that there are six families with over 100 species with four of those families having over 1000 species. Orchidaceae are one of the most diverse families of the angiosperms, and have more species (18,000–35,000) than the rest of Asparagales combined. In fact, the orchid family alone accounts for 10% of flowering plant diversity and up to 50% of the species of monocots. Other diverse families include Iridaceae (ca. 1900 species: P. Goldblatt pers. comm.) and the combined Xanthorrhoeaceae s.l., Asparagaceae s.l., and Alliaceae s.l. (APG II 2003) clade includes several thousand species. All six of these families have a wide range of chromosome numbers and genome sizes (Chase et al. 2000*a*; discussed below), but it is not clear if the lability of these genomes is a cause or consequence of the evolutionary diversification seen in these families.

Chromosome and genome size evolution in Asparagales.—Asparagales genomes differ spectacularly in chromosome number and genome size. Chromosome numbers are now available for all the recognized families in Asparagales except the monotypic Lanariaceae (Flory 1977; Index to Plant Chromosome Numbers 1984 onward; Tamura 1995; Brummitt et al. 1998; Chase et al. 2000*b*; Hanson et al. 2001, 2003; Bennett and Leitch 2003). Figure 3 illustrates the wide range of chromosome numbers (reported as $2n$) within and among families of Asparagales, which collectively have a 58-fold difference in chromosome number. The families with the lowest chromosome numbers are Asparagaceae s.l. (*Ornithogalum tenuifolium* Gren. & Godr., $2n = 4$) and Iridaceae (*Crocus* L. and *Lapeirousia* Thunb. species, $2n = 6$). The families with the highest chromosome numbers are Iridaceae (*Libertia* Lej., $2n = 228$; Tamura 1995), Asteliaceae (*Astelia* Banks & Sol. ex R. Br., $2n = 210$; Hanson et al. 2003) and Hypoxidaceae (*Hypoxis obtusa* Burch., $2n = 200$; Nordal et al. 1985), with several other families having taxa with over 100 chromosomes (Alliaceae s.l., Asparagaceae s.l., and Orchidaceae). Chromosome numbers vary spectacularly within families, as seen in Asparagaceae s.l. (48-fold), Iridaceae (38-fold), Orchidaceae (16-fold), and Xanthorrhoeaceae s.l. (9-fold).

Genome size estimates are now available for all the recognized families in Asparagales except Boryaceae and Lanariaceae (Hanson et al. 2001, 2003). Figure 3 illustrates the wide range of genome sizes among the families of Asparagales, with each range given as a haploid genome value (1 C-value). Asparagales have a remarkable 158-fold difference in genome size, from the smallest measured genomes found in Iridaceae (*Hesperantha bachmannii* Baker, 0.48 pg; *Sisyrinchium tinctorium* Kunth, 0.50 pg), Orchidaceae (e.g., *Oncidium flexuosum* Lindl. and *Notylia barkeri* Lindl., 1.10 pg; Chase et al. 2005), and Asparagaceae (*Aphyllanthes monspeliensis* L., 0.65 pg; *Asparagus plumosus* Baker, 0.67 pg; Stajner et al. 2002); to the largest measured genomes in Asparagaceae s.l. (*Scilla mordakiae* Speta, 75.9 pg) and Alliaceae (*Allium validum* S. Watson, 74.5 pg). Genome sizes also vary dramatically within families, as seen in Asparagaceae s.l. (116-fold), Iridaceae (65-fold), Orchidaceae (35-fold), and Xanthorrhoeaceae s.l. (45-fold).

Few analyses have mapped chromosome number or genome sizes across families of flowering plants (but see Bharathan et al. [1994] for comparisons of genome sizes in monocots; Leitch et al. [1998] who show that "weeds" possess small genomes across angiosperms; and Leitch and Hanson [2002] and Soltis et al. [2003] who report and reconstruct ancestral genome sizes for major clades in angio-

sperms). However, caution must be taken when comparing chromosome numbers or genome sizes among the well-studied families with other families in Asparagales in which only a few representative chromosome counts or DNA content measurements have been made (Hanson et al. 2001, 2003). Thus, it is almost certain that Fig. 3 underestimates both chromosome number variation and genome size variation within some families. Similarly, caution must be exercised when considering the chromosome number differences within genera (e.g., *Ornithogalum tenuifolium* $2n = 4$; *Ornithogalum umbellatum* L. $2n = 44–104$) or when considering the genome size differences within genera (e.g., *Scilla albescens* Speta, 3.8 pg; *Scilla mordakiae,* 75.9 pg) because these genera may be polyphyletic (Manning et al. 2004; M. F. Fay pers. comm.). Despite these cautions, the available data indicate that dynamic change in chromosome number and genome size is characteristic of Asparagales, with taxa with the fewest and most chromosomes and the smallest and largest genomes found in the most diverse families in three clades: Orchidaceae, Iridaceae, and a clade composed of Alliaceae s.l., Asparagaceae s.l., and Xanthorrhoeaceae s.l.

Despite extensive research on chromosome biology in the petaloid monocots, previous reviews on base chromosome number evolution relied on antiquated concepts of phylogenetic relationships. For example, Tamura (1995) pointed out that Raven (1975) hypothesized that Liliaceae s.l. (e.g., Cronquist 1988) all had basic chromosome numbers from $x = 7$ to $x = 11$. However, since Raven (1975), Liliaceae have been circumscribed much more narrowly to exclude many taxa with genera formerly assigned to Liliaceae now placed in numerous families across several orders. Chromosome numbers have been mapped onto phylogenetic trees for a number of families in Asparagales, including Agavaceae s.s. (Bogler et al. 2006), Amaryllidaceae s.s. (Meerow et al. 1999, 2000, pers. comm.), Asphodelaceae s.s. (Chase et al. 2000*a*; Devey et al. 2006), Iridaceae (Reeves et al. 2001; P. Goldblatt pers. comm.), and Ruscaceae s.s. (Yamashita and Tamura 2000; Rudall et al. 2000). However, hypothesizing base chromosome numbers remains elusive even for these well-studied families due to either lack of phylogenetic resolution or lack of chromosome counts in key early diverging taxa. To date, there are only two examples of base chromosome numbers providing synapomorphies among families in Asparagales: Asparagaceae and Ruscaceae s.s. (but not Laxmanniaceae) share $x = 10$; and Ixioliriaceae and Tecophilaeaceae share $x = 10$ (Tamura 1995). Perhaps this is no surprise given that base chromosome numbers vary substantially even within monocot families, as seen in the grasses (Gaut 2002). Regardless, the range of Asparagales chromosome numbers in Fig. 3 indicates that their genomes are evolutionarily labile.

Genome size measurements are beginning to be mapped onto phylogenetic trees because they play a role in plant systematics at several taxonomic levels, from intraspecific variation within species to comparisons among genera and families (Grime 1998; Ohri 1998; Soltis et al. 2003). Hence, robust phylogenetic trees are essential for both global (genome size) and local (fine-scale) inferences of past genomic evolutionary events (Wendel et al. 2002*b*). Within Asparagales, examples of such studies include the slipper orchids and Asphodelaceae. In the slipper orchids (Cypripedioideae), DNA content varies 5.7-fold, with this change in genome size variation occurring independently of Robertsonian changes in chromosome fragmentation (Cox et al. 1998). Such observations have been seen in other lineages of the monocots, such as Commelinaceae, in which Robertsonian changes are well known (Jones 1998). A similar pattern is found in Asphodelaceae, in which DNA amounts vary twofold just among diploid species of *Aloe* with the same number of chromosomes (Brandham and Doherty 1998). Our understanding of other genera with numerous existing DNA content measurements (e.g., *Allium,* Ohri et al. 1998; Ohri and Pistrick 2001) will benefit from species-level phylogenetic analyses that may help clarify the dynamics of genome size evolution and their relationships to life-history traits. On a cautionary note, optimizations of genome sizes on trees sampling only a few species out of perhaps hundreds or thousands are likely to be misleading if the variation in genome size within the group is not well characterized. This caution applies to some extent to this study because in many cases only a small percentage of the species have been sampled. Thus, we can only speak about general trends and cannot come to firm conclusions.

Even given our limitations about knowledge of mechanisms for chromosomal change, we can revisit Darlington's hypotheses on the origin of the bimodal karyotype in the "desert-loving genera" of Asparagales (Darlington 1963, 1973; Fig. 1). The genera that particularly excited Darlington included *Yucca, Agave,* and *Hosta,* which all have $2n = 60$ ($x = 30$, with bimodal karyotype of 5 large and 25 small pairs of chromosomes, or in shorthand, 5L + 25S). *Aloe, Ornithogalum* L., *Gasteria* Duval., and *Eucomis* also have bimodal karyotypes (*Aloe* with 4L + 3S chromosomes; *Ornithogalum umbellatum* with 5L + 25S; *Gasteria bayfieldii* Baker with 4L + 3S; and *Eucomis bicolor* Baker with 4L+ 11S). Darlington puzzled over several criteria, including morphology, biogeography, and karyotypes. For example, *Agave* and *Aloe* shared xeric habits and *Hosta* had mesic habits, but *Agave* and *Hosta* shared the same bimodal karyotype (5L + 25S) whereas *Aloe* differed (4L + 3S). So, did particular bimodal karyotypes evolve once or multiple times? Darlington's answer was that there were multiple origins of bimodality, and he hypothesized that Hutchinson's phylogenetic scheme (1959) was correct, with *Agave* and *Yucca* united by both karyotype and biogeography, but with *Hosta* having a closer association with *Aloe* despite their different karyotypes. However, Darlington qualified his hypothesis by saying that all of these taxa (along with *Eucomis* and others) may be related by ancient polyploid events. Thus, Darlington evoked mechanisms of shared ancestral polyploidy followed by loss of chromosomes to explain how the *Agave* and *Aloe* types of bimodal karyotypes are related, but that *Agave*-like karyotypes found in such genera as *Ornithogalum, Hyacinthus* L., and *Scilla* L. arose in parallel. Finally, Darlington hypothesized that *Doryanthes* in Australia and *Phormium* in New Zealand evolved from the *Agave–Yucca* group centered in Mexico, thus showing a correlation between biogeographic migration and chromosome number (Fig. 1). In terms of mechanisms, Darlington proposed a base chromosome evolution sequence from $x = 7$ to 14/15–30 and subsequent losses to $x = 19$, 16, and 12. Superimposed on this broad pattern was intrageneric and intraspecific

polyploidy. Although Darlington considered chromosome number to be a character of immense weight, he still allowed morphological and biogeographical criteria to weigh in, as seen in his decision to align *Hosta* with *Aloe* instead of *Agave* (Fig. 1).

Bimodal karyotypes are found in several families of Asparagales (Fig. 3), including Orchidaceae (e.g., tribe Orchideae; Stebbins 1971; Greilhuber 1995), Iridaceae (e.g., tribe Tigridieaae; Kenton et al. 1990), Xanthorrhoeaceae s.l. (e.g., Asphodelaceae s.s., Hemerocallidaceae s.s.; Brandham and Doherty 1998; Adams et al. 2000*a*, *b*; Chase et al. 2000*a*; Devey et al. 2006), Asparagaceae s.l. (e.g., Agavaceae s.s. and Hyacinthaceae s.s.; Darlington 1973; Stedje 1988, 1989; Bogler and Simpson 1996; Pedrosa et al. 2001; Bogler et al. 2006), and Alliaceae s.l. (e.g., *Lycoris*; Darlington 1973; A. W. Meerow pers. comm.). Within these families, the bimodal karyotype is often found in several clades, indicating that it has evolved more than once even within a family. For example, within Asparagaceae s.l., the bimodal karyotype is found in several genera of Hyacinthaceae s.s. and Agavaceae. Given that *Agave* and *Yucca* are related, Darlington's scenario was problematic because we now infer that *Hosta* is related to *Agave* and *Yucca* (Agavaceae s.s., now including *Hesperocallis,* see Pires et al. 2004; and Bogler et al. 2006). Darlington's other hypotheses are also off the mark because *Aloe* and *Phormium* (Xanthorrhoeaceae s.l.) and *Doryanthes* (Doryanthaceae) are relatively distantly related to the Mexican genera in Agavaceae s.s. and Hyacinthaceae s.s.

Darlington's philosophy toward plant systematics was a synthetic one, as he tried to link experimental breeding studies of a few hundred cultivated species to chromosomal studies diffused over many thousands of wild species. More recently, botanists have asserted that "cytology is not useful at higher levels" (Greilhuber 1995), but studies of synteny across the grass family (Gaut 2002) and even across the monocot/magnoliid-eudicot divide (Vision et al. 2000; Bowers et al. 2003) indicate that large chromosomal blocks can be evaluated across deep branches in the phylogeny of the monocots. To date, these bioinformatic evaluations have been conducted among taxa used as models in genome sequencing (e.g., *Arabidopsis* and *Oryza*). What are needed now are genomic tools to evaluate mechanisms of chromosomal evolution in Asparagales.

Mechanisms of Chromosomal and Genome Evolution

Polyploidy and chromosomal evolution play fundamental roles in the formation and evolution of both plant and animal species (Stebbins 1950, 1971; Grant 1971, 1981; Bush 1981; Soltis and Soltis 2000*b*; Wendel 2000; Rieseberg 2001; Noor et al. 2002; Delneri et al. 2003; Navarro and Barton 2003; Osborn et al. 2003), but we have little data on how changes in chromosome number come about. In plants, polyploidy is common, as are the mechanisms that lead to rearrangements and rediploidization of polyploid genomes (reviewed in Wendel 2000). Polyploidy has been documented in every family of Asparagales (sensu APG II 2003; Fig. 3) except Blandfordiaceae (four species), Lanariaceae (monotypic), and Xeronemataceae (two species) as summarized by Tamura (1995). In addition to polyploidy, chromosome number can change by the "loss and gain" of single chromosomes and Robertsonian changes. One possibility is that Robertsonian rearrangements could promote dysploidy in taxa with large and medium-sized chromosomes (Jones 1998). Tamura (1995) reported that Robertsonian rearrangements occurred in Alliaceae s.l. (*Allium, Crinum* L., *Lycoris* Herb., *Nothoscordum* Kunth), Iridaceae (*Crocus, Galaxia* Thunb.), and Orchidaceae (*Paphiopedilum* Pfitzer). Genera in Asparagales with small chromosomes can also vary in base chromosome number (*Cyanastrum* Oliv., *Dracaena, Lomandra* Labill.) as seen in taxa with small chromosomes outside Asparagales (*Carex* L., *Drosera* L., *Luzula* DC.). However, those last genera have non-localized centromeres, which are unknown in Asparagales (Tamura 1995). At this point, our knowledge of mechanisms that change chromosome number in Asparagales is limited.

Mechanisms of genome size evolution in plants and animals have been recently reviewed (Bennetzen 2000, 2002; Petrov 2001, 2002; Betran and Long 2002; Gaut 2002; Gregory 2002, 2003; Hancock 2002; Kidwell 2002; Sternberg 2002; Wendel et al. 2002*a*, *b*; Zuckerkandl 2002; Casacuberta and Santiago 2003; Gallardo et al. 2003; Lynch and Connery 2003; Lynch and Kewalramani 2003). In brief, three primary mechanisms increase genome size: (1) polyploidy = whole genome duplication; (2) segmental duplication (via unequal recombination or non-reciprocal translocations with selection bias to genome size increase); and, (3) transposable element (TE) insertion. Similar processes can also decrease genome size: (1) dysploidy = whole chromosome loss; (2) rapid gene loss after polyploidization (e.g., Song et al. 1995; Shaked et al. 2001); (3) unequal recombination coupled to selection bias to genome size decrease (e.g., gene family shrinkage); and, (4) deletions of numerous kinds (e.g., bias to small intron sizes).

Given any phylogenetic comparison for change in genome size, it is likely that more than one mechanism is at play. Previously, it had been thought that genome sizes in plants have a "one-way ticket" to genomic obesity as seen in the grass genomes (Bennetzen and Kellogg 1997). For example, the amplification of transposable elements in the grass family (reviewed in Bennetzen 2002) indicates higher transposition rates in those taxa. However, Gaut (2002) cautioned that there are numerous mechanisms that increase genome size in the grasses (e.g., ancient and recent polyploidy), and almost nothing is known about mechanisms for genome size decrease. Another recent idea is that large genome sizes are related to population bottlenecks (Lynch and Connery 2003), which corresponds with the observation that rare plants often have large genomes (due to more selfish DNA) in comparison to more common related taxa (Vinogradov 2003). This has led some researchers toward investigations of mechanisms such as unequal intrastrand recombination and illegitimate recombination that may unidirectionally increase or decrease genome size (e.g., between large regions that lack homology; Devos et al. 2002; Orel and Puchta 2003) . Thus, future genome size studies should: (1) focus on mechanisms, especially for genome size decrease in plants; and, (2) use phylogenetic patterns to uncover ancestral character states and directions of change (Bennetzen 2002; Wendel et al. 2002*a*, *b*; Soltis et al. 2003). In Asparagales, we are only beginning to understand these mechanisms, but in addition

to polyploidy there is evidence for transposable element amplification in Iridaceae (Kentner et al. 2003) and intrachromosomal duplication in *Allium* (Havey 2002).

Genomic Tools Needed to Decipher Mechanisms of Chromosomal Evolution

Understanding genome evolution in Asparagales, or any plant group, will require detailed knowledge of both phylogenetic patterns and mechanisms of chromosomal evolution. To make further progress in phylogenetics, we will need to move beyond sequences of plastid and nuclear ribosomal DNA, particularly to reconstruct hybrid and polyploid speciation. Because the plastid genome is predominantly inherited as a single haploid linkage group, and nuclear ribosomal DNA (nrDNA) sequences can be homogenized by concerted evolution/gene conversion, a series of low-copy nuclear genes is needed to detect reticulation and to increase phylogenetic resolution (Sang 2002; Lawton-Rauh 2003; Small et al. 2004). Whereas the phylogenetic utility of low-copy nuclear genes is confounded by the need to distinguish orthologs from paralogs, copy number can be estimated by Southern blotting or fluorescent in situ hybridization (FISH) (Jackson et al. 1998; Small and Wendel 2000; Small et al. 2004; Walling et al. 2005). To unravel ancient genome duplications and use nuclear genes as physical markers on chromosomes, one would ideally choose nuclear genes in separate linkage groups. In eudicots, one can use the complete sequence of the *Arabidopsis* genome in a bioinformatics approach to identify putatively single- or low-copy nuclear genes in related model taxa such as tomato or soybean, and in fact such conserved ortholog set (COS) markers have been constructed (Fulton et al. 2002). So how can we identify both copy number and location of putatively low-copy nuclear genes in Asparagales?

One efficient approach is to use small-genome species to study related large-genome species (Bennett 2000; Bancroft 2001). For example, Jackson et al. (2000) used six BACs as FISH probes to identify a 431-kb region of the *Arabidopsis thaliana* (L.) Heynh. genome on chromosome 2, and then used the same BACs to find the homologous region in four to six areas of the *Brassica rapa* L. genome. This result is consistent with the hypothesis that the "diploid" *Brassica rapa* has experienced ancient polyploid events relative to the *Arabidopsis* genome. Similarly, small genome *Sorghum* L. has been used as a foundation for integrating genetic and physical maps across grass genera with larger genomes (Zwick et al. 1998; Draye et al. 2001; Islam-Faridi et al. 2002; Kim et al. 2002; Koumbaris and Bass 2003). The key genomics tool in both of these sets of studies was a BAC library made at a reasonable cost from a small-genome species (*Arabidopsis* and *Sorghum*). Studies that integrate comparative genetics at the gene and chromosomal levels have been carried out between rice (*Oryza*) and wildrice (*Zizania* Gronov. ex L.) (Haas et al. 2003), but to date no study has integrated nuclear gene phylogenetic studies with BAC-FISH based studies of chromosomal evolution.

In Asparagales, BACs could be used to decipher which mechanisms are at play to create bimodal karyotypes, such as ancient polyploidy or fission-fusion events (Fig. 4). Thus, comparative BAC-FISH mapping can be used to study chromosomal duplications and expansions of genomes (Walling et al. 2005). Given a modest investment in a few BAC libraries derived from taxa across the Asparagales phylogenetic tree, a host of questions could then be addressed. What is the distribution of genes on large and small chromosomes? Do the tempo and mode of molecular gene evolution differ on large vs. small chromosomes? Does polyploidy "accelerate" evolution? In animals, it appears that there is "accelerated evolution" in lineages that have rearranged chromosomes (chromosomal speciation; Navarro and Barton 2003). Thus, phylogenetic questions and cytogenetic questions can be integrated, with BAC libraries offering approaches that could be used to do both things. BAC libraries, by providing a window for examination of blocks of chromosomes, serve as both markers for physically mapping chromosome evolution and can be tied to nuclear gene phylogenetic trees by assisting in homology assessment.

Phylogenomics of Asparagales: Necessity and Criteria for Developing Model Taxa

Chromosome and genome-size changes occur frequently in evolution, and even the most closely related species can show differences in their karyotypes. Among animals, phyla with high rates of chromosomal change also have high rates of speciation (Bush 1981; Navarro and Barton 2003) and the same appears to be true in angiosperms (Greilhuber 1998; Bennett et al. 2000; Rieseberg 2001). Polyploidization and chromosomal rearrangements are thought to generate reproductive isolation or prevent recombination in linkage groups that contain ecologically important loci, thereby playing a role in speciation (Rieseberg 2001; Lonnig and Saedler 2002). Fluctuations in chromosome number and genome size have also been correlated with a number of morphological and environmental variables in plants, which can generate novel phenotypes that lead to habitat divergence (Levin 1983; Bennett 1987; Grime 1998; Ohri et al. 1998; Watanabe et al. 1999; Bennett et al. 2000; Givnish et al. 2000; Gregory 2002; Osborn et al. 2003).

Despite the rapid progress in understanding grass genomes (Gaut 2002), our understanding of Asparagales genomes is rudimentary. We might postulate that Asparagales genomes evolve similarly to grass genomes. However, grass genomes may not be representative of other genomes of the monocots, and in fact, recent evidence indicates that in some ways Asparagales genomes are more like that of *Arabidopsis* (Kuhl et al. 2004; see Havey 2006). Given this, deciphering genome evolution in Asparagales is critical in understanding monocot evolution because of: (1) the incredible diversity of Asparagales (e.g., orchids); (2) the economic importance of Asparagales (e.g., onion, garlic, asparagus, aloe, yucca, ornamentals); and, (3) the phylogenetic position of Asparagales within monocots, because they bridge the gap between the well-studied Poales and *Acorus* L., the sister of the rest of the monocots, which is also now becoming a model taxon (Soltis et al. 2002).

We argue that BAC libraries should be made for several lineages of Asparagales. Other genomic studies (Mandoli and Olmstead 2000; Hall et al. 2002; Pryer et al. 2002) have established criteria for choosing model taxa. These include phylogenetic position, clade diversity, economic importance,

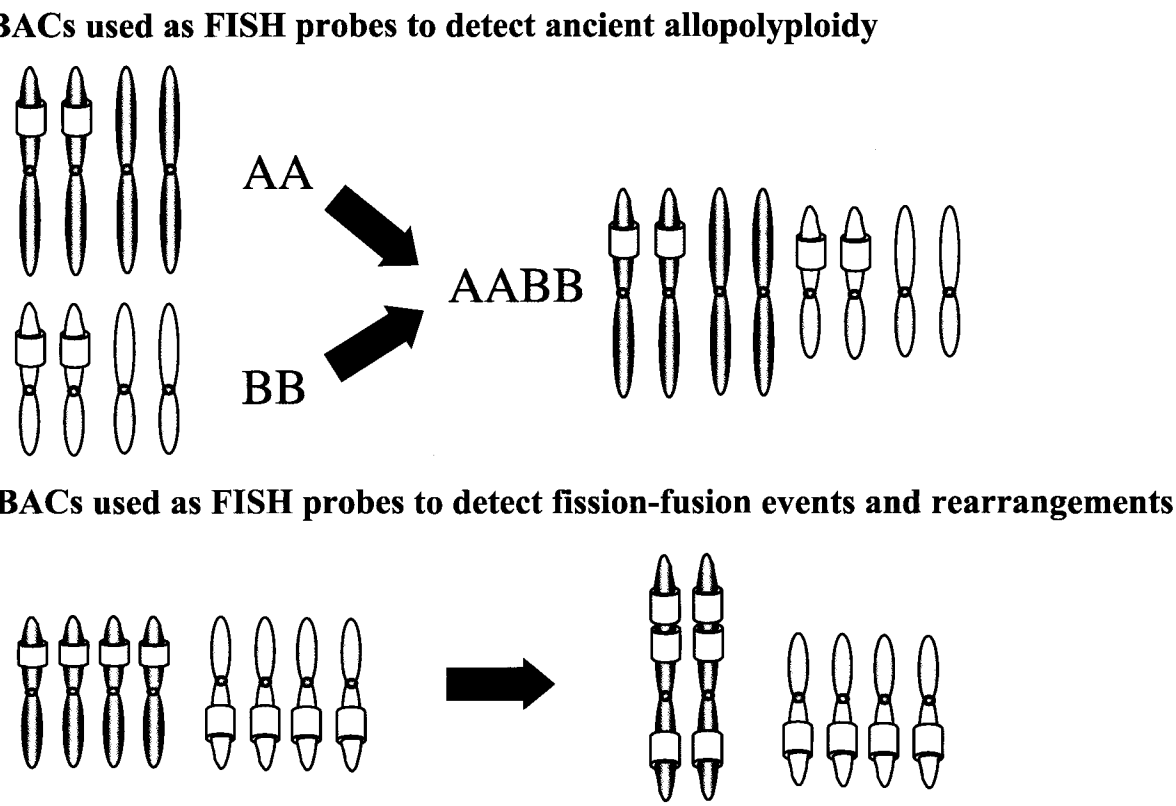


Fig. 4.—Bacterial artificial chromosomes (BACs) can be used to integrate the studies of chromosomes with evolutionary studies of nuclear DNA. BACs can be used as molecular cytogenetic markers to infer mechanisms that may have caused bimodal karyotypes, such as ancient polyploid events or chromosomal rearrangements.—A. An ancient allopolyploid event between two genomes with different size chromosomes (AA with large shaded chromosomes and BB with small open chromosomes) can be detected by using BACs (white bars) that have homeologous regions in each of the two parental genomes.—B. Chromosomal rearrangements have led to fission-fusion events among the small-shaded chromosomes to create large chromosomes in the bimodal karyotype. These fission-fusion events are detected by BACs (white bars) that are specific to a set of chromosomes prior to the rearrangements.

and experimental amenability (e.g., annual life cycle, transformability, etc.). Given these criteria, six clades are species-rich: Alliaceae s.l., Asparagaceae s.l., Hypoxidaceae, Iridaceae, Orchidaceae, and Xanthorrhoeaceae s.l. Both *Asparagus* (Lee et al. 1997; Rudall et al. 1998*b*; Pavesi et al. 2000) and *Allium* are promising models because of their economic importance and existing genetic maps (Suzuki et al. 2001; Fritsch and Friesen 2002; Havey 2002; Klaas and Friesen 2002; Shibata and Hizume 2002; see Havey et al. 2006). Additional choices for models would depend on questions of interest, but likely candidates would be species with small genome sizes from either Iridaceae or Orchidaceae given both their diversity, horticultural interest, and wide range of chromosome numbers (e.g., Kenton et al. 1986; Rudall et al. 1986; Reeves et al. 2001; van Doorn et al. 2003).

It is clear that Asparagales genomes evolve dynamically with polyploidy, chromosomal rearrangements, and changes in genome size through time. Our robust phylogenetic tree, derived from molecular data and consistent with morphological and genomic synapomorphies, provides an evolutionary framework to examine patterns of change in genome size and chromosome number. Our knowledge of phylogeny and chromosome numbers does not yet provide extensive insights into base chromosome number for several families, and changes in DNA content and chromosome number likely reflect numerous genomic mechanisms that we are only beginning to understand. In any case, our first look at phylogenetic patterns, chromosome evolution, and genome size indicates that Asparagales genomes are dynamic, rapidly evolving entities.

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