

Phylogenetics and chromosomal evolution in the Poaceae (grasses)

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Abstract. The wide range in basic chromosome number ($x = 2$ –18) and prevalence of polyploidy and hybridisation have resulted in contrasting views on chromosomal evolution in Poaceae. This study uses information on grass chromosome number and a consensus phylogeny to determine patterns of chromosomal evolution in the family. A chromosomal parsimony hypothesis is proposed that underscores (1) the evolution of the Joinvilleaceae/Ecdeiocoleaceae/Poaceae lineage from Restionaceae ancestors with $x = 9$, (2) aneuploid origin of $x = 11$ in Ecdeiocoleaceae and Poaceae (*Streptochaeta*, Anomochlooideae), (3) reduction to $x = 9$, followed by chromosome doubling within Anomochlooideae to generate the $x = 18$ in *Anomochloa*, and (4) aneuploid increase from the ancestral $x = 11$ to $x = 12$ in Pharoideae and Puelioideae, and further diversification in remaining taxa (Fig. 3b). Higher basic chromosome numbers are maintained in basal taxa of all grass subfamilies, whereas smaller numbers are found in terminal species. This finding refutes the ‘secondary polyploidy hypothesis’, but partially supports the ‘reduction hypothesis’ previously proposed for chromosomal evolution in the Poaceae.

Introduction

Variation in basic chromosome number, high incidence of polyploidy, frequent hybridisation and wide range of variation in genomic size are prominent features of grass-genome evolution. These features are evident in the estimated 80% polyploidy, basic chromosome numbers of $x = 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14$ and 18 (deWet 1987; Hunziker and Stebbins 1987), and 2C DNA contents of 0.7 in *Chloris gayana* to 27.6 in *Lygeum spartum* (Bennett *et al.* 2001). The distribution of these basic chromosome numbers in the various grass subfamilies has resulted in wide disagreements on the polarity and trends of chromosome evolution. High frequency of polyploidy, particularly allopolyploidy and aneuploidy, a tendency for rediploidisation and frequent hybridisation between closely and distantly related species compound the difficulties in deducing the trends. Above all, lack of a robust phylogeny based on empirical approaches has resulted in equivocal assertions on chromosomal evolution in Poaceae. Consequently, contrasting views have emerged on chromosomal evolution in the family (Avdulov 1931; Flovik 1938; Hubbard 1948; Raven 1975; Clayton 1981; Stebbins 1982, 1985; deWet 1987; Hunziker and Stebbins 1987). Currently, a general consensus on grass phylogeny does exist and the identity of the most basal lineages is well documented, making prediction and interpretation of

chromosomal evolution more attainable. In this study, available knowledge of chromosome variation and its patterns of distribution in grass subfamilies and tribes will be evaluated and superimposed on a well supported consensus phylogeny. Previous hypotheses on chromosomal evolution in Poaceae will be critically discussed and a new assessment will be formulated. Information on chromosome numbers is obtained from Dahlgren *et al.* (1985), Clayton and Renvoize (1986), Watson and Dallwitz (1992), Kubitzki (1998), and several other references cited here.

Current hypotheses on chromosomal evolution in Poaceae

The ancestral chromosome number and trends in chromosomal evolution in grasses have been the subject of considerable speculation. Avdulov (1931) proposed the ‘reduction hypothesis’ which maintains that $x = 12$ is the ancestral number from which lower numbers originated through aneuploidy. Raven (1975) agreed with this hypothesis and used as evidence the prevalence of $x = 12$ in the Bambusoideae, which were presumed to be the most ancestral grasses. He concluded that $x = 6$, found in some advanced genera, is of aneuploid origin. Stebbins (1982, 1985) disagreed with the ‘reduction hypothesis’ and presented the ‘secondary polyploidy hypothesis’ which suggests $x = 12$ and 11 as secondary chromosome numbers

derived from $x = 5$ and 6. He used the countervailing arguments that $x = 6$ and 7 are found in the most primitive genera of their tribes, such as *Danthonia* and *Pentaschistis* of the Danthoniaceae and *Sorghum* of the Andropogoneae.

Hubbard (1948), Stebbins (1985) and deWet (1987) proposed independent origins for the basic chromosome numbers in the major groups of grasses from species complexes with $x = 5, 6$ and 7. DeWet (1987) argued that if one followed Clayton's (1981) proposed scheme of grass evolution, the Arundoideae with its unspecialised genera and broad diversity in basic chromosome numbers ($x = 6, 7, 9, 10, 11$) would have given rise to the $x = 7$ of the Pooideae, $x = 9$ and 10 of the Panicoideae and Chloridoideae, whereas $x = 10-12$ of the Bambusoideae and $x = 12$ of Oryzoideae and Centothecoideae would be derived from a pre-arundinoid grasses. This evolutionary pattern was not convincing to him and, thus, he speculated that the major groups of Poaceae were independently derived from an original species complex with $x = 5, 6$ and 7. He asserted that hybridisation and chromosome doubling, followed by diploidisation at the tetraploid level, gave rise to secondary diploids, with $x = 10, 12$ and 14. DeWet also indicated that aneuploidy and further cycles of polyploidy resulted in somatic chromosome numbers ranging from $2n = 6$ to $2n = 265$. Mehra *et al.* (1968), Sharma (1979) and Hunziker and Stebbins (1987) argued in favour of $x = 6$ as the most ancestral chromosome number. Hunziker and Stebbins (1987) speculated that from this number, the secondary basic number of $x = 12$ originated and subsequently gave rise to higher polyploids. Aneuploid events in both directions gave origin to $x = 7$ and 5. They also proposed that $x = 5$ is ancestral to $x = 10$, and from the latter evolved $x = 11, 9, 8$, and again 7 (as in *Olyra*).

Patterns of variation in Poaceae chromosome numbers

The basic chromosome numbers of $x = 2-18$ in the Poaceae have generated somatic numbers that vary between $2n = 4$ and $2n = 263-265$ (deWet 1987). The most common basic chromosome numbers in the family are 7, 9, 10 and 12 (Stebbins 1982). The number $x = 2$ is reported for the Pooideae species *Colpodium versicolor* (tribe Poeae) and *Zingeria biebersteiniana* (tribe Aveneae) (Tsvelev and Zhukova 1974; Sokolovskaya and Probatova 1977). *Colpodium* contains species with $x = 2, 4, 5, 6, 7$ and 9, and *Zingeria* species with $x = 2, 4$ and 6; both series may represent aneuploid reduction derived from the pleisomorphic number $x = 7$ that is predominant in these tribes. These chromosomal series underscore the remarkable flexibility in genomic evolution in the Poaceae.

Various patterns of variation at the subfamily level are detectable (Fig. 1). The subfamily Anomochlooideae, encompassing *Anomochloa* and *Streptochaeta*, is based on $x = 18$ and 11, respectively (Pohl and Davidse 1971; Davidse and Pohl 1972; Hunziker and Stebbins 1987; Hunziker *et al.* 1989). The Pharoideae have $x = 12$ (Davidse and Pohl 1972;

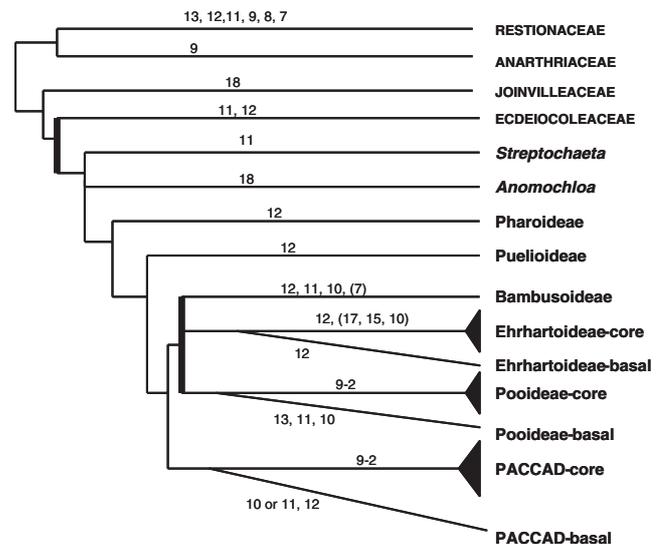


Fig. 1. A consensus tree for the Poaceae based on Hilu *et al.* (1999) and GPWG (2001) molecular and non-molecular data. Basic chromosome numbers are mapped on the tree. Dark lines represent potentially unresolved lineages and parentheses enclose infrequent chromosome numbers in taxa.

Clayton and Renvoize 1986). In the Bambusoideae *s.s.*, the basic number $x = 12$ is characteristic of woody members, although a few species with $x = 10$ have been reported (Clayton and Renvoize 1986). Herbaceous members of the Bambusoideae, however, have basic numbers of $x = 7, 10, 11$ and 12 (Tateoka 1955; Kammacher *et al.* 1973; Hunziker *et al.* 1982; Clayton and Renvoize 1986; Hunziker and Stebbins 1987). Homogeneity in chromosome number is a trademark of the oryzoid grasses, with $x = 12$ appearing in all but *Zizania* and *Microlaena*. In contrast, the Pooideae stands out with the highest diversity in chromosome number, displaying a wide range of numbers from $x = 2$ to $x = 13$. Diversity in basic chromosome number is also evident in the Arundoideae ($x = 6, 9, 12$), Panicoideae ($x = 5, 7, 9, 10, 12, 14$) and Chloridoideae ($x = 7, 8, 9, 10$). However, these subfamilies tend to have predominant basic numbers (such as $x = 9$ and 10 in the Panicoideae and Chloridoideae) and some rare numbers.

Current views on grass evolution

Cladistic analyses of morphological, anatomical and molecular information in the past two decades have refined our understanding of grass phylogeny (reviewed in Hilu *et al.* 1999; GPWG 2001) and a consensus phylogeny has emerged (Fig. 1). Recent molecular studies in particular have provided critical testing of the various proposed hypotheses on grass evolution. A general agreement on the monophyly of subfamilies and tribes and their phylogenetic position are evident (Barker *et al.* 1995; Clark *et al.* 1995; Hilu *et al.* 1999; GPWG 2001). Owing to the high congruence between the two recent comprehensive treatments of the family by

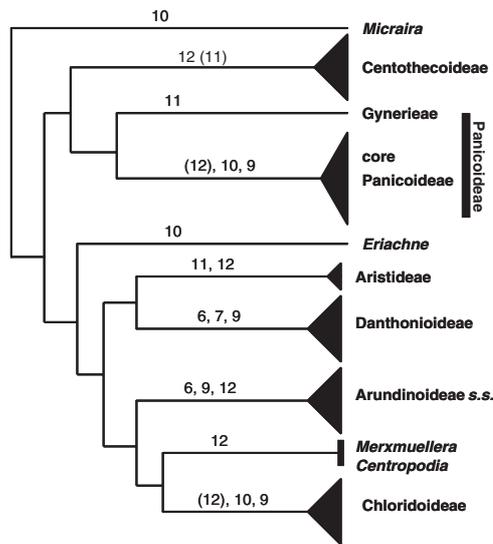


Fig. 2. A consensus tree of the PACCAD clade based on Hilu *et al.* (1999) and GPWG (2001), with basic chromosome numbers mapped on the tree. Solid triangles represent core groups in the lineages, lines depict basal taxa and parentheses enclose infrequent chromosome numbers in taxa.

using *matK* data (Hilu *et al.* 1999) and molecular and structural information (GPWG 2001), this paper will follow the taxonomic treatment proposed by the latter study.

The Anomochlooideae (*Anomochloa* and *Streptochaeta*) is a sister taxon to remaining Poaceae; diverging next in a grade are the Pharioideae, Puelioideae, and then the remaining grasses (Barker *et al.* 1995; Clark *et al.* 1995; Soreng and Davis 1998; Hilu *et al.* 1999; GPWG 2001). Although the monophyly of *Anomochloa* and *Streptochaeta* has been questioned (Hilu and Alice 1999; Hilu *et al.* 1999, Zhang 2000), their most basal position in the phylogeny is not. Further support for the basal lineages is evident in the lack of a 1-bp deletion that caused a frame shift at the 3' end of the *matK* gene in all grasses but *Streptochaeta* and *Anomochloa* (Hilu and Alice 1999). The lack of this mutation is shared with the outgroup Joinvilleaceae but not Restionaceae (Hilu and Alice 1999).

The subfamilies Arundinoideae, Centothecoideae, Chloridoideae and Panicoidae form a strongly supported lineage named PACC (Esen and Hilu 1989; Davis and Soreng 1993; Barker *et al.* 1995; Clark *et al.* 1995; Hilu and Alice 1999; Hilu *et al.* 1999). Recently, subfamilies Aristidoideae and Danthonioideae have been recognised to accommodate the isolated tribe Aristideae and a danthonioid segregate from the Arundinoideae, respectively, modifying the acronym to PACCAD (Fig. 2). The following two major groups are evident in the PACCAD lineage: (1) panicoid and centothecoid, and (2) aristidoid, danthonoid, arundinoid and chloridoid (GPWG 2001). In addition to the PACCAD, Clark *et al.* (1995) recognised the BOP lineage that comprises bambusoid, oryzoid and pooid taxa (the acronym is changed

to BEP because the name Oryzoideae is replaced by Ehrhartoideae). Although the alliance among these clades is not strongly supported, they are phylogenetically distinct from the PACCAD subfamilies (Fig. 1). Another important finding that will also have an impact on interpreting chromosome evolution in grasses is the solidification of the phylogenetic positions of some tribes whose affinities have been uncertain. Outstanding among these are the Diarrheneae, Ehrharteae, Lygeae, Micraireae, Nardeae and Stipeae. It is also to be noted that the identities of basal lineages in a number of subfamilies are well ascertained and the phylogenetic positions of various tribes are well resolved (Hilu *et al.* 1999; GPWG 2001).

Sister-group relationships of Poaceae

Another issue crucial to understanding chromosomal evolution in the Poaceae is the status of the sister-group relationship to the family. Poales as defined by Dahlgren *et al.* (1985) and confirmed in recent studies (see Briggs *et al.* 2000 and Michelangeli *et al.* 2003) comprises the families Flagellariaceae, Centrolepidaceae, Anarthriaceae, Restionaceae, Joinvilleaceae, Ecdeiocoleaceae and Poaceae. Flagellariaceae is generally considered as sister to remaining Poales (Briggs *et al.* 2000; Linder *et al.* 2000; Michelangeli *et al.* 2003). The precise position of Anarthriaceae and Centrolepidaceae in Poales has been debated; however, the two families are closer to the Restionaceae than Joinvilleaceae, Ecdeiocoleaceae and Poaceae (Briggs *et al.* 2000; Linder *et al.* 2000; Bremer 2002; Michelangeli *et al.* 2003). Bremer (2002) and Michelangeli *et al.* (2003) showed Anarthriaceae as sister to Restionaceae. Among Poales families, it has been demonstrated that Joinvilleaceae and Ecdeiocoleaceae are most closely related to the Poaceae (Doyle *et al.* 1992; Kellogg and Linder 1995; Hilu and Alice 1999; Briggs *et al.* 2000; Bremer 2002; Michelangeli *et al.* 2003). This assessment is based on analyses of morphological and molecular data. In particular, two inversions found in the plastid genome of the Poaceae (Hiratsuka *et al.* 1989; Shimada and Sugiura 1991; Doyle *et al.* 1992; Katayama and Ogihara 1996; Michelangeli *et al.* 2003) have been effective in ascertaining phylogenetic relationships in Poales. These inversions are called the 28-kilobase (kb) and 6-kb inversions (a third inversion, called *trnT* inversion, is confined to Poaceae). Outside the Poaceae, the 28-kb inversion was detected only in the plastid genomes of the Joinvilleaceae, Ecdeiocoleaceae and some Restionaceae (Doyle *et al.* 1992; Katayama and Ogihara 1996; Michelangeli *et al.* 2003). Doyle *et al.* (1992) found the 6-kb inversion in Joinvilleaceae and Restionaceae, but were unable to detect it in the Ecdeiocoleaceae. However, the presence of the 6-kb inversion in the Ecdeiocoleaceae was recently demonstrated by Michelangeli *et al.* (2003). Therefore, the 28-kb inversion defines the Restionaceae, Joinvilleaceae, Ecdeiocoleaceae and Poaceae clade in Poales, whereas the 6-kb inversion is a

synapomorphic character uniting Joinvilleaceae, Ecdeiocoleaceae and Poaceae (designated here as JEP). Hilu *et al.* (1999, 2003), using *matK* sequence data, resolved Joinvilleaceae as sister to Poaceae, with Restionaceae being sister to both. This sister-group relationship was further confirmed by identifying base substitutions and an insertion at the 3' end of the plastid *matK* gene that resulted in a longer reading frame in these two families. Restionaceae lacked that indel but emerged as sister to both. Ecdeiocoleaceae was not examined in these studies.

Insight into the phylogenetic relationship among the JEP families comes from recent analyses of molecular and morphological information (Briggs *et al.* 2000; Bremer 2002; Michelangeli *et al.* 2003). Analysis of combined sequence data from *rbcL* and *trnT-F* regions (Briggs *et al.* 2000) resolved Ecdeiocoleaceae and Poaceae in a clade with 99% jackknife (JK) support (*trnT-F* data were not available for Joinvilleaceae); *rbcL* data alone showed Joinvilleaceae + Ecdeiocoleaceae sister to Poaceae (no jackknife values were reported). However, strongest support for the sister-group relationship of Ecdeiocoleaceae and Poaceae was evident in a parsimony and Bayesian analyses of sequence information from *rbcL* and *atpB* (Bremer *et al.* 2002) where the two families formed a clade supported by 99% JK and 1.00 posterior probability; Joinvilleaceae appeared as the sister to this clade (100% JK and 1.00 posterior probability). A similar topology emerged in an analysis of combined structural data and sequence information from *rbcL* and *atpA* (Michelangeli *et al.* 2003). Although the Ecdeiocoleaceae/Poaceae clade received low jackknife support (49%), the two alternative topologies, Joinvilleaceae/Poaceae and Ecdeiocoleaceae/Joinvilleaceae, were found to be least likely (<20% jackknife support).

The basic chromosome numbers in the families concerned are the following: Flagellariaceae, $x = 19$ (Appel and Bayer 1998); Anarthriaceae, $x = 9$ (Linder *et al.* 1998); and Joinvilleaceae, $x = 18$ (Bayer and Appel 1998). In Restionaceae, basic numbers of $x = 6, 7, 9, 11, 12$ and 13 have been reported for Australian taxa (Linder *et al.* 1998) and $2n = 16$ and 20 for African taxa (Krupko 1962, 1966). Chromosome numbers in Ecdeiocoleaceae are not precise as chromosome counts of $2n = 64-66$ for *Georgeantha* and $2n = \sim 48$ for *Ecdeiocolea* have been reported (B. Briggs, unpubl., cf. Linder *et al.* 1998). Centrolepidaceae is poorly known, with reports of $n = 10$ and 13 in two *Centrolepis* species, and $n = 20$ and ~ 24 in another two (Goldblatt 1980), implying that $x = 10$ for the former and $x = 12$, or most likely 13 , for the latter.

Results

Trends in chromosome evolution

To establish a robust theory on chromosomal evolution of any taxonomic group, three crucial points have to be

unequivocally determined: (1) monophyly of the ingroup, (2) the identity of the sister group, and (3) a robust phylogeny, with most basal lineages well defined. The monophyly of the Poaceae has been well ascertained in several studies (Barker *et al.* 1995; Clark *et al.* 1995; Duvall and Morton 1996; Liang and Hilu 1996; Soreng and Davis 1998; Hilu *et al.* 1999; GPWG 2001). The sister-group relationship of Poaceae to Ecdeiocoleaceae and Joinvilleaceae is well documented and is detailed above. In terms of the identity of most basal lineages in the Poaceae, the subfamilies Anomochloideae and Pharoideae, respectively, are the first taxa to diverge in phylogenies, based on different types of molecular and non-molecular data (Clark *et al.* 1995; Hilu and Alice 1999; Hilu *et al.* 1999; GPWG 2001). Therefore, all three basic criteria for a critical assessment of chromosome evolution for the grass family are met.

Chromosomal evolution at the base of grasses

The basic chromosome number in the Joinvilleaceae and *Anomochloa* is $x = 18$. Such a number is generally considered to be a rediploidised ancient polyploid (Masterson 1994) and, therefore, it is derived from $x = 9$. *Streptochaeta* is based on $x = 11$. The chromosome counts in Ecdeiocoleaceae of $2n = 64-66$ and ~ 48 are most likely based on $x = 11$ (hexaploid) and $x = 12$ (tetraploid), respectively. Assuming a basic chromosome number of $x = 9$ for the Ecdeiocoleaceae would imply heptaploidy for $2n = 64-66$ and pentaploidy for $x = \sim 48$; both are unlikely as they would require either overcoming meiotic irregularities or acquiring consistent apomixis. Considering the chromosome numbers reported for the Restionaceae and the sister-group relationship of this family to the JEP clade, both $x = 9$ and $x = 11$ have to be considered in assessing chromosomal evolution from Joinvilleaceae to Ecdeiocoleaceae and Poaceae. On the basis of these numbers, two hypotheses are proposed and illustrated in Fig. 3a, b. In both hypotheses, aneuploidy at $x = 9$ and 11 but not at $x = 18$ is assumed, and $x = 18$ in Joinvilleaceae and *Anomochloa* has arisen independently by chromosome doubling of $x = 9$.

If the parsimony principal is invoked here, the choice between these two chromosomal hypotheses ($x = 9$ v. $x = 11$) will depend on ascertaining the ancestral chromosome number for the Anomochloideae ($x = 9$ or 11) should the subfamily be considered monophyletic or whether *Streptochaeta* ($x = 11$) or *Anomochloa* ($x = 18$) is the sister taxon to remaining Poaceae should paraphyly be assumed for the subfamily. Information on this point may come from the three cases where the Anomochloideae appeared paraphyletic; in all these cases, *Streptochaeta* emerged sister to *Anomochloa* plus remaining Poaceae (Hilu *et al.* 1999; Hilu and Alice 1999; Zhang 2000). Considering the ancestral chromosome number in Anomochloideae, and consequently Poaceae, as $x = 11$, the hypothesis depicting $x = 9$ as ancestral basic chromosome number for the JEP

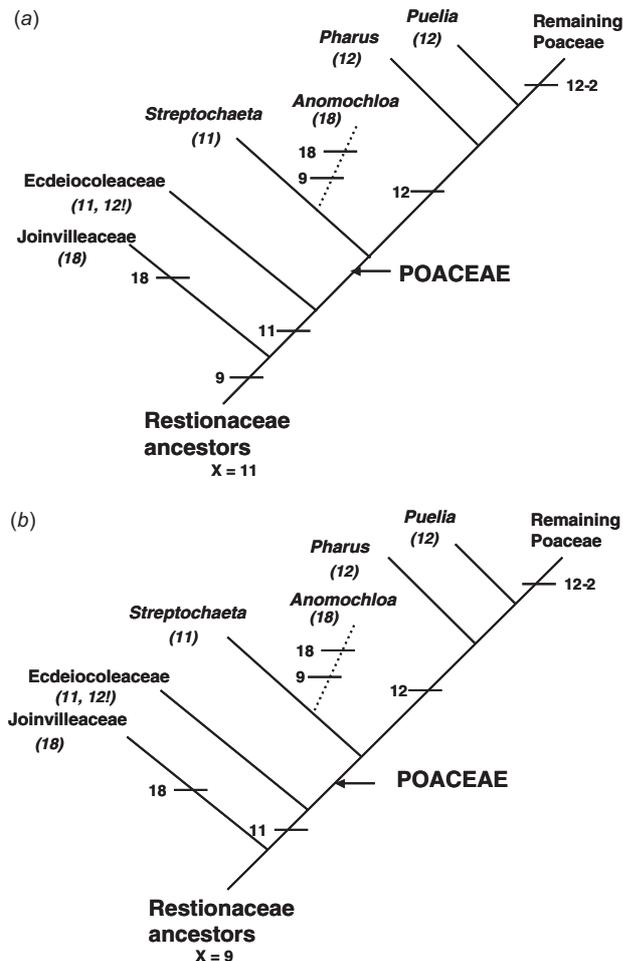


Fig. 3. Diagrammatic representations of two proposed hypotheses for chromosomal evolution from Restionaceae to Poaceae, with $x = 11$ (Fig. 3a) and $x = 9$ (Fig. 3b) as ancestral basic chromosome numbers. Changes in chromosome number are mapped on the trees.

lineage is more parsimonious, with Joinvilleaceae sister to Ecdeiocoleaceae plus Poaceae. In this case, only one aneuploidy event from $x = 9$ to $x = 11$ is required to the base of the grass tree (Fig. 3). In contrast, aneuploidy from $x = 11$ to $x = 9$ and a reversal to $x = 11$ is required should one assume $x = 11$ as an ancestral number for JEP.

Further support for a chromosomal evolution from $x = 9$ in Joinvilleaceae to $x = 11$ in Ecdeiocoleaceae and *Streptochoeta* in Poaceae comes from the position of Anarthriaceae ($x = 9$) in Poales. Bremer (2002) showed Anarthriaceae and Restionaceae as sister taxa (93% JK and 1.0 posterior probability). In the molecular-structural study of Michelangeli *et al.* (2003), Anarthriaceae emerged sister to Centrolepidaceae plus Restionaceae (87% JK). Similar topologies were also evident in the molecular study of Briggs *et al.* (2000) and were inferred from flavonoid data (Williams *et al.* 1997). Considering the sister-group relationship of Restionaceae/Anarthriaceae to the JEP clade and the basic chromosome number of $x = 9$ in Anarthriaceae and $x = 18$ in

Joinvilleaceae, the JEP and the Anarthriaceae/Restionaceae clades may share the ancestral basic chromosome number of $x = 9$.

I propose the chromosomal parsimony hypothesis to explain chromosomal evolution in the Poaceae. This hypothesis asserts that (1) the ancestral chromosome number for the Poaceae is $x = 11$, derived from a common ancestor with Ecdeiocoleaceae, (2) aneuploidy led to $x = 9$ and then chromosome doubling to $x = 18$ in *Anomochloa*, followed by (3) an aneuploid increase from $x = 11$ to $x = 12$ of *Pharus* and *Puelia* (Fig. 3b). This proposed theory is congruent with current views on grass phylogeny (Fig. 1), regardless of the definition of the Anomochlooideae.

Chromosomal evolution of terminal grass lineages

Next to diverge after these basal lineages are the BEP and PACCAD groups (Fig. 1). The former includes the Bambusoideae, Ehrhartoideae and Pooideae as currently defined (Hilu *et al.* 1999; GPWG 2001). Although monophyly of the group is not well substantiated and its topology is inconsistently reported in different studies, it is significant to note that high basic chromosome numbers are predominant in its subfamilies or at least their basal taxa. The number $x = 12$ prevails in woody Bambusoideae and is found in all ehrhartoid grasses except for *Zizania* ($x = 15$ and 17). Herbaceous bambusoids also have high chromosome numbers ($x = 10, 11$ and 12); the exception here is the Olyreae in which there is an aneuploid series of $x = 7$ and 9, in addition to $x = 10-12$ (Hunziker *et al.* 1982). Moreover, chromosome numbers of $x = 10, 11$ and 13 are characteristic of basal genera and tribes of the Pooideae (Fig. 1). Therefore, high (≥ 10) basic chromosome numbers remained prevalent in the family during its early stages of evolution. Only in the Pooideae, the second largest grass subfamily, did smaller chromosome numbers ($x = 2-7$) appear later in the subfamily (Fig. 1), probably in concert with accelerated speciation and radiation into new habitats.

Within the PACCAD clade (Fig. 2), Micraireae is considered most basal (GPWG 2001). This Australian monogeneric tribe of 13 species possesses a basic chromosome number of $x = 10$ (Clayton and Renvoize 1986). *Eriachne* appears basal to one of the two PACCAD lineages that include the Aristidoideae, Arundinoideae, Danthonioideae and Chloridoideae. This mainly Australian genus also has $x = 10$ (Clayton and Renvoize 1986). Therefore, it is possible that the ancestral basic number for the PACCAD lineage is $x = 10$. This number is quite prevalent among members of the PACCAD group. It is, however, lacking in the basal Anomochlooideae, Pharioideae and Puelioideae lineages and is quite uncommon and evidently derived in the Bambusoideae. Therefore, a chromosomal link between the PACCAD clade and the basal lineages is not evident, and the $x = 10$ appears to have emerged in the PACCAD via aneuploidy from $x = 12$.

Chromosome evolution within subfamilies

Basal subfamilies

Among the basal-grade subfamilies, the Anomochlooideae has $x = 11$ and 18. In the Pharoideae, Davidse and Pohl (1972) reported $x = 12$ for *Pharus*, and Clayton and Renvoize (1986) cited $x = 12$ for the Phareae, stating that four species have been counted for three genera of the Phareae, without identifying those genera. For the Puelioideae, a subfamily comprising *Puelia* and *Guaduella* and sister to remaining grasses, the basic chromosome number $x = 12$ has been reported for *Puelia* (GPWG 2001). Therefore, except for the disputable Anomochlooideae, homogeneity in basic chromosome number within subfamilies appears to be the norm at the base of the Poaceae.

Subfamily Bambusoideae

In the Bambusoideae *s.s.*, $x = 12$ is characteristic of woody Bambuseae, although a few species with $x = 10$ have been reported (Clayton and Renvoize 1986; Pohl and Clark 1992). Diploidy is rare in this group and polyploidy is the norm (Pohl and Clark 1992). Herbaceous members of the Bambusoideae, however, have basic numbers of $x = 10$, 11 and 12 (Hunziker *et al.* 1982; Clayton and Renvoize 1986; Hunziker and Stebbins 1987). The Olyreae have $x = 11$ for 16 species, $x = 10$ for four, and $x = 9$ and 7 for one species each (Hunziker *et al.* 1982; Clayton and Renvoize 1986; Hunziker and Stebbins 1987). Other herbaceous bambusoids are Parianeae (two genera and ~40 species) that have $x = 11$ and 12 (Clayton and Renvoize 1986) and Phaenospermateae that possess $x = 12$ (Kammacher *et al.* 1973). Therefore, $x = 11$ or $x = 12$ are ancestral chromosome numbers in the Bambusoideae, and lower aneuploidy series in the Olyreae and other herbaceous bambusoids are secondarily derived. This hypothesis is phylogenetically sound, considering the pattern of divergence and basic chromosome number of the basal grade in the Poaceae ($x = 18$, 11 and 12; Fig. 1). The burst in chromosomal evolution in the Olyreae, the largest of the herbaceous bambusoid tribes, could be correlated with their shorter life cycle than for woody species that reproduce sexually only once every several years. Shorter life cycle allows for more frequent opportunities of meiotic recombination and subsequent possibilities for aneuploidy. What also sets the Olyreae aside from other herbaceous bambusoids is its extensive species and habitat diversity. Clayton and Renvoize (1986) indicated that the tribe not only successfully occupies the special environment of the forest floor, but seems to be undergoing adaptive radiation in that habitat. Chromosomal repatterning through aneuploidy, coupled with increased genetic heterozygosity, could provide the genetic means for radiation and habitat exploitation.

Subfamily Ehrhartoideae

This relatively small subfamily includes 12 genera, occupying primarily aquatic or wet habitats. It includes the

core tribes Oryzeae and Zizanieae and the basal tribes Streptogyneae and Ehrharteae. The subfamily consistently maintained $x = 12$ from basal to terminal taxa, with the exception of the monoecious *Zizania* with its aneuploidy-derived $x = 15$ and 17 (Clayton and Renvoize 1986; Watson and Dallwitz 1992). The increased basic number in *Zizania* could not be attributed to floret unisexuality and its obligate outbreeding because *Zizaniopsis* and *Luziola* have a similar reproductive system but maintain $x = 12$. Homogeneity in basic chromosome number in the oryzoid grasses could be a consequence of the buffering effect of their aquatic habitat.

Subfamily Pooideae

The Pooideae is remarkable among grass subfamilies in its chromosome-number diversity. It is one of the major grass subfamilies, occupying temperate regions worldwide. Recent studies have placed the Brachyelytreae, Lygeae and Nardeae as first diverging Pooideae, and the Meliceae, Diarrheneae and Stipeae near the base (Catalan *et al.* 1997; Hilu *et al.* 1999; GPWG 2001). Brachyelytreae, a monotypic tribe, has $2n = 22$ (Tateoka 1955). The monogeneric Diarrheneae possesses $2n = 38$ and 60 (Macfarlane and Watson 1980; Clayton and Renvoize 1986), and is possibly based on $x = 10$. A basic chromosome number of $x = 10$ has been reported for the only species of the Lygeae (Clayton and Renvoize 1986). *Nardus* has $2n = 26$ –30, and may be based on $x = 13$ (Watson and Dallwitz 1992). The Stipeae has an extensive aneuploid series ($2n = 22$ –66) that may be based on $x = 11$ (Clayton and Renvoize 1986). In the Meliceae, *Melica* has $x = 9$, whereas *Glyceria* and *Anthoxanthum* have $x = 10$ (deWet 1987). In the monotypic Brylkinieae, a tribe allied to the Meliceae, $2n = 40$ was reported for *Brylkinia* (Clayton and Renvoize 1986) and is probably based on $x = 10$. *Brachyelytrum* alone (GPWG 2001), or in association with *Nardus* (Hilu *et al.* 1999), forms the most basal clade in the Pooideae. Therefore, $x = 11$ or higher (possibly 13) is ancestral in the Pooideae, from which $x = 2$ –10 were derived higher in the tree (Fig. 1).

The Brachypodieae represents the first lineage of the terminal pooid taxa that has undergone diversification in chromosome number. *Brachypodium* contains species with basic chromosome numbers of $x = 5$, 7 and 9 (Clayton and Renvoize 1986). Next to diverge are the Triticeae (wheat tribe) and Bromeae; both maintain a remarkable consistency of $x = 7$ but achieved diversity via hybridisation and polyploidy. It is not until the Poeae and Aveneae that chromosomal diversity reaches its peak with $x = 2$, 4, 5, 6, 7, 8 and 9. The Aveneae, although characteristically having $x = 7$, possesses genera in which all species have $x = 5$ (*Briza* and *Anthoxanthum*) or $x = 4$ (*Aiopsis*, *Holcus* and *Periballia*), and others where only some of their species deviate, such as the case of $x = 8$ found in three species of *Phalaris*, in one species of *Sphenopus* (*S. divaricatus*) and

two species of *Catapodium* (Hunziker and Stebbins 1987). In *Milium* (Aveneae), $x = 4, 5, 7$ and 9 have been cited (Watson and Dallwitz 1992); the perennials have $x = 7$, whereas the annuals have $x = 5$ and 4 (Stebbins 1982; Hunziker and Stebbins 1987; Watson and Dallwitz 1992). This suggests that the annuals and their chromosome numbers are derived from the perennials.

It may not be coincidental that in the pooid lineage the basal taxa are mostly homogeneous in basic chromosome number and are not speciose, whereas increase in the diversity of chromosome numbers in the terminal taxa is accompanied by explosive speciation. This parallelism in chromosome number and species diversity is also evident in the pooid *Stipa*, a genus with ~300 species, where basic chromosome numbers of $x = 9, 10, 11$ and 12 were reported along with somatic numbers of $2n = 22-96$ (Watson and Dallwitz 1992). The chromosomal diversity in *Stipa* represents speciation enhanced by various levels of euploidy and aneuploidy.

Subfamily Centothecoideae

This subfamily encompasses 10 genera that characteristically have $x = 12$. The exception is *Zeugites* where $2n = 46$ is cited (Watson and Dallwitz 1992); the latter number, if correctly counted, is probably an aneuploidy, derivative of $x = 12$. The monotypic *Thysanolaeneae*, a tribe that used to be allocated to the Arundinoideae, has been reported to have $x = 11$ or $x = 12$ (Watson and Dallwitz 1992). Therefore, $x = 12$ is the hallmark of this subfamily.

Subfamily Panicoideae

The monotypic *Gynerieae* ($x = 11$) is most basal in the Panicoideae (GPWG 2001). The Arundinelleae, a basal tribe in the Panicoideae (Hilu *et al.* 1999; GPWG 2001), has $x = 9, 10$, and 12 , with $x = 9$ being least common (Phipps and Mahon 1970; Kammacher *et al.* 1973). Among the smaller Panicoideae tribes, a chromosome count is available only for the Isachneae ($x = 10$; Tateoka 1962). In the core Panicoideae, $x = 9$ and $x = 10$ predominate. DeWet (1987) pointed out a striking pattern of basic chromosome numbers in the two major tribes, Paniceae and Andropogoneae. The former contains $x = 10$ in 21% of the species and $x = 9$ in most of the remaining taxa, whereas the exact opposite is true for the Andropogoneae, with 85% of the species having $x = 10$, compared with the less common $x = 9$. Basic chromosome number of $x = 5$ is found in *Parasorghum*, *Stiposorghum*, *Thelopogon*, *Sorghum*, *Zea* and *Elionurus* (deWet 1987; Hunziker and Stebbins 1987; Melak *et al.* 1993). Giussani *et al.* (2001) have resolved in a *ndhF*-based tree three major clades with 'largely identical' basic chromosome numbers, one clade representing Paniceae members with $x = 9$, whereas the other two comprise Andropogoneae and Paniceae with $x = 10$ (latter two appearing as weakly supported sister lineages).

Therefore, $x = 11$ appears as the ancestral basic number for the Panicoideae from which $x = 9, 10$ and other numbers emerged. Considering the sister relationship of *Micraira* to remaining PACCAD grasses (Fig. 2), $x = 10$ should theoretically be regarded as an ancestral number in this lineage. This suggests that $x = 11$ and 12 of the Centothecoideae and $x = 11$ of *Gynerium* are derived, and that $x = 10$ of the Panicoideae represents a reversal. In this case, $x = 5$ found in some Andropogoneae is an aneuploid secondary derivative of a larger number ($x = 9$ or 10). The 20 chromosomes of maize (*Zea mays* L.) that were considered as a diploid appear as two duplicated sets of five chromosomes each (Moore *et al.* 1995). This most likely represents a secondary number derived by an allopolyploid event involving two closely related taxa with $x = 5$. This overall pattern implies chromosomal evolution via aneuploid reduction, followed by allopolyploidy that restored the ancestral number ($x = 10 \rightarrow 9 \rightarrow 5 \rightarrow 10$).

Subfamilies Aristidoideae, Danthonioideae and Arundinoideae

With *Eriachne* being basal to the second PACCAD clade, $x = 10$ appears ancestral (Fig. 2). The Aristidoideae, comprising *Aristida*, *Stipagrostis* and *Sartidia*, is basal to the remaining PACCAD (Soreng and Davis 1998; Hilu *et al.* 1999; *Eriachne* was not included in these studies) or sister to the Danthonioideae clade (Fig. 2; GPWG 2001). The basic chromosome numbers of $x = 11$ and $x = 12$ have been reported for the subfamily (Tateoka 1962; Clayton and Renvoize 1986; Watson and Dallwitz 1992). In the Danthonioideae, $x = 6, 7$ and 9 were noted (Hunziker and Stebbins 1987; Watson and Dallwitz 1992). The Arundinoideae *s.s.* characteristically have $x = 6, 9$ and 12 (Watson and Dallwitz 1992). Because $x = 10$ is not reported for these three subfamilies, aneuploidy in both directions from the ancestral number $x = 10$ seems to have played a role in their chromosomal evolution.

Subfamily Chloridoideae

As for the Panicoideae, the Chloridoideae have $x = 9$ and 10 as the common basic numbers, but the pattern is similar to that of the Paniceae where $x = 9$ is found in 86% of the species and $x = 10$ in 13% (deWet 1987). Chromosome numbers of $x = 7$ and 10 have been reported for *Spartina* (Reeder 1977; Gould and Shaw 1983; Watson and Dallwitz 1992) and *Blepharidachne* (Clayton and Renvoize 1986), $x = 8$ for *Blepharoneuron*, *Erioneuron* and *Munroa*, and $x = 12$ for *Sporobolus* and a few *Muhlenbergia* (Clayton and Renvoize 1986). Unlike the Panicoideae, the basic number of $x = 5$ has not been reported for the Chloridoideae. The sister-group relationship between the arundinoid *Centropodia* and the Chloridoideae has been well established (Barker *et al.* 1995, 1999; Hilu *et al.* 1999; Hilu and Alice 2001). *Centropodia* has $x = 12$ (Watson and Dallwitz 1992).

Parsimony analysis of *matK*-sequence data resulted in a polytomy at the base of the Chloridoideae that included *Triraphis* and two major clades (Hilu and Alice 2001). However, neighbour-joining analysis of that dataset and new molecular information (Neves, L. A. Alice and K. W. Hilu, unpubl. data) places *Triraphis* at the very base of the Chloridoideae, followed by a clade encompassing Uniolineae, Pappophoreae and *Eragrostis*. A basic chromosome number of $x = 10$ has been reported for *Triraphis* (deWet 1954), $x = 9$ or 10 for the Pappophoreae and $x = 10$ for all four genera of the Uniolineae (Watson and Dallwitz 1992). Consequently, it is evident that aneuploid reduction from $x = 12$ of *Centropodia* to $x = 10$ and 9 appeared early in the evolutionary history of the subfamily.

Conclusions

Mapping chromosome numbers on a robust phylogeny is a valuable approach that can provide strong evidence and important information on chromosome evolution. The proposed chromosomal-parsimony approach to assess chromosome evolution at various taxonomic levels should take into consideration the number of steps required to explain the changes, number of potential reversals, and extent of changes in chromosome number. This study has demonstrated that the ancestral chromosome number in the Poaceae appears to be $x = 11$. Aneuploidy from $x = 11$ to $x = 9$, followed by chromosome doubling, gave rise to the rediploidised paleopolyploid number $x = 18$ of *Anomochloa*. Direct aneuploid shift from $x = 11$ to $x = 18$ is less parsimonious and cytologically less feasible. Further aneuploidy from the ancestral number $x = 11$ resulted in $x = 12$ found in Pharoideae and Puelioideae and ancestral basic chromosome numbers of remaining grass subfamilies.

This finding is in partial agreement with the 'reduction hypothesis' of Avdulov (1931), supported by Raven (1975), that proposed $x = 12$ as the primitive number from which lower numbers originated by aneuploidy. In this case, $x = 11$, not $x = 12$, is the ancestral chromosome number. It is, however, in sharp discordance with Stebbins' (1982, 1985) 'secondary polyploidy hypothesis' and its modification by deWet (1987) which maintains that $x = 11$ and 12 are secondary chromosome numbers derived from $x = 5$ and 6.

Further chromosomal diversity observed in grasses was achieved via combinations of aneuploidy, euploidy and hybridisation. Aneuploid reduction can be achieved via chromosomal rearrangements and loss of the centromeric regions. Such events are quite deducible from genomic-mapping studies that used syntomy and circulation approaches of grass genome (Devos *et al.* 1993; Moore *et al.* 1995). It is apparent from that approach that rearrangements of linkage groups and translocations among chromosomes, coupled with the loss of centromeric region, could result in reduction in chromosome numbers from the $x = 12$ in rice to $x = 7$ in wheat and $x = 5$ in maize. In contrast, when

chromosomal rearrangements are accompanied by divisions in centromeric regions, an increase in the basic chromosome number will be the outcome. Chromosomal rearrangements, as well as division or loss of centromeric regions, are common events in genome evolution (Devos *et al.* 1993; Moore *et al.* 1995; Zang *et al.* 1998). The Poaceae genome is evidently particularly amenable for such evolutionary events.

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