**Original article**

**Inferring hypothesis-based transitions in clade-specific models of chromosome number evolution along the sedges’ phylogeny (Cyperaceae, Poales).**

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**Introduction**

Chromosomal rearrangements are usual in eukaryotes and have been proved to lead species differentiation (Coghlan et al. 2005). These rearrangements could be produced by a sole mechanism or a combination of translocations, inversions, duplications, deletions, dysploidies (fusions and fissions of parts or complete chromosome sets) and polyploidies (whole genome duplication –WGD–) (Coghlan et al. 2005). Whereas some of these events could produce changes in the linkage disequilibrium of genes (Butlin 2005), others could affect directly in the amount of gene content either erasing (i.e. deletions and fissions –except for some lineages–) or increasing it (i.e. duplications and polyploidies) (Coghlan et al. 2005). These events aid speciation by provoking changes in species fitness, adaptability to new habitats, reproductive isolation or shifts in recombination rates (Otto and Whitton 2000a; Rieseberg 2001; Navarro and Barton 2003a, b; Coyne and Orr 2004; Coghlan et al. 2005; Butlin 2005; Soltis et al. 2009).

In angiosperms, it has been especially discussed the role of polyploidy and its consequences on speciation, with a remarkably interest in ancient polyploid events in some of the richest lineages (Debodt et al. 2005; Soltis et al. 2009; Soltis and Soltis 2016; Smith et al. 2017), which has led to understand polyploidization as a possible driver for lineage radiation (Levin 1983; Soltis and Soltis 2000, 2016; Otto and Whitton 2000b; Comai 2005; Hegarty and Hiscock 2007, 2008; Otto 2007; Van de Peer 2011). On the other hand, although dysploidy is more frequent than polyploidy and aneuploidy (duplication or deletion of a chromosome) in angiosperm (Grant 1981), its consequences in diversification have been disregarded, despite some authors pointed out dysploidy as important events in species diversification (Lee and Namai 1992, 1993; Vickery 1995; Orellana et al. 2007; Weiss‐Schneeweiss et al. 2009; Vallès et al. 2012; Gitaí et al. 2014). Nonetheless, its importance has been recently suggested to be neutral in terms of lineage diversification, probably because these events do not involve changes in genetic material content (Escudero et al. 2014).

The cosmopolitan family of sedges (Cyperaceae, ca. 5500 species; Govaerts et al. 2017) is the tenth richest angiosperm family and it is mainly diversified on the tropics, with exception of genus *Carex* L. that is distributed mostly along the temperate regions (Reznicek 1990). Moreover, sedges are the angiosperm family with highest chromosomal variation (2n=4–224; Roalson 2008). Because of its high species richness and wide range of chromosome numbers, Cyperaceae constitutes a model taxa for implementing studies on biodiversity, evolution and systematics, especially the genus *Carex* (e.g. Hipp 2007). This genus also present a wide variation of chromosome numbers (2n=12–124; Roalson 2008) which have encourage important works on this matter (e.g. Hipp 2007 and Roalson 2008b). Variation in the number of chromosomes and changes in the mode of evolution have been suggested as a possible driver of species richness increment in *Carex* (Escudero et al. 2012, 2014), the most diversified genus of the family (ca. 40%; Govaerts et al. 2017).

Shifts in diversification have been detected in four main nodes of Cyperaceae. Previously, Escudero et al. (2012) had already found an increment in diversification rates in the node that comprises Core *Carex*, Caricoid *Carex* and *Carex* subgenus *Vignea*, which has been confirmed in a recent study by Spalink et al. (2016). Escudero and Hipp (2013) found the node including the tribes Scirpeae, Dulichieae, and Cariceae plus *Khaosokia* *caricoides* (SDC clade) and the tribes Fuireneae, Abildgaardieae, Eleocharideae, and Cypereae (FAEC clade) to present changes in the diversification rate based on Hinchliff and Roalson (2013) phylogeny. Spalink et al. (2016), showed shifts in three different lineages inside the clade reported by Escudero et al. (2013), obtaining the same node as Escudero et al. (2012). Moreover, Spalink et al. (2016) also found a shift in the FAEC clade and in the represented taxa of the C4 photosynthetic pathway *Cyperus* within Cypereae 2 clade.

The huge continuous variation in chromosome number of this family is explained by the presence of holocentric chromosomes, which means that the kinetochoric activity is present along the chromosomes, contrary to those monocentric, that present a clear primary constriction in which kinetochoric activity is concentrated (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013). In lineages that present holocentric chromosomes (see revision in Márquez-Corro et al. 2017), fusions and fissions (named symploidy and agmatoploidy, respectively) are more common (Grant 1981). This occur even within species level, due to the characteristics of the kinetochoric plate (Mola and Papeschi 2006; Melters et al. 2012; Hipp et al. 2013) and the neutral balance in DNA content (Escudero et al. 2014).

As stated above, dysploidy (Lee and Namai 1992, 1993; Vickery 1995; Orellana et al. 2007; Weiss‐Schneeweiss et al. 2009; Vallès et al. 2012; Gitaí et al. 2014) and polyploidy (Levin 1983; Soltis and Soltis 2000, 2016; Otto and Whitton 2000b; Comai 2005; Hegarty and Hiscock 2007, 2008; Otto 2007; Van de Peer 2011) have been considered as possible diversification drivers. Cyperaceae family present lineages with prevalence of different modes of chromosomal evolution (e.g. *Carex* karyotype evolves mainly via agmatoploidy and symploidy; Heilborn 1924; Davies 1956). Thus, this hyperdiverse family and its wide range of karyotypic variation constitute a perfect taxon to study how changes in diversification rates and chromosome evolution are related. In light of these studies, we hypothesize that some shifts in lineage diversification could be caused, at least in part, by changes in the mode of chromosome evolution, probably leading to different mechanism of adaptation or reproductive isolation (Otto and Whitton 2000a; Rieseberg 2001; Navarro and Barton 2003a, b; Coyne and Orr 2004; Coghlan et al. 2005; Butlin 2005; Soltis et al. 2009).

Recently (Mayrose et al. 2010; Glick and Mayrose 2014), some hypothesis-testing probabilistic models of chromosome number evolution have been formulated. These models include different parameters, with the simplest ones calculating the rate of gains, losses and ploidy augments in chromosome number along a phylogeny. Complexes models allow identifying linear dependency between the starting number of chromosomes and the rate of ascending and decreasing dysploidy. Because our hypothesis is focused on the shifts of gains/losses of specific lineages chromosome number, and not in the relation between the latter and the parameters, we overlook the linear models. We applied these models to the latest sedges phylogenies (Hinchliff and Roalson 2013; Spalink et al. 2016) and the different pruned lineages with a treatment similar to that proposed by O’Meara et al. (2006) and replicated by Hipp (2007) on north American *Carex* sect. *Ovales* species.

The aim of this study is (i) to elucidate the role of chromosome evolution in diversification of sedges family by the implementation of probabilistic models in an unprecedented manner, and (ii) to evaluate the potential usability of the nested models treatment in studies of chromosome evolution for high species richness lineages in order to discern different evolution patterns within a phylogeny. The null hypothesis is that chromosome number changes along the family at constant rate, independent of the diversification rates. On the other hand, the alternative hypothesis is that there are transitions in the mode of chromosome evolution, somewhat followed by a shift in diversification rates.

Thus, lineages with these chromosomes might present different modes of chromosome evolution and diversification along its species groups. Several shifts in the mode of chromosome of evolution have been registered in this family. Polyploidy is frequent in *Rhynchospora* (Luceño et al. 1998; Vanzela et al. 2000), *Eleocharis* (Bureš 1998; Maximiano da Silva et al. 2008), as well as in some *Carex* species (Hipp et al. 2009; Rotreklová et al. 2011): *C. siderosticta* (sect. *Siderostictae*), *C.* *dolichostachya* (sect. *Mitratae*), *C. parciflora* (sect. *Paniceae*), *C. roraimensis* (which has been proposed to be an allotretraploid of some species of sections *Ovales* and *Stellulatae*), and the *Kobresia* clade (Seeber et al. 2014). However polyploidy has been proved in these previous cases, it has been suggested for other *Carex* taxa (see Rotreklová et al. 2011) such as *C. pediformis* complex *–C. macroura*, *C. rhizina* and *C. lanceolata–* and *C. humilis* complex–*C. callitrichos* and *C. humilis* complex– (both groups from sect. *Digitatae*), *C. capillaris* (sect. *Capillares*); as well as some taxa of *Fimbristylis* (Nijalingappa 1975), *Bulbostylis* (López et al. 2017) and *Cyperus* (*C. rotundus*; Arias et al. 2011).

For this purpose, (i) we carry out counts of chromosome number, some of them news to science, and together with previous counts we place them in both phylogenies of the family (Hinchliff and Roalson 2013; Spalink et al. 2016); and (ii) in order to seek for correspondences between changes in the mode of chromosome evolution and shifts in diversification rates of clades, we generated alternative inputs of trees pruned by the clades in which diversification changed. Statistical results from ChromEvol software (Mayrose et al. 2010) were compared in order to discern the model that best fit each input. If the mode of chromosome evolution of the entire phylogeny presents less explanatory statistical power than the sum of each subtree separately, a shift in diversification rate might be related to a change in the mode of chromosome evolution.

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