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Evolution of haploid chromosome numbers in the sunflower family. Are genomic duplications associated to ancient climate changes?

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*“Era preciso agradecer às flores
Terem guardado em si,
Límpida e pura,
Aquele promessa antiga
Duma manhã futura.”*

Sophia de Mello Breyner Andresen

“En croyant à des fleurs, souvent on les fait naître.”

Edmond Rostan

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Abstract

The remarkable diversity of land plants is associated with immense genetic variation manifested also by a wide range of chromosome numbers. Changes of chromosome number during evolution of angiosperms are likely to have played a role in speciation, being their study of utmost importance, especially at the present time when a probabilistic model is available to study chromosome evolution within a phylogenetic framework. In the present study likelihood models of chromosome number evolution were fitted to the largest family of flowering plants, the Asteraceae family. Specifically, two phylogenetic supertrees of this family were used to reconstruct the ancestral chromosome number and infer genomic events, as whole genome duplications and dysploidies. In addition, we tested if genomic duplications were linked with periods of ancient climate changes. The results of this Thesis evidenced that $n = 9$ was the most probable ancestral chromosome number of the family, irrespectively of the supertrees used. Also, our models supported that genomic duplications, as well as, descending dysploidy, were common genomic events in the evolution of Asteraceae. The increase in the number of chromosomes through polyploidy events was related with a high frequency of chromosome losses which was the most frequent event in the chromosome number evolution. The exploratory approach applied in this Thesis provided a first insight about the linkage that may exist between genome doubling processes and periods of climate changes. More than a half of the branches with polyploidization events coincided with these stressful periods. Further phylogenetic studies and genetic investigations focused in obtaining more complete phylogenetic trees will help to more accurately date the time of occurrence of these ancient genomic duplication, and therefore will allow a better assessment of the causal link between climate changes and the success of polyploid lineages.

Key words: Asteraceae, chromosome number evolution, climate changes, polyploidy, probabilistic models.

Resumo

A extraordinária diversidade das plantas terrestres está associada a uma imensa variação genética manifestada também por uma grande variedade de números cromossômicos. As alterações no número cromossômico que ocorreram durante a evolução das angiospérmicas tiveram provavelmente um papel fundamental no processo de especiação, sendo o seu estudo de uma elevada importância, especialmente agora que existem métodos probabilísticos que possibilitam o estudo da evolução cromossômica num contexto filogenético. Na presente Tese, estes modelos foram aplicados à maior família de plantas com flor, a família das Asteraceae. Especificamente, foram usadas duas super-árvores filogenéticas desta família de modo a reconstruir o número cromossômico ancestral e inferir o número de eventos genômicos como duplicações e disploidias. Adicionalmente, testou-se a ligação entre duplicações genômicas e períodos ancestrais de alterações climáticas. Os resultados desta Tese evidenciaram que $n = 9$ foi o número cromossômico ancestral mais provável para a família, independentemente da super-árvore utilizada. Foi igualmente notório que as duplicações genômicas e as disploidias descendentes foram eventos genômicos comuns durante a evolução da família Asteraceae. O aumento no número de cromossomas causado pelos eventos de duplicação está relacionado com a elevada frequência de redução no número de cromossomas, a qual constitui o tipo de evento mais comum durante a evolução do número de cromossomas. Também, a abordagem aplicada nesta Tese fornece uma primeira visão sobre a ligação que pode existir entre processos de duplicação genômica e períodos de alterações climáticas. Mais de metade dos ramos com eventos de poliploidização coincide com esses períodos de *stress*. Estudos filogenéticos e investigações genéticas futuras que permitam obter árvores filogenéticas mais completas ajudarão a datar com mais precisão o momento em que estas duplicações genômicas ocorreram, e conseqüentemente permitirão uma melhor avaliação da ligação causal entre as alterações climáticas e o sucesso de linhagens poliplóides.

Palavras-chave: alterações climáticas, Asteraceae, evolução do número cromossômico, modelos probabilísticos, poliploidia.

1. Introduction

The remarkable diversity of land plants is associated with immense genetic variation manifested also by a wide range of genome sizes and chromosome numbers (Lysák & Schubert, 2013). Whereas genome size of land plants varies more than 2,300-fold, from 64 Mbp (*Genlisea aurea*, Greilhuber et al., 2006) to approximately 150,000 Mbp (*Paris japonica*; Pellicer et al., 2010), chromosome numbers vary from $n = 2$ in six angiosperm species (Vanzela et al., 1996; Cremonini, 2005) to $n > 320$ in *Sedum suaveolens* (Uhl, 1978). This large variation of chromosome numbers found in angiosperms is driven by two main mechanisms operating in opposite directions: increases through polyploidy (whole genome duplications, WGD) and decreases (or increases) through structural chromosomal rearrangements (dysploidy). Indeed, polyploidy seems to be one of the main mechanisms responsible for the evolutionary success of many species, mainly those unable to disperse naturally or through human-mediated translocation to climatically suitable habitats (Hegarty & Hiscock, 2008). For example, the recurrent occurrence of polyploids in different habitats from that of their diploid progenitors constitutes a proof of the ability of polyploids to colonize new environmental niches (Hegarty & Hiscock, 2008). Still, the evolutionary success of polyploids have been a controversial and a much debated topic, with some authors considering that polyploidy is most often an evolutionary dead-end (Mayrose et al., 2011), while others defend its fundamental role on the evolution of flowering plants (Hegarty & Hiscock, 2008; Lim et al., 2008; Soltis & Soltis, 2000; Soltis et al., 2014). Despite of this, several studies have suggested that 47% to 100% of flowering plants can be traced to a polyploid event at some point within its diversification or had a polyploid ancestry (Van de Peer et al., 2009; Wood et al., 2009; Fawcett & Van de Peer, 2010; Vanneste et al., 2014;). Therefore, changes of chromosome number during evolution of angiosperms are likely to have played a role in speciation, being their study of utmost importance, especially at the present time when a specific probabilistic model is available to study chromosome evolution within a phylogenetic framework (Mayrose et al., 2010).

The recurrent observations of a high frequency of polyploids in harsh and unstable environments (Fawcett & Van de Peer, 2010), such as high altitudes and latitudes (e.g., arctic areas; Brochmann et al., 2004) has been stated to suggest that the success of some ancient WGD might be linked with periods of climatic change. During a climate change, the increase of empty niches due to the extinction of many species that were not able to deal with the environmental changes might bring an opportunity to recently formed

polyploids to persist at the first critical stages (Van de Peer et al., 2009). Indeed, several studies based on more or less complex study-systems have shown the highest ability of the polyploids to cope with specific periods of climate change (Comes & Kadereit, 1998; Antonelli et al., 2010; Couvreur et al., 2010; Fawcett & Van de Peer, 2010; Vanneste et al., 2014).

The study of ancient WGDs represents a challenge. Until now, the available studies employed threshold techniques to infer the occurrence and the location of polyploidy events (e.g. Stebbins, 1938); still these studies suffered from a large degree of extrapolation, and did not take into account the phylogenetic relationships and the possible occurrence of aneuploidy or dysploidy during evolution (Glick & Mayrose, 2014). In addition, as the knowledge about the evolutionary changes in chromosome numbers is incomplete, in angiosperms the base number (' x ') has been commonly used (Cusimano et al., 2012). However, a misunderstanding between the definition of base number and other concepts such as the monoploid chromosome number (i.e, the number of apparently originally unique chromosomes in a haploid set) has often been observed (Langlet, 1927 *cited in* Cusimano et al., 2012; Roy & Manton, 1964; reviewed in Glick & Mayrose, 2014). To clarify the nomenclature, it has been proposed that the base number should be estimated inferring the haploid ancestral chromosome number of the most recent common ancestor (MRCA) of the group examined (Cusimano et al., 2012; Glick & Mayrose, 2014). With that purpose a new mathematical model has been recently developed to fit the evolution of chromosome numbers in a given lineage working under a robust probabilistic inference framework (Mayrose et al., 2010). Both dysploidies and polyploidies are considered on this method, allowing to test the importance of these genomic processes along the evolution. Hence, this model represents a great opportunity to explore the occurrence of ancient WGD and its evolutionary implications (Glick & Mayrose, 2014). In the present study we fit these likelihood models of chromosome number evolution to the largest family of flowering plants, the sunflower family.

The sunflower family (Asteraceae) comprises the largest number of described species of any plant family, 24,000-30,000 species distributed in 1,600-1,700 genera (Funk et al., 2009). Its members occur on all continents except Antarctica, existing in a great range of habitats and presenting many different habits (Funk et al., 2005). Considering the incredibly large number of species and its comparatively young age (Barreda et al., 2012), it is not surprising that this family possesses one of the highest rates of diversification

among all flowering plants, being also indicative of the ecological success and evolutionary ability of its members (Funk et al., 2009).

So far, a very large range of chromosome numbers has been described in Asteraceae: $n = 2$ to $n = \text{ca. } 216$ chromosomes, being $n = 9$ the most frequent number reported in this family (Semple & Watanabe, 2009). Many authors suggested $x = 9$ as the base number of this family (Stebbins 1950; Solbrig 1977; Cronquist, 1981, Bremer, 1994 *cited in* Semple & Watanabe 2009; Santosh & Raghbir 2013), but $x = 8$ has also been reported (Vallès et al., 2005). Furthermore, several paleoploidization events have been suggested along the evolution of this family. Barker et al. (2008) examined gene duplication and retention in Asteraceae and found that at least three ancient WGD have occurred in this family. Thus, ancient polyploidization may be, in part, responsible for the evolutionary success of the family (Funk et al., 2009). However, to date, the evolution of chromosome numbers has not been studied under a probabilistic phylogenetic method that could provide robust estimations of ancient WGD in this successful lineage.

Thus, the general aim of this work was to explore the evolution of haploid chromosome numbers along the history of the Asteraceae. In particular, the ancestral chromosome number of this family, as well as the ancestral chromosome numbers of its main lineages were inferred using two different phylogenetic hypotheses. Furthermore, events of chromosomal changes, as duplications and dysploidies that occurred along the evolution were estimated and located in a phylogenetic tree. Finally, it was assessed if WGD were linked with periods of ancient climate change, mainly those that occurred during the Cenozoic Era, the age of origin of the sunflower family.

2. Materials and Methods

Chromosome numbers collection

The number of chromosomes of genera and species of Asteraceae and of the outgroup family's Calyceraceae and Goodeniaceae were collected from the website: Index to Chromosome numbers in Asteraceae (http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e). To start, we searched the taxa included in the supertrees published by Funk et al. (2005; 2009; 403 and 757, respectively) including the outgroup families. Both supertrees represent mainly phylogenetic relationships between genera, however, some problematic species were also considered. These supertrees cover approximately 24% (with 2005 supertree) and 46% (with 2009 supertree) of the ca. 1650 genera of the Asteraceae family. Still, as there was no chromosome number information for 91 and 198 taxa of the 2005 and 2009 supertrees, respectively, the final total coverage of chromosome number data was of 77.4% (2005) and 73.8% (2009) of the taxa included in the supertrees.

Chromosome numbers were coded using the following approach: first, all reported chromosome numbers of each genus were searched, regardless of their frequency in different species, but excluding B chromosomes data, odd numbers, and situations when chromosome counts were given as intervals of numbers. Then, the available chromosome information of the diploid level was converted into haploid chromosome numbers, keeping the same frequency. After this conversion, 125 monomorphic (40.06%) and 187 polymorphic genera (59.94%) were obtained for the 2005 data, and 293 monomorphic (52.42%) and 266 polymorphic (47.58%) genera for the 2009 data. The evolution of haploid chromosome numbers was then analysed considering both data sets: with (hereafter *polymorphic data*) or without chromosome number polymorphism (hereafter *single data*). In the *single data*, only one chromosome number was selected from the polymorphic dataset. For that, the following criteria were used: the most frequent chromosome number, and, when more than one chromosome number had the same frequency, the lowest chromosome number. This second criterion was used because the lowest haploid chromosome numbers are typically assumed to represent the nonpolyploidized state, i.e., the base chromosome number (Mayrose et al., 2010).

Phylogenetic hypotheses

As revealed above, two different phylogenetic hypotheses for the Asteraceae family were used: Funk et al. (2005) and (2009). For the 2005 supertree, the branch length modifications of Torices (2010) were used. By other way, for the 2009 supertree, time-calibrated branch lengths were estimated using the BLADJ function of Phylocom v.4.0.1b software (Webb et al., 2008). Basically, this software enables to fix the root node at a specified age and fixes the other nodes for which age estimates are already available. The remainder branch lengths can then be assessed by placing the nodes between dated nodes, or between dated nodes and terminals. Finally, BLADJ presents a new phylogeny with adjusted branch lengths.

The 2009 supertree was first translated into a Newick tree file format. Then, nodes with known age were fixed based on bibliographic review of information on clade age estimates. These age estimates were mainly selected based on molecular dating in which fossil calibration had been previously used, although other dating methods were also considered (e.g., geological dating; Table S1 in Appendix I). As clade ages estimates are usually given as time intervals, and as BLADJ function only accepts one age for each node, the mean value of the minimum and the maximum time estimates was calculated and used as calibration age (Table S1 in Appendix I). In some cases, the age clade data was not consistent among sources; in these situations, the most reliable age estimation (i.e., the most consistent with the other estimations) was selected.

Evolutionary models of haploid chromosome number change

For both trees, the evolution of haploid chromosome numbers of Asteraceae was inferred using chromEvol software v.2.0 (Glick & Mayrose, 2014), through both the maximum likelihood (ML) and the Bayesian phylogenetic inference (Bayes) methods. This software is based on a probabilistic model of chromosome number evolution that assumes that changes in chromosome number over time result from a combination of polyploidy (demi-duplication and duplication events) and dysploidy (ascending or descending, by chromosome fission or fusion events, respectively) along branches of a phylogeny (Mayrose et al., 2010). By comparing the fit of the different models to biological data, it is possible to test the probability of those events and therefore to understand the pathways

by which chromosome numbers evolve (Mayrose et al., 2010) and to estimate the ancestral chromosome numbers at internal nodes of the tree (Glick & Mayrose, 2014). This software offers the possibility to test 10 models based on different combinations of nine parameters: chromosome loss rate (δ), which considers that the number of chromosomes might decrease by one, with rate δ ; chromosome gain rate (λ) that assumes that the number of chromosomes might increase by one, with rate λ ; chromosome duplication rate (ρ) that considers that the number of chromosomes might double, with rate ρ ; chromosome demi-duplication rate (μ), which assumes the union of reduced and unreduced gametes leading to, for example, triplication events, with rate μ ; linear chromosome loss rate (δ_1), that considers that the chromosome loss rate depends on the current number of chromosomes; linear chromosome gain rate (λ_1), that considers that the chromosome gain rate depends on the current number of chromosomes; base chromosome number (β) which is the monoploid chromosome number; base chromosome number rate (ν) and base chromosome number optimized by the program. We did not allow chromEvol optimizing base number as the software's authors suggest for complex data sets (Glick & Mayrose, 2014). Then, for those models including a base chromosome number rate, we fixed the hypothetical base number of Asteraceae at 9, according to the previous known data about the Asteraceae's base number (Stebbins 1950; Solbrig 1977; Cronquist, 1981, Bremer, 1994 *cited in* Semple & Watanabe 2009; Santosh & Raghbir 2013). Four of the models have only constant rates (Mc1, Mc2, Mc3, Mc0), whereas the other four include two linear rate parameters (M11, M12, M13, M10; Table S2 in Appendix I). Both model sets have a null model (Mc0 and M10) that assumes no polyploidization events. Finally, two Mb models (Mb1 and Mb2) consider that the evolution of chromosome number can be influenced by the base number and by the base number rate (Table S2 in Appendix I). The M1 models (Mc1 and M11) assume that the number of chromosomes might increase (by chromosome gain) or decrease (by chromosome loss) by one or might double (polyploidization), having, therefore, three parameters: chromosome gain, chromosome loss and polyploidization rates (Table S2 in Appendix I). The M2 models (Mc2 and M12) consider that the rate of demi-polyploidization (or demi-duplication) is equal to that of polyploidization, including the possibility of chromosome gains and losses (three parameters). In M3 models (Mc3 and M13), the demi-polyploidization is treated as an additional free parameter, considering that the rate of demi-polyploidization is different than that of polyploidization (four parameters: gain chromosome, loss chromosome, demi-polyploidization and polyploidization rates). The

Mb1 considers four parameters: constant chromosome gain rate, constant chromosome loss rate, base chromosome number rate and base chromosome number. The Mb2 adds the chromosome duplication rate, thus considering five parameters in its analysis.

Two approaches were used to estimate the model parameters of chromosome evolution in Asteraceae and thus disentangle which genetic events might have occurred during the evolution of this family. First, all models were fitted and compared using the Akaike Information Criteria (AIC) value (Burnham and Anderson, 2004 *cited in* Bolker, 2007). The model with lowest AIC value was considered the best model. Second, model averaged parameters were estimated by weighting each rate parameter by the AIC weights of each model (Bolker, 2007); then each parameter's value was compared to those obtained with the best model. All models were fitted twice considering single and polymorphic data. The minimum chromosome number was set to 2, and the maximum number was set to 5-fold higher than the empirical data. The branch lengths were scaled by 0.01 to get parameters values below the bounds established for the algorithms included in chromEvol software. To compute the expected number of changes along each branch, as well as, the ancestral haploid chromosome numbers at nodes, the best-fit model for both supertrees and for both datasets (single and polymorphic data) was rerun computing 20,000 simulations.

Ancestral chromosome number of Asteraceae

Considering the results of the previous section: 2, 8, 9 or 10 were tested as likely ancestor haploid numbers of Asteraceae, using polymorphic data. These haploid numbers were inferred with the highest PP at the root of the Asteraceae (see results). For that, the best models were rerun with each one of them, either 8, 9 or 10 being fixed at the node of the most recent ancestor of Asteraceae. The obtained AIC values were compared with those obtained previously without fixing the ancestral value.

Polyploidization events and climate changes

To evaluate if polyploidization events (WGD) occurred preferentially associated with ancient climate changes, genomic duplications and demi-duplications that occurred along

the evolution of Asteraceae family were mapped. By this way it was possible to calculate the proportion of polyploidization events occurring near ancient climate change events. According to Zachos et al. (2001), four main periods of climate changes occurred during the evolution of the Asteraceae family: between Mid-Paleocene and Early Eocene (59-52 My), between Early-middle-Miocene and Early-Oligocene (49-32 My), between Late-middle-Miocene (17-15 My) and between Late-Miocene and Early-Pliocene (6-3.2 My). The genomic duplications and demi-duplications events inferred in the best-fitted models (see above) were mapped on both phylogenetic trees. The probability of each genomic event type was mapped according with these probabilities groups: ≥ 0.5 , ≥ 0.8 and ≥ 0.95 . As ChromEvol only enables to identify those branches in which a polyploidization event occurred with a probability higher than 0.5, but not the exact period time along the branch, it was impossible to determine exactly when this events occurred within each branch. Thus, polyploidization events were simply depicted in the middle of their respective branches. Afterwards, it was evaluated if those branches in which a polyploidization event had been reconstructed, also presented at least one climate change event along the whole branch evolution. Thereby, the percentage of branches associated with polyploidization events in each period of climate change was estimated.

3. Results

Models of chromosome evolution in Asteraceae

Regardless of the phylogenetic hypothesis and the coding scheme used, the best models were always Mc2 or Mc3 (Table 1). Both models consider the same three parameters on their analysis, i.e., chromosome gain rate, chromosome loss rate and chromosome duplication rate, with the difference that the Mc2 model considers that duplication and demi-duplication rates are equal, whereas Mc3 model assumes that the demi-duplication rate is an additional free parameter. This result supports that genomic duplications, together with dysploidy, were very important events in the evolution of Asteraceae. In addition, the Mc0 model, that considers no polyploidization events on the evolution of haploid chromosome number, was the model with the worst score in any of the four set of analyses (Table 1).

The comparison of the rate parameter values obtained by the best model and by the averaging model revealed that the rates of chromosome loss, gain and duplication were equal or very near ($\Delta = 0.01$), in both approaches, irrespective of the phylogenetic hypotheses and coding scheme (Table 2). However, for the demi-duplications the rate was lower for the averaging models than for the respective best model (Table 2). Also, in the averaged models, the linear rate parameters (i.e., linear chromosome loss and linear chromosome gain rates) and the base chromosome number rate had very low values (Table 2).

The ancestral haploid chromosome number in Asteraceae

The two methods used in the ancestral chromosome number analyses provided very different results. The ML method always inferred $n = 2$ as the most likelihood ancestral for Asteraceae, whereas that Bayesian analysis led to $n = 8, 9$ or 10 depending on the coding scheme and the phylogenetic tree (Table 3). Nevertheless, $n = 9$ was always the ancestral chromosome number with the highest posterior probability (*PP*), while $n = 8$ and $n = 10$ were the second best haploid ancestral chromosome numbers inferred by the Bayesian analyses (Table 3). Fixing the most recent ancestor of Asteraceae family with each one of these haploid numbers ($n = 2, 8, 9$ or 10) resulted in $n = 9$ as the best ancestral number, with the lowest AIC value, for both phylogenetic hypotheses (Table 4).

However, in 2009 supertree, the $n = 10$ model had an AIC value very similar to that obtained using $n = 9$ (Δ AIC = 1.38).

For most of the main lineages, the estimated ancestral haploid number was mainly $n = 9$ and $n = 10$, with some exceptions, which ranged from $n = 12$ to $n = 22$ (Table 3). Also, in most of cases, the Bayesian inference and the ML method reconstructed the same ancestral number (Table 3).

Table 1 - Goodness of fit of the ten different models of chromosome number evolution, applied to both polymorphic and single chromosome data. In bold, the lowest AIC value for each data set indicates the best model. In brackets, the numbers indicate the descending order of the best model. ML – Maximum Likelihood; AIC – Akaike Information Criterion.

Phylogenetic hypothesis	2005						2009					
	Polymorphic data			Single data			Polymorphic data			Single data		
	AIC	ML		AIC	ML		AIC	ML		AIC	ML	
Mc0	1919.32 (10)	-957.66	1931.81 (10)	-963.91	3758.82 (10)	-1877.41	3836.15 (10)	-1916.07				
Mc1	1711.13 (6)	-852.57	1772.71 (6)	-883.36	3021.70 (7)	-1507.85	3156.25 (4)	-1575.12				
Mc2	1679.41 (1)	-836.70	1732.85 (2)	-863.43	2863.98 (1)	-1428.99	3021.40 (1)	-1507.7				
Mc3	1680.50 (2)	-836.25	1730.76 (1)	-861.38	2865.74 (2)	-1428.87	3807.53 (9)	-1899.76				
MI0	1858.10 (8)	-925.05	1873.30 (9)	-932.65	3502.08 (9)	-1747.04	3546.47 (6)	-1769.24				
MI1	1708.53 (5)	-849.26	1758.80 (5)	-874.40	2993.74 (6)	-1491.87	3549.09 (8)	-1769.54				
MI2	1684.95 (3)	-837.47	1734.71 (3)	-862.35	2881.82 (3)	-1435.91	3548.96 (7)	-1769.48				
MI3	1685.58 (4)	-836.79	1740.11 (4)	-864.06	2884.16 (4)	-1436.08	3030.43 (2)	-1509.21				
Mb1	1868.58 (9)	-930.29	1861.74 (8)	-926.87	3292.42 (8)	-1642.21	3302.70 (5)	-1647.35				
Mb2	1715.26 (7)	-852.63	1776.91 (7)	-883.46	2992.68 (5)	-1491.34	3131.09 (3)	-1560.55				

Table 2 – Rate parameters values and frequency of the four possible event types with a posterior probability (PP) > 0.5 in the best model and by averaging all models, for each data coding scheme and phylogenetic hypotheses. The values of rate parameters were multiplied by 0.01, according to the branch length modifications made to scale the phylogenetic tree. single: single data; polym.: polymorphic data; dupl.: duplications; δ – constant chromosome loss rate; δ_l – linear chromosome loss rate; λ – constant chromosome gain rate; λ_l – linear chromosome gain rate; ρ – chromosome duplication rate; μ – chromosome duplication or triploidization rate; β – base number; ν – base number rate.

Phylogenetic hypothesis	Coding scheme	Best model	LogLik	AIC	Rate parameters										Events inferred with PP > 0.5			
					δ	λ	ρ	μ	δ_l	λ_l	ν	Losses	Gains	Dupl.	Demi-dupl.			
2005	polym.	Mc2	-836.70	1679.41	0.037	0.033	0.014	0.014	0.014	-	-	-	-	177.40	154.41	68.53	62.54	
		Averaging			0.036	0.033	0.015	0.004	2.040e-4	4.400e-5	1.518e-13							
	single	Mc3	-861.38	1730.76	0.060	0.039	0.014	0.024	-	-	-	-	-	282.94	183.24	64.99	113.01	
		Averaging			0.060	0.038	0.015	0.017	-5.000e-5	-4.100e-5	1.245e-22							
2009	polym.	Mc2	-1428.99	2863.98	0.043	0.022	0.017	0.017	-	-	-	-	299.78	154.40	118.67	117.30		
		Averaging			0.043	0.022	0.017	0.005	2.490e-7	2.150e-7	9.390e-33							
	single	Mc2	-1507.70	3021.40	0.064	0.031	0.021	0.021	-	-	-	-	441.05	218.52	144.61	145.85		
		Averaging			0.064	0.031	0.021	2.190e-4	-3.000e-6	1.300e-5	5.349e-30							

Table 3 - Chromosome number of the most recent common ancestor of the Asteraceae family and of the main tribes in the 2005 and 2009 supertrees, under the best model of chromosome evolution for each coding scheme. The two most probable ancestral chromosome numbers obtained through the Bayesian phylogenetic inference (Bayes), as well as, the result of the maximum likelihood (ML) are provided, with the probability (PP) of occurrence being given in parentheses. When available, the base number information available in the literature is also given.

Tribes	2005				2009				Base numbers
	Bayes best <i>n</i> (PP);		Bayes best <i>n</i> (PP);		Bayes best <i>n</i> (PP);		Bayes best <i>n</i> (PP);		
	Polymorphic data	Single data	Polymorphic data	Single data	Polymorphic data	Single data	Polymorphic data	Single data	
Asteraceae	9 (0.80); 8 (0.09); 2	9 (0.51); 10 (0.26); 2	9 (0.60); 10 (0.21); 2	9 (0.22); 10 (0.18); 2	9^a, 8^b				
Barnadesieae	9 (0.52); 8 (0.30); 8	9 (0.36); 8 (0.26); 8	9 (0.74); 8 (0.19); 9	9 (0.40); 8 (0.27); 6	8, 9, 12, 27 ^c ***				
Stiffueae	9 (0.84); 10 (0.08); 2	9 (0.54); 10 (0.29); 2	9 (0.27); 18 (0.15); 12	9 (0.21); 10 (0.14); 9	9*				
Onoserideae	9 (0.41); 18 (0.21); 9	9 (0.45); 10 (0.21); 6	9 (0.36); 18 (0.18); 18	9 (0.50); 10 (0.33); 9	No data				
Mutisieae	13 (0.23); 12 (0.15); 12	10 (0.18); 15 (0.15); 8	15 (0.57); 12 (0.17); 12	15 (0.51); 16 (0.27); 16	9 ^d *				
Nassauvieae	13 (0.39); 10 (0.15); 12	10 (0.32); 9 (0.27); 9	10 (0.50); 9 (0.16); 12	10 (0.56); 9 (0.22); 9	No data				
Hyalideae	-	-	27 (0.32); 18 (0.23); 18	27 (0.19); 18 (0.16); 18	-				
Wunderlichieae	-	-	27 (0.18); 18 (0.18); 12	18 (0.12); 27 (0.11); 12	No data				
Gochnatieae	22 (0.35); 23 (0.18); 22	22 (0.22); 23 (0.21); 24	9 (0.69); 10 (0.15); 2	9 (0.45); 10 (0.31); 2	4; 9; 23 ^e ***				
Hecastocleideae	9 (0.89); 10 (0.07); 2	9 (0.61); 10 (0.28); 2	9 (0.85); 10 (0.13); 2	9 (0.57); 10 (0.35); 2	8 ^f ***				
Dicomeae	10 (0.44); 9 (0.24); 11	10 (0.41); 9 (0.25); 11	9 (0.56); 10 (0.37); 10	9 (0.53); 10 (0.37); 10	10; 11*				

Oldenburgieae	9 (0.57); 10 (0.28); 12	9 (0.48); 10 (0.28); 12	18 (0.52); 9 (0.33); 18	9 (0.71); 10 (0.21); 9	9*
Tarconanthaeae	9 (0.44); 18 (0.42); 18	9 (0.64); 10 (0.13); 12	18 (0.76); 9 (0.20); 18	9 (0.89); 10 (0.06); 9	9*
Cardueae	9 (0.45); 10 (0.36); 12	9 (0.42); 10 (0.27); 12	10 (0.43); 9 (0.40); 10	9 (0.68); 8 (0.18); 9	10*; 7 – 16 ^{gl} ***, 17 ^{g2}
Pertyeae	13 (0.31); 9 (0.23); 13	9 (0.23); 13 (0.20); 13	13 (0.28); 9 (0.26); 6	9 (0.22); 14 (0.22); 6	13 ^h ***
Gymnarrheneae	9 (0.93); 10 (0.05); 11	9 (0.65); 10 (0.26); 11	9 (0.92); 10 (0.08); 2	9 (0.70); 10 (0.29); 2	10*
Cichorieae	9 (0.60); 8 (0.33); 9	9 (0.44); 8 (0.28); 9	9 (1.00); 9	9 (0.96); 10 (0.03); 9	9*/****
<i>Heterolepis</i>	-	-	9 (0.91); 8 (0.08); 9	9 (0.86); 8 (0.11); 9	6 ⁱ ***
Eremothamneae	-	-	9 (0.35); 8 (0.19); 9	9 (0.25); 8 (0.18); 9	-
Arctotideae	9 (0.74); 8 (0.22); 9	9 (0.43); 8 (0.23); 9	-	-	9*
Arctotideae_Arct	-	-	9 (0.91); 8 (0.06); 9	9 (0.81); 8 (0.10); 9	-
Arctotideae_Gort	-	-	8 (0.55); 9 (0.36); 8	8 (0.49); 9 (0.39); 8	-
Plathy carpheae	-	-	9 (0.82); 10 (0.12); 9	9 (0.71); 10 (0.19); 9	-
Liabeae	9 (0.97); 10 (0.03); 9	9 (0.87); 10 (0.11); 9	9 (1.00); 9	9 (0.98); 10 (0.02); 9	7; 9*; 12; 14; 16; 18 ^k ***
<i>Distephanus</i>	-	-	9 (0.60); 10 (0.40); 9	10 (0.52); 9 (0.47); 9	No data
Moquinieae	-	-	10 (0.41); 9 (0.28); 9	10 (0.74); 9 (0.25); 9	-
Vernonieae	9 (0.77); 10 (0.22); 9	10 (0.61); 9 (0.37); 9	10 (0.97); 9 (0.03); 10	10 (0.96); 9 (0.03); 10	10*
Corymbieae	9 (0.94); 10 (0.05); 10	9 (0.65); 10 (0.27); 10	9 (0.90); 10 (0.10); 13	9 (0.67); 10 (0.33); 13	8 ⁱ ***
Senecioneae	9 (0.57); 10 (0.42); 10	10 (0.59); 9 (0.35); 10	10 (1.00); 10	10 (1.00); 10	5; 10 ^m *

Calenduleae	9 (0.63); 10 (0.17); 9	9 (0.43); 10 (0.37); 9	9 (0.77); 10 (0.21); 10	9 (0.56); 10 (0.39); 10	8; 9; 10*
Gnaphalieae	9 (0.89); 8 (0.08); 9	9 (0.71); 10 (0.22); 9	9 (0.51); 8 (0.45); 8	9 (0.52); 8 (0.41); 8	6; 7; 10 ^{o*} ; 11; 12; 13; 14 ⁿ */***
Anthemideae	9 (0.92); 10 (0.08); 9	9 (0.64); 10 (0.32); 9	9 (0.89); 10 (0.10); 9	9 (0.74); 10 (0.25); 9	9 ^o ; 10*/***
Astereae	9 (0.96); 10 (0.02); 9	9 (0.75); 10 (0.22); 9	9 (0.96); 10 (0.04); 9	9 (0.83); 10 (0.16); 10	9 ^p */***
Inuleae	9 (0.60); 10 (0.37); 10	10 (0.46); 9 (0.44); 10	9 (0.71); 10 (0.28); 10	9 (0.51); 10 (0.47); 10	5, 8, 9, 10 ^{o1} *, 7 – 11 ^{o2} /**
Athroismeae	10 (0.72); 9 (0.23); 10	10 (0.65); 9 (0.18); 10	10 (0.76); 9 (0.18); 11	10 (0.66); 9 (0.17); 12	7; 10 ^r */***
Feddeae	-	-	9 (0.89); 10 (0.09); 15	9 (0.66); 10 (0.31); 15	No data
Helenieae	9 (0.76); 10 (0.09); 9	9 (0.49); 10 (0.28); 9	9 (0.90); 10 (0.05); 13	9 (0.67); 10 (0.23); 12	18**
Coreopsideae	12 (0.29); 13 (0.20); 12	9 (0.24); 12 (0.18); 9	9 (0.75); 10 (0.09); 12	9 (0.61); 10 (0.35); 10	12; 16*
Neurolaeneae	9 (0.80); 10 (0.17); 9	9 (0.72); 10 (0.20); 9	9 (0.76); 10 (0.15); 11	9 (0.69); 10 (0.26); 9	11*
Tageteae	9 (0.87); 10 (0.08); 9	9 (0.89); 10 (0.04); 9	9 (0.77); 12 (0.12); 12	9 (0.87); 10 (0.09); 9	18**
Chaenactideae	8 (0.54); 9 (0.37); 8	8 (0.41); 7 (0.37); 8	9 (0.45); 8 (0.42); 9	8 (0.40); 7 (0.35); 8	9*
Bahieae	10 (0.76); 9 (0.19); 10	10 (0.58); 11 (0.17); 10	10 (0.65); 11 (0.15); 11	10 (0.48); 11 (0.18); 10	17**
Polymnieae	9 (0.89); 10 (0.09); 9	9 (0.80); 10 (0.16); 9	9 (0.91); 10 (0.08); 10	9 (0.77); 10 (0.22); 9	15 ^s
Heliantheae	18 (0.83); 9 (0.10); 18	18 (0.71); 9 (0.12); 18	9 (0.96); 10 (0.04); 9	9 (0.84); 10 (0.16); 9	8, 9, 17 – 19, 18 ^t
Millerieae	9 (0.53); 8 (0.36); 9	9 (0.43); 8 (0.41); 9	9 (0.55); 8 (0.34); 9	9 (0.47); 8 (0.38); 9	9; 10; 11; 12; 14 ^u
Perityleae	18 (0.87); 9 (0.12); 18	18 (0.85); 9 (0.11); 18	18 (0.83); 9 (0.15); 18	18 (0.76); 9 (0.19); 18	18 ^v **

Eupatorieae	9 (0.97); 10 (0.03); 9	9 (0.90); 10 (0.06); 9	9 (0.78); 10 (0.21); 9	9 (0.65); 10 (0.30); 9	10; 17; 18 ^w
Madieae	9 (0.91); 8 (0.09); 9	9 (0.43); 7 (0.29); 9	9 (0.97); 8 (0.01); 9	9 (0.86); 10 (0.07); 9	19 ^{**}

Arct. = Arctotidinae subtribe; Gort. = Gorteriinae subtribe. ^a Solbrig (1977), Cronquist (1981), Bremer (1994), Santosh and Raghbir (2013). ^b Vallès et al. (2005). ^c Stuessy et al. (2009), chapter 13; x = 27 (Watanabe et al., 2007). ^d x = 9 seems to be available for all or nearly all the base numbers listed. ^e x = 4, x = 9, x = 23 (reviewed by Sancho & Freire (2009), chapter 15. ^f Based on a single count of 2n = 16 (Funk & Hind, 2009), chapter 16. ^{g1} Susanna & Garcia-Jacas (2009), chapter 20; ^{g2} in Watanabe et al. (2007). ^h (Freire, 2009), chapter 21. ⁱ in Raven et al. (1960); Funk & Chan (2009), chapter 23. ^j Funk & Karis (2009), chapter 31. ^k Dillon et al. (2009), chapter 27. ^l Based on a single count of 2n = 16 in Corymbium congestum (Nordenstam & Funk, 2009), chapter 32. ^m Raven et al. (1960) and Vallès et al. (2005); Watanabe et al. (1999) cited in Watanabe et al. (2007); Ward et al. (2009), chapter 36. ⁿ Vallès et al. (2005); Watanabe et al. (2007); Oberprieler et al. (2009), chapter 38. ^p Raven et al. (1960); Watanabe et al. (2007); Brouillet et al. (2009), chapter 37. ^{q1} Raven et al. (1960) and Vallès et al. (2005); ^{q2} Watanabe et al. (2007). Robinson et al. (1997) presented x = 10 and Anderberg (2009) presented the base numbers of x = 9 or x = 10, chapter 39. ^r Anderberg (2009a), chapter 40. ^s Estes & Beck (2011). ^t x = 9 or its multiples (Raven et al., 1960) or also x = 18 (Watanabe et al., 2007); x = 17-19 (Smith, 1975) and x = 8 (Stuessy, 1977) cited in Robinson (1981). ^u Blösch et al. (2009). ^v Robinson (1981) and Baldwin et al. (2002). ^w x = 10 (Robinson et al., 1997), x = 17 (Watanabe et al., 1995), x = 18 (Watanabe et al., 1999) cited in Watanabe et al. (2007).

* Estimated by Funk et al. (2009). ** Estimated by Baldwin et al. (2002). *** In Funk et al. (2009)

The dash (-) present on the base number indicates the absence of data, according to Funk et al. (2009).

Table 4 – Akaike Information Criterion (AIC) and Maximum Likelihood (ML) values obtained with and without fixing the Asteraceae root with a certain haploid chromosome number, on each phylogenetic hypothesis. For the root fixed analyses, the two best ancestral numbers as inferred by the Bayesian and maximum likelihood methods were considered. All analyses were performed with the polymorphic data only, according to the best model (Mc2).

	2005 supertree		2009 supertree	
	AIC	ML	AIC	ML
<i>Root not fixed</i>	<i>1679.41</i>	<i>-836.70</i>	<i>2863.98</i>	<i>-1428.99</i>
Root fixed at 2	1689.51	-841.75	2860.98	-1427.49
Root fixed at 8	1678.98	-836.49	-	-
Root fixed at 9	1661.62	-827.81	2846.58	-1420.29
Root fixed at 10	-	-	2847.96	-1420.98

The expected number of changes along each branch

Regardless of the phylogenetic hypothesis and the coding scheme, the most common inferred events with a $PP > 0.5$ were the chromosome losses (Table 2). The number of events of chromosome number change were, in general, higher for the single data than for the polymorphic data, irrespective of the type of event (Table 2). Only the number of duplications events in 2005 supertree were higher for the polymorphic data than for the single data (Table 2).

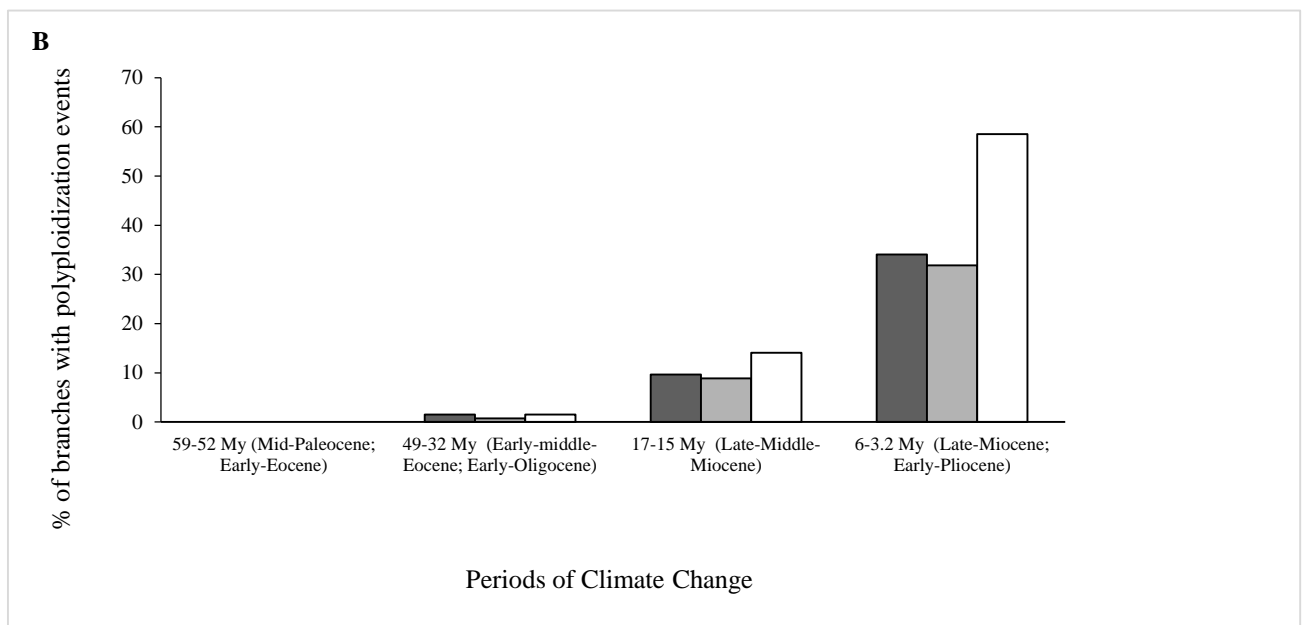
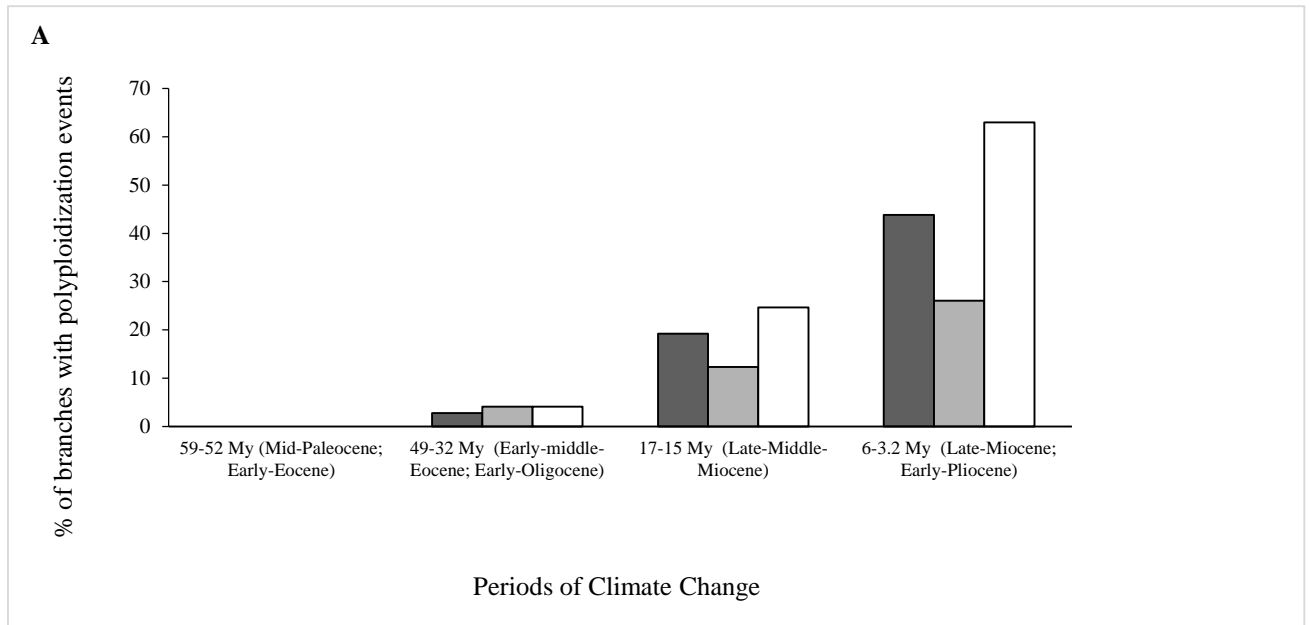
The number of chromosome duplications was higher than the number of chromosome demi-duplications for polymorphic data, whereas the opposite was observed for the single data, irrespective the phylogenetic hypothesis (Table 2). Nevertheless the differences between the number of duplications and demi-duplications were very low with the exception of the best model for single data in 2005 supertree (Table 2).

Polyploidization events and climate changes

Many branches in which a polyploidization event was inferred coincided with ancient climate change periods (Figure 1). The polyploidization events were associated more frequently to the most recent period of climate change, namely the Late-Miocene and Early-Pliocene (6.0 - 3.2 Mya). This association was weaker in older climate change events (Figure 1).

In detail, during the period of Mid-Paleocene and Early Eocene, there were no branches with polyploidization events (Figures 1A and 1B). During the Early-middle-Miocene and the Early-Oligocene, the number of branches with polyploidization events was very low, with duplications (2.74%) coinciding with climate change events in a lower proportion than demi-duplications (4.11%) for the 2005 supertree (Figure 1A), whereas the opposite was observed in the 2009 supertree (Figure 1B). In Late-middle-Miocene, an increase in polyploidization events was observed (from 4.11% to 24.66% - 2005 supertree; from 1.48% to 14.07% - 2009 supertree), with the number of branches with duplications events being higher than the number of branches with demi-duplications. The same trend was observed for the period of Late-Miocene and Early-Pliocene but with a higher number of polyploidization events (63.01% and 58.52% of total polyploidization events in the 2005 and 2009 supertrees, respectively), in both phylogenetic hypotheses.

Figure 1 – Percentage of branches with polyploidization events during the periods of climate change occurred along the evolution of the Asteraceae family. A – 2005 supertree; B – 2009 supertree; My – million years. Dark grey bars – duplication; light grey bars – demi-duplication; white bars – any chromosome duplication type.



4. Discussion

Models of chromosome evolution in Asteraceae

The analysis of the chromosome number evolution in Asteraceae revealed that the haploid chromosome number shifted frequently along the evolution of the Asteraceae family. The best evolutionary models obtained (Mc2 and Mc3 models) showed that dysploidy and polyploidy were very important events, being frequently associated (Table 1). More exactly, the descending dysploidies through chromosome fusion were the most common genetic mechanism along the evolution of this family (Table 2). These results are similar to those obtained for the Araceae (Cusimano et al., 2012), Melanthiaceae (Pellicer et al., 2014), and Colchicaceae (Chacón et al., 2014) families. In addition, previous studies in specific tribes of the Asteraceae family using other approaches have also shown that dysploidies (more precisely, descending dysploidy) and genome duplications were two frequent and important processes of chromosomal number change (Ito et al., 2000; Anderberg, 2009b; Funk & Chan, 2009; Semple & Watanabe, 2009; Susanna & Garcia-Jacas, 2009; Ward et al., 2009); whereas in other tribes, polyploidy seems to have played the most important role (Robinson, 1981; Vallès et al., 2005; Baldwin, 2009; Kilian et al., 2009; Oberprieler et al., 2009; Sancho & Freire, 2009; Stuessy et al., 2009). A recent study exploring karyotypic changes in fifteen clades of angiosperms also highlighted the co-occurrence of dysploidy and polyploidy (Escudero et al., 2014). Thus, our results and previous studies emphasize the importance of these phenomena and their association in the evolution of chromosome number of flowering plants, and of Asteraceae in particular.

The differences observed in the number of events that occurred along the branches, between coding schemes and phylogenetic hypotheses, may be explained by the complexity and diversity of the Asteraceae family. This family comprises a vast number of species, and phylogenetic uncertainties still exist for some tribes, as the Heliantheae, Senecioneae and Mutisieae tribes. Furthermore, for several genus and species no chromosomal data was available (Semple & Watanabe, 2009). These sampling difficulties suggest both the need for more phylogenetic studies to solve the uncertain position of some tribes, as well as further cytological investigations. Finally, the inclusion of polymorphic data seemed to provide more reliable inferences than that chromosome number reduction, and thus, future studies are encouraged to follow the same approach and consider chromosome number polymorphisms.

Ancestral chromosome numbers

Our models of chromosome number evolution allowed to infer for first time the ancestral haploid numbers for the sunflower family using a statistical approach (for further and more precise information of all the ancestral numbers inferred, see Figure S1 in Appendix 1). Overall, our results agreed with previous hypotheses of ancestral chromosome numbers for the family and for many of its tribes (Table 3), with $n = 9$ (under Bayesian inference) being the most probable ancestral chromosome number of the Asteraceae family (Solbrig, 1977; Cronquist, 1981, Bremer, 1994 *cited in* Semple & Watanabe, 2009; Santosh & Raghbir, 2013). Unexpectedly, the ancestral number for Asteraceae obtained under ML was $n = 2$, a very low and unreliable number (Table 3). Considering that other ancestral ML estimations across the tree were very consistent with the Bayesian approach, it is difficult to envisage the causes for this large disagreement in the root of the family.

Still, several disagreements were observed between the ancestral inferences of this study and those of previous works for ancestral and base chromosome number estimations (e.g., Anthemideae, Mutisieae, Hecastocleideae, and Corymbieae; Table 2). Two main factors might have contributed for the observed disagreement between previous estimations: 1) the use of different approaches, mainly basic chromosome numbers instead of the haploid chromosome number; and 2) incomplete taxon sampling. First, previous estimations have been frequently based in algebraic inferences or, considered solely the lowest available haploid count as the ancestral condition instead of using specific models of chromosome evolution (Powell et al. (1974) *cited in* Funk & Hind (2009); Weitz (1989) *cited in* Nordenstam & Funk (2009). Second, in spite our analysis makes use of the largest phylogenetic trees of the family, they still represent an incomplete data set. Future studies with more complete phylogenetic trees will allow to get even better inferences of ancestral states and to solve if the disagreements that may be related with the lack of complete sampling.

Polyploidy and climate changes

The present study revealed that it is very likely that several WGD occurred in the evolution of different lineages of Asteraceae. For instance, a WGD event with a high probability (≥ 0.8) was observed near the base of the Heliantheae alliance (for further and more precise information of all the duplication and demi-duplication events inferred, see

Figure S2 in Appendix 1). This result seems to be in accordance with previous evidences from genomic and cytological analyses that also revealed independent genome duplications near the base of this lineage (Baldwin et al., 2002; Barker et al., 2008). The origin of the Heliantheae might have occurred during the Late-Middle Miocene (Figure S2). Although our analyses agree with the results of Barker et al. (2008) in this particular case, the two other suggested paleopolyploidization events inferred by their genome analyses, i.e., 1) a paleopolyploidization placed near the origin of the family just prior to the rapid radiation of its tribes; and 2) an independent genome duplication near the base of the tribe Mutisieae, were not confirmed by our study. This disagreement may be related with the different approaches followed. While Barker et al. (2008) performed a comparative study of thousands of expressed sequence tag (ESTs) from 18 Asteraceae species and two outgroups (*Solanum lycopersicon* and *Arabidopsis thaliana*), our study focuses on inferring the evolution of chromosome numbers using data from more than 500 genera. Therefore, the lack of congruence might be produced mainly because of the very low sampling effort in Barker et al. (2008), which might have led to an incorrect localization of the WGD events that were found. Indeed, considering the outgroups used in Barker et al. (2008), the WGD attributed to the origin of Asteraceae might have occurred before the origin of this family. In addition, these authors suggest that there was another WGD at the base of the tribe Mutisieae. However, only one species of this tribe, *Gerbera hybrida*, was included in that study. In our study, no WGD was discovered at the origin of the Mutisieae, but rather within the tribe in the origin of the genus *Gerbera* (Figure S1).

Ancient polyploidization events may be harder to detect than recent ones, because of the genomic changes and restructuring that follows polyploidization events. The majority of the WGD reconstructed were observed mainly towards the tips of the tree (Figure S2). The same pattern was observed in the Araceae (Cusimano et al., 2012) and Melanthiaceae (Pellicer et al., 2014) families. Further studies combining haploid chromosome number evolution and genome wide analyses will help to assess the reliability of these results.

The lack of accuracy to find the exact place of WGD events makes it difficult to assess whether ancient climate changes could trigger the success of old WGD in the evolution of plant lineages. Dynesius and Jansson (2000) suggested that species formed by abrupt mechanisms such as polyploidy may be present on higher proportions in harsh and

unstable environments, as are the periods of climatic change (Fawcett & Van de Peer, 2010). Our exploratory approach provided a first insight in this issue. More than a half of the branches with polyploidization events underwent periods of climate change, suggesting that WGD may be linked with stress conditions. No WGD was found during the first period of climate changes that was considered, mainly because the origin of Asteraceae was estimated on 50 million years (Funk et al., 2005), much later than the mentioned period. Most of the WGDs were related with the most recent period of climate change, namely the period between the Late Miocene and the Early Pliocene (Figure 1). This period of time was marked by cooling conditions and a subtle warming trend, which may explain its instability (Zachos et al., 2001). The high occurrence of polyploids (in particular, of recently evolved polyploids) at present time in arctic areas is a well-known example of polyploid distribution in harsh areas of the Earth (Brochmann et al., 2004). Van de Peer et al. (2009) argued that most of the ancient WGD that survived - and according to these authors, a few - did so because they occurred at specific times, e.g. during major ecological upheavals and periods of extinction, when the competition with diploids was reduced and when new ecological niches became available. In stable ecosystems, the competition may be much higher than in severely perturbed environments (Van de Peer et al., 2009; Fawcett & Van de Peer, 2010). The same authors defended that the availability of ecological niches could be the single most important determinant for the survival and long-term evolutionary success of a newly arisen polyploid. To fully assess the potential effect of ancient climate changes on the evolutionary success of polyploids future studies should focus in exploring in detail the WGD found in these analyses, in particular to get more accurate estimations of their age, and therefore be able to more systematically assess the validity of the correlations that were found.

The evolutionary significance of polyploidy has long been a controversial subject. The results obtained by Mayrose et al. (2011) suggested that the newly formed polyploid lineages generally fail to persist, indicating that polyploidy is most often an evolutionary dead-end; however, the authors considered that the expanded genomic potential of those polyploids that do persist drives longer-term evolutionary success. On the other hand, Soltis & Soltis (2000) defended that the pattern of divergent speciation at the polyploid level contradicts the view of polyploids as evolutionary dead-ends, and even considered that polyploidy has a major role on the evolution and speciation, because genomic studies indicate numerous ancient WGD events across the angiosperms, being its genomes

fundamentally polyploids (Soltis et al., 2014). This Thesis revealed that the evolution of the Asteraceae family was marked by a considerable number of polyploidy events, with some tribes being of polyploid origin (e.g., all the tribes included on the Heliantheae alliance have a tetraploid ancestor). Furthermore, our analyses provided the first macroevolutionary observation of a possible link between climate changes events and the probability of genome duplications. Still, further studies are needed to fully understand this relation, as well as, the importance of that these genomic mechanisms have along the evolution of the Asteraceae family.

5. References

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6. Appendix I

Supplementary Tables

Table S1 - Estimated age of branching events.

Node label ¹	Branching event	Dating Method ²	Estimated time (Mya) ³	Reference	Calibration age ⁴ (Mya)
n0	Supertree root	MD/FD	73 (62–79)	Bell et al. (2010)	73
n1	Origin of Goodeniaceae	MD	64.5 (49 - 80)	Kim et al. (2005)	64.5
n2	Goodeniaceae/ Calyceraceae and Asteraceae	MD	50	Funk et al. (2005)	
	Origin of Asteraceae crown-group ^a	MD	42-36	Kim et al. (2005)	
	Origin of Asteraceae older Crown-group ^a	MD/FD	49 (52-48)	Beaulieu et al. (2013)	49
	Origin of Asteraceae older Crown-group ^a	*	(71.1-) 52.6,47.4 (-45.4)	Swenson et al. (2012)	
	Origin of Asteraceae	MD	45.5 (42 - 49)	Kim et al. (2005)	
	Origin of Asteraceae	**	48 (43 - 52)	Devore & Stuessy (1995)	
	Origin of Asteraceae	MD	41-50	Funk et al. (2009)	
	Major radiations of Asteraceae	MD	35-25	Funk et al. (2009)	
n3	Barnadesioideae / rest of Asteraceae	MD	39.0 (36 - 42)	Kim et al. (2005)	
	Barnadesioideae/rest of Asteraceae	FD	47.5	Barreda et al. (2012)	
	Barnadesioideae genera diversification	MD	28.5 (22 - 35)	Kim et al. (2005)	28.5
n4	Origin of Barnadesieae clade	FD	23	Katinas et al. (2007) ; Stuessy et al. (2009) ⁵	23

n50	Origin of Nassauvieae clade	FD	20-23	Katinas et al. (2007)	21.5
n5	Origin of Gochnatioideae ^a	MD	38-32	Kim et al. (2005)	35
n6	Origin of extant genera of Gochnatioideae	FD	23-28	Katinas et al. (2007)	
	Origin of Carduoideae ^a	MD	40	Barres et al. (2013)	40
	Origin of Carduoideae ^a	MD	29-24	Kim et al. (2005)	
n7	Origin of Cardueae	MD/FD	24-5	Cox and Moore (2004) ⁶	14.5
n8	Origin of Gymnarrhenoideae ^a	MD	(31-)28,24(-21)	Wikström et al. (2001)	
	Origin of Gymnarrhenoideae ^a	MD/FD	(37-)29,27(-19)	Bell et al. (2010)	28
n9	Origin of Cichorieae	MD	28	Zhang et al. (2011)	
	Origin of Cichorieae	MD/FD	26 (23-30)	Tremetsberger et al. (2012)	26
n10	Origin of Cichorieae - <i>Cichorium intybus</i>	FD	22-28.4	Hochuli (1978) ⁷	25.2
n11	Origin of Cichorieae – <i>Scorzonera hispanica</i>	FD	3.4	Blackmore et al. (1986)	3.4
n12	Origin of Cichorieae – <i>Sonchus oleraceus</i>	FD	5.4	Blackmore et al. (1986)	5.4
n13	LAVL ⁸ tribes / Asteroid tribes	MD	35 (32 - 38)	Kim et al. (2005)	
	Origin of LAVL tribes	MD	27.5 (24 - 31)	Kim et al. (2005)	27.5
n14	Origin of Liabeae	MD	10 (5 - 15)	Funk et al. (2005,2009)	10
n15	Origin of Asteroideae sub-family	MD	29-30	Hershkovitz et al. (2006)	
	Origin of Asteroideae sub-family	MD	26-29	Kim et al. (2005)	

n16	Origin of Asteroideae sub-family	MD	35-39	Kim et al. (2005)	37
	Origin of Asteroideae ^a	MD	39-26	Pelser & Watson (2009)	
	Diversification of Asteroideae ^a	MD	(56.6-43.0(-29.6)	Bergh & Linder (2009)	
	Origin of Asteroid tribes	MD	32.5 (26 - 39)	Kim et al. (2005)	32.5
	Origin of Asteroid tribes	MD	43.0 (56.6 - 29.6)	Bergh & Linder (2009)	
n18	Heliantheae Alliance/ <i>Ambrosia</i> -type pollen	FD	35-25	Graham (1994)	30
	Origin of helianthoid tribes	MD	19 (17 - 21)	Kim et al. (2005)	
n19	Origin of Hecastocleidoideae	MD	35 (32 - 38)	Kim et al. (2005)	35
n20	Origin of Gnaphalieae	MD	34.5 (52.3 - 20.6)	Bergh & Linder (2009)	34.5
n21	Origin of Australasian Gnaphalieae	MD	14.6 (20.6 - 8.3)	Bergh & Linder (2009)	14.6
n22	Diversification of Anthemideae	MD	23.1 (19.0 - 27.2)	Oberprieler (2005); Oberprieler et al. (2009)	23.1
n23	Origin of <i>Coreocarpus</i>	MD	1	Kimball et al. (2003)	1
n24	Origin of <i>Cousinia</i> genera	MD	8.72	Barres et al. (2013)	8.72
n25	<i>Leontodon</i> / <i>Hypochoeris</i>	MD	6.58	Tremetsberger et al. (2006)	6.58
n26	<i>Helianthus</i> / <i>Tagetes</i>	MD	17.4 (15.1 - 22.3)	Tremetsberger et al. (2006)	17.4
n27	Origin of <i>Abrotanella</i>	MD	19.41(17.1 - 21.9)	Wagstaff et al. (2006)	19.41
n28	Diversification of Subtribe Chrysantheminae	MD	8	Oberprieler (2005)	8
	Diversification of Subtribe Chrysantheminae	MD	2.75 (2.5 - 3.0)	Francisco-Ortega et al. (1995)	

n29	Origin of <i>Argyranthemum</i>	MD	0.26 - 2.1	Francisco-Ortega et al. (1997)
	Origin of <i>Argyranthemum</i>	MD	5	Oberprieler (2005)
n30	Origin of Hawaiian silverword alliance	MD/FD	5.2 (4.4 - 6.0)	Baldwin & Sanderson (1998)
n31	Origin of Scolyminae	MD/FD	20	Tremetsberger et al. (2012)
n32	Origin of Scorzonerinae	MD/FD	18.5 (13.3-23.5)	Tremetsberger et al. (2012)
n33	Origin of Microseridinae crown-group	MD/FD	9	Tremetsberger et al. (2012)
	Radiation of Microseridinae	MD/FD	8.9 (5.6-12.6)	Tremetsberger et al. (2012)
	Origin of Microseridinae and Cichoriinae stem-group	MD/FD	11-12 (8-16)	Tremetsberger et al. (2012)

¹Branching events selected to estimate branch lengths on supertree; ²Fossil Data (FD)/ Molecular Data (MD); ³Mya = Million years ago; ⁴ The mean value was utilised for the minimum and maximum time estimates, when the age estimative was given as time interval; ⁵ Analyses of fossil pollen in Patagonia document (Katinas et al., 2007; Palazzesi et al. 2009); ⁶ cited in Susanna and Garcia-Jacas (2009), chapter 20; ⁷ cited in Tremetsberger et al. (2012); ⁸ LAVL= Lactuceae (= Cichorieae), Arctoteae, Liabeae, and Vernoniaeae tribes; * Obtained by historical biogeography of the trans-Pacific Abrotanella genus, analysed with cladistic methods; ** Obtained by molecular and fossil results reviewed by the authors; ⁴ Data from Angiosperm Phylogeny Website.

Table S2 - Models of chromosome number evolution considered in chromEvol 2.0 software of Mayrose et al. (2013), indicating the parameter estimates and the number of parameters included in each model. δ – constant chromosome loss rate; δ_1 – linear chromosome loss rate; λ – constant chromosome gain rate; λ_1 – linear chromosome gain rate; ρ – chromosome duplication rate; μ – chromosome demi-duplication or triploidization rate; β – base number; ν – base number rate.

Model name	Model code	Parameters	No. of parameters
Constant rate no duplication	Mc0	$\lambda; \delta$	2
Constant rate	Mc1	$\lambda; \delta; \rho$	3
Constant rate demi-duplication	Mc2	$\lambda; \delta; \rho=\mu$	3
Constant rate demi-duplication estimated	Mc3	$\lambda; \delta; \rho; \mu$	4
Linear rate no duplication	Ml0	$\lambda; \lambda_1; \delta; \delta_1$	4
Linear rate	Ml1	$\lambda; \lambda_1; \delta; \delta_1; \rho$	5
Linear rate demi-duplication	Ml2	$\lambda; \lambda_1; \delta; \delta_1; \rho=\mu$	5
Linear rate demi-duplication estimated	Ml3	$\lambda; \lambda_1; \delta; \delta_1; \rho; \mu$	6
Base number	Mb1	$\lambda; \delta; \beta; \nu$	4
Base number duplication	Mb2	$\lambda; \delta; \rho; \beta; \nu$	5

Supplementary Figures

Figure S1. Chromosome number evolution in the 2005 supertree using the polymorphic data set, according to the Mc2 model. Pie charts at nodes represent the probabilities of the inferred chromosome number(s); numbers inside charts show the chromosome number with the highest probability. Numbers at the tips are the most common haploid chromosome number for each taxa. The colour coding of chromosome numbers is explained in the inset.

Given the very large size of this tree, this figure is available in electronic format at this site: <http://cfe.uc.pt/files/figs1.pdf>

Figure S2. Duplication and demi-duplication events inferred and mapped in the 2005 supertree. The periods of climate changes occurred during the evolution of the Asteraceae family are represented. The colours symbolize the temperatures that characterized each period of climate changes: red – warm temperatures; blue – cold temperatures; purple – oscillations between warm and cold temperatures. The star symbol symbolizes whole genomic duplication events and the circle symbolizes demi-duplication events. The colour coding of the duplications and demi-duplications is explained in the inset.

Given the very large size of this tree, this figure is available in electronic format at this site: <http://cfe.uc.pt/files/figs2.pdf>

