Polyploidy and other changes at chromosomal level and in genome size: Its role in systematics and evolution exemplified by some genera of Anthemideae and Cardueae (Asteraceae)

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Abstract Polyploidy is one of the major evolutionary forces in plants and in particular in the largest angiosperm family, the Asteraceae. This chromosome set multiplication directly impacts the nuclear DNA contents, in terms of variation at holoploid and monoploid levels. Other karyological changes such as aneuploidy or dysploidy might produce genome size alterations as well, therefore playing also a relevant role as evolutionary forces. All these factors may promote speciation, thus having systematic implications. In this paper we review the mechanisms associated with genome size variation, as well as their evolutionary consequences at phylogenetic, systematic and even taxonomic levels. To do so, C-values and chromosome numbers have been compiled and complemented with other molecular cytogenetic data to be discussed within their respective molecular phylogenetic frameworks. The case studies come from several genera of the Asteraceae belonging to tribes Anthemideae (*Artemisia*) and Cardueae (*Cheirolophus*, *Echinops* and members of *Rhaponticum* group), which cover a wide range of life strategies (e.g., life cycles, ecology, geographical distribution).

Keywords aneuploidy; *Artemisia*; *Cheirolophus*; Compositae; dysploidy; *Echinops*; genome size variation; polyploidy; *Rhaponticum* group

Supplementary Material Table S1 is available in the free Electronic Supplement to the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

Amongst the mechanisms inducing chromosome number variation, polyploidy is probably the most important and certainly the most studied one. In fact, genome multiplication episodes are considered key factors in plant diversification (Jiao & al., 2011, and references therein). This phenomenon has occurred throughout the history of the vast majority (around 70%) of extant angiosperms (Masterson, 1994). Indeed, taxa with very small genomes have been claimed to be ancient polyploids (such as the putative paleohexaploid *Arabidopsis thaliana* (L.) Heynh., Blanc & al., 2003). Dysploidy and aneuploidy describe karyotype changes less frequent and less studied in plants (Siljak-Yakovlev, 1996). Dysploidy is a change (increase or decrease) of basic chromosome number usually coupled with chromosomal rearrangements, and is stable within populations and taxa, whereas aneuploidy is the loss or addition of one to a few (most frequently one or two) chromosomes to a chromosomal complement by abnormalities in cell division, and is usually unstable, even lethal, within taxa (Ehrendorfer, 1980; Rieger & al., 1982; Guerra, 2008). Other more marginal (in terms of evolutionary significance) chromosomal change events are aneusomaty (mixoploidy), i.e., unequal chromosome numbers in the cells of one individual (Duncan, 1945), and the presence of B-chromosomes (Jones & Rees, 1982; Camacho & al., 2000; Camacho, 2005).

All these chromosome number changes impact genome size, through variation in the holoploid nuclear DNA content (the so-called C-value, see Swift, 1950 and Greilhuber & al., 2005, for accurate genome size terminology) and frequently in the monoploid value as well (Pellicer & al., 2010a, and references therein). Assessing changes that affect chromosomes and/or genome size, the mode and tempo of their occurrence across a phylogeny, and relationships with morphological, ecological and other plant traits, is a powerful tool for deciphering evolutionary histories (Favarger, 1984; Crawford, 2000; Stace, 2000; Dobigny & al., 2004; Buggs & al., 2011). Besides alterations of chromosome number, differential chromosome staining and mapping methods provide valuable insights in biosystematic issues. Intraspecific variation at the genomic level often precedes interspecific variation, which makes it crucial for the understanding of microevolutionary processes and speciation

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phenomena (Favarger, 1978, 1981; Siljak-Yakovlev, 1996; Crawford, 2000). For all these reasons we believe that cytotaxonomic and cytobiogeographic approaches are invaluable for evolutionary studies (Stebbins, 1971; Stace, 2000; Guerra, 2008, and references therein).

The present paper is based on talks delivered at the XI IOPB congress (International Organization of Plant Biosystematists, Aurangabad, India, September 2010) on the "Evolution of plants from tropical to high mountain ecosystems" and at the XIII OPTIMA meeting (Organisation for the PhytoTaxonomic Investigation of the Mediterranean Area, Antalya, Turkey, March 2010) on "The role of Irano-Turanian elements in the evolution of Mediterranean Flora". Following Grant (1984) in the IOPB seminal book *Plant Biosystematics* based on the symposium held in Montréal in 1983, we here emphasize the importance of cytogenetics as one of the sustaining pillars of biosystematic studies. We focused on several representatives of the Asteraceae, since this large plant group—the largest family of angiosperms—has been widely studied cytogenetically (Watanabe, 2002, 2004; Garnatje & al., 2010, 2011). The following case studies have been selected from tribes Anthemideae Cass. and Cardueae Cass. *Artemisia* L., with more than 500 species, has a Central Asian origin, and is distributed across the whole Northern Hemisphere, including some landscape-dominating species; it is closely related to a dozen small genera, many of them of unclear taxonomy (Vallès & al., 2011). *Echinops* L. comprises more than 100 species distributed in tropical Africa, the Mediterranean basin and other temperate regions of Eurasia (Sánchez-Jiménez & al., 2010). The *Rhaponticum* Vaill. group contains ca. 40 species distributed in Eurasia, Africa and Australia (Hidalgo & al., 2006). Finally, *Cheirolophus* Cass. includes around 20 western Mediterranean and Macaronesian species (Garnatje & al., 2007).

■ POLYPLOIDY

Polyploidy, present in all the major clades of the Asteraceae, has been reported for ca. 570 genera of the family (58.3% of the 978 genera with chromosome counts), and an astonishing range of ploidy levels, from 2*x* to 48*x*, has been found (Semple & Watanabe, 2009).

Particularly *Artemisia* is a karyologically rather well-known genus. With 1400 chromosome counts, it is 12th in the family ranking (Semple & Watanabe, 2009). Of the species counted, 44% are exclusively diploid, 30% exclusively polyploid, and approximately 26% are known to be both diploid and polyploid (Pellicer & al., 2010a, and references therein). This means that more than 50% of the species show polyploidy. To date, 42 different chromosome numbers have been reported for the genus (see Fig. 1; Table S1 in the Electronic Supplement for tribe Artemisiinae). Categorized by base chromosome number, we find taxa from 2*x* to 16*x*—one single report, $2n = 144$, in *Artemisia medioxima* Krasch. (Pellicer & al., 2007)—for $x = 9$ and from 2*x* to 6*x* for $x = 8$. Within these ranges, every even ploidy level has been documented with the exception of 14*x*, whereas odd levels have rarely been reported (and only 3*x*, 5*x* and 7*x*).

Polyploidy is highly prevalent in some species, such as *Artemisia dracunculus* L., with ploidy levels ranging up to 10*x*, including 3*x*, which is uncommon in the genus (Kreitschitz & Vallès, 2003; Eisenman & Struwe, 2011, and references therein). Much more frequent are polyploid series involving three levels, such as those of the North American endemics *A. arbuscula* Nutt., *A. cana* Pursh and *A. rothrockii* A. Gray (McArthur & Sanderson, 1999) and the *A. vulgaris* L. complex (Vallès, 1987; James & al., 2000). Polyploidy appears also in taxa that are basically diploid: in *A. absinthium* L. 70 diploid chromosome counts ($2n = 18$) are available and one polyploid ($2n = 4x = 36$) record exists (Watanabe, 2002; Kreitschitz & Vallès, 2003). This exceptional tetraploid population may represent the starting point of an evolutionary differentiation in this species. Findings such as these strongly demonstrate the need for extensive chromosome counts as a general procedure, even in taxa considered well-known, in which new cytotypes can still be detected (e.g., *Melampodium* L.; Stuessy & al., 2004).

All *Artemisia* subgenera have undergone polyploidy, and several of the closely related genera also contain polyploids (Vallès & al., 2005; Garcia & al., 2006a; Sánchez-Jiménez & al., 2009), some of which have been re-included in *Artemisia* (*Mausolea* Poljakov, *Neopallasia* Poljakov and *Turaniphytum* Poljakov of subgenus *Dracunculus* (Besser) Rydb.). However, other segregate genera are still recognized on the basis of molecular studies (such as *Ajania* Poljakov, *Hippolytia* Poljakov and *Leucanthemella* Tzvelev; Sanz & al., 2008, and references therein). Polyploid representatives of the genus are scattered throughout its phylogeny, and do not form strictly polyploid lineages, thus confirming that genome duplications have arisen in independent episodes (Sánchez-Jiménez & al., 2009).

While some of these closely related genera such as *Artemisiella* Ghafoor (1 sp.), *Brachanthemum* DC. (10 spp.) and *Nipponanthemum* (Kitam.) Kitam. (1 sp.), among others, have $2n = 18$ as the only chromosome number (Watanabe, 2004), others such as *Ajania* (39 spp.), with six different chromosome numbers recorded, contain polyploid series (Fig. 1). The remaining genera of Artemisiinae with polyploid representatives reported to date exhibit large polyploid series. These are *Chrysanthemum* L. and *Dendranthema* (DC.) Des Moul. (together 37 species, sometimes merged; Oberprieler & al., 2009), with, 55 and 15 different chromosome numbers known, respectively. Aneuploid numbers have been noticed within this group, possibly due to the existence of several cultivars, the relatively high frequency of mixoploidy/aneusomaty, B-chromosomes and hybridisation.

In contrast to the Anthemideae discussed above, polyploidy characterizes whole genera and even subtribes of Cardueae. Its base number of $x = 10$ is considered ancestral (Semple & Watanabe, 2009), but the first-diverging genera in Cardueae have other base numbers, with $x = 9–12$ in Carlininae Dumort., $x = (7)13–18$ in Echinopsinae (Cass.) Dumort. and $x = 18$ in Cardopatiinae Less. Such high numbers suggest a polyploidy event in the early evolution of the tribe. Barker & al. (2008) indicated a single paleopolyploidisation event predating the Cardueae. This implies that no additional polyploidisation took place between the root of Cardueae and Centaureinae (Cass.) Dumort.

Especially, a closer view at *Echinops*, with all but one species having around $2n = 30$ chromosomes, suggests an ancient polyploidisation event at the base of the genus (Sánchez-Jiménez & al., 2009). However, the single exception to this chromosome number, *E. acantholepis* Jaub. & Spach (=*Acantholepis orientalis* Less.) with $2n = 14$ chromosomes, can be interpreted differently. It can either be the only strict diploid in the genus, or $2n = 14$ may have arisen through strong descendent disploidy, which is supported by the derived position of *E. acantholepis* within a clade of annual species (Sánchez-Jiménez & al., 2009, 2010). Annuals tend to have diverging features, including reduced chromosome numbers (e.g., within Cardueae, *Oligochaeta divaricata* (Fisch. & C.A. Mey.) K. Koch, Hidalgo & al., 2007, 2008; *Xeranthemum* L. group, Garnatje & al., 2004b), which is also the case in the sister species of *E. acantholepis*, *E. gmelinii* Turcz., that has the second lowest chromosome number of the genus, $2n = 26$ (Sánchez-Jiménez & al., 2009, 2010).

Polyploidy also is an important evolutionary mechanism in other Cardueae genera, with ploidy levels ranging from $4x$ to $7x$ ($2n = 112$–114 in *Cirsium quercetorum* Jeps.), $9x$ ($2n = 80$ in *Carduus pycnocephalus* L.), $10x$ to $11x$ ($2n = 110$ in *Centaurea kunkelii* N. Garcia), $11x$ ($2n = 110$ in *Centaurea spruneri* Boiss. & Heldr.). The most important genera in terms of species number such as *Centaurea* L. and *Cirsium* Mill. (both with ca. 250 species) display frequent polyploidy, as does to a lesser extent *Cousinia* Cass. (600–700 species; Watanabe, 2002, 2004). Polyploid series span two ploidy levels, less frequently three (e.g., *Cirsium vulgare* (Savi) Ten. $x = 17$ [$2x$, $4x$, $6x$]; *Carthamus lanatus* L. $x = 11$ [$4x$, $5x$, $6x$]), four (e.g., *Centaurea toletana* Boiss. & Reut. $x = 10$ [$2x$, $4x$, $5x$, $6x$]), or even six (e.g., *Carduus pycnocephalus* $x = 9$ [$2x$, $3x$, $4x$, $6x$, $7x$, $9x$]) (Watanabe, 2002, 2004).

Soltis & Soltis (1999) hypothesised that polyploidy is followed by a period of transilience similar to what Templeton (1980) proposed for diploid speciation, during which the genome is more amenable to change. Polyploidy is related to different biological parameters, of which ecological adaptation is particularly relevant for evolutionary processes. Polyploid taxa apparently have new evolutionary potential, allowing them to colonise new habitats and consequently expand geographically (Stebbins, 1971; Levin, 1983; Stuessy & al., 2004; Pellicer & al., 2010a, b, and references therein).

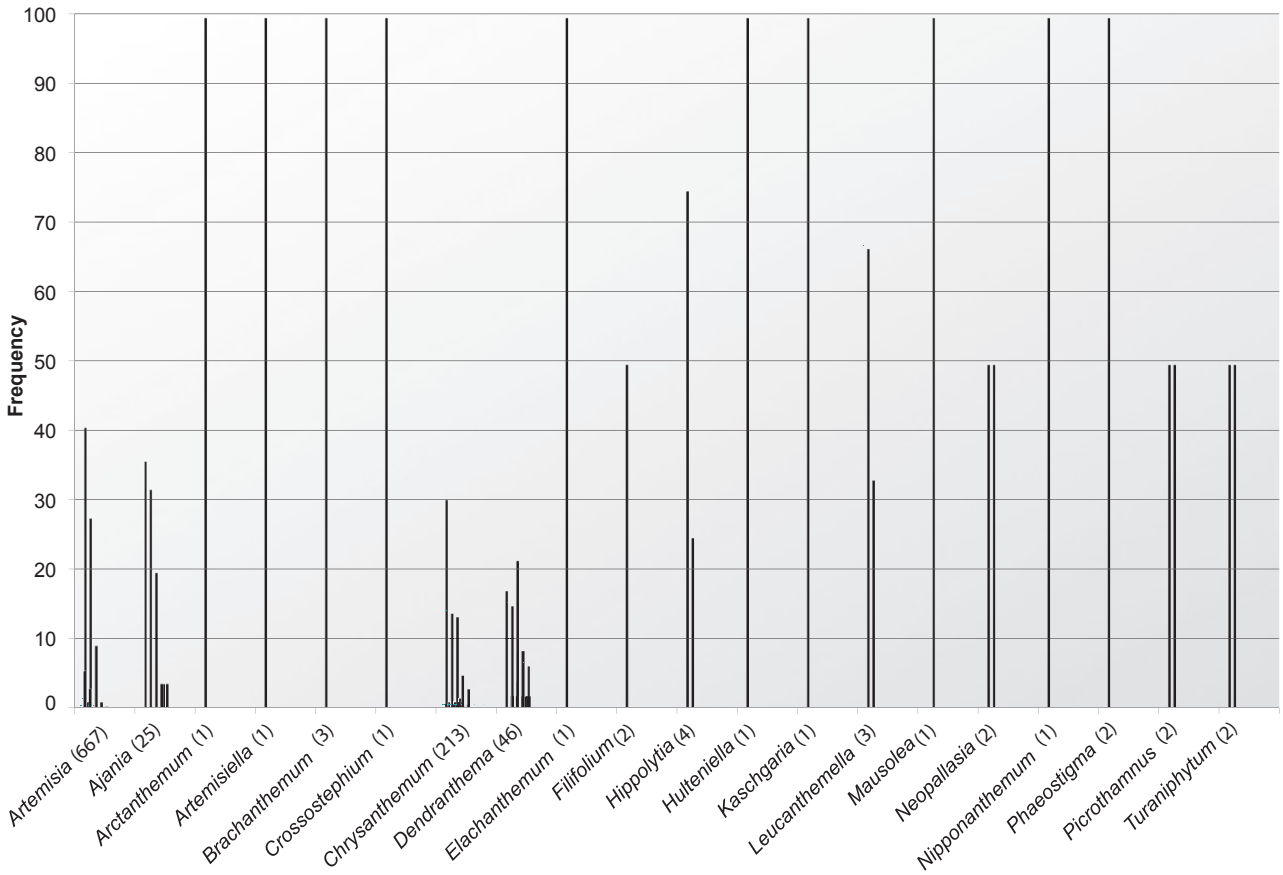

Fig. 1. Frequency plot of chromosome numbers in the genera of subtribe Artemisiinae. For every genus, each bar represents a published chromosome number, and its height represents the frequency (%) of this count in that genus (i.e., genera with one known chromosome count have a single bar of maximum height, 100). The numbers of observations are in brackets behind genus names. Data extracted from the Index to Chromosome Numbers in Asteraceae (Watanabe, 2002; http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e, accessed 4–7 Oct. 2011) and other available literature. Data for hybrids, cultivars or breeding lines have been excluded. A summary of the numerical data can be found in Table S1 (Electronic Supplement).

■ DYSPLOIDY (WITH ANEUPLOIDY AND ANEUSOMATY CASES)

Dysploidy is another powerful evolutionary mechanism in Asteraceae, although to a much lesser extent than polyploidy. It affects 214 genera, i.e., 21.9% of the genera with chromosome counts available (Semple & Watanabe, 2009). Solbrig (1977) stated that roughly 30% of chromosome numbers known for Asteraceae species are based on $x = 9$, but that whether this base number was the ancestral one in the family would require further study. Semple & Watanabe (2009) showed that the basal clades of Asteraceae and its sister families also are based on $x = 9$, supporting Solbrig's (1977) assumption, and hypothesised that ascending dysploidy to $x = 10$ should have occurred, coupled with geographical changes. Tribes Anthemideae and Cardueae are considered to have $x = 10$ as ancestral base number, although $x = 9$ has also been postulated as base number in the former, where it is the most frequent chromosome number (Heywood & Humphries, 1977; Schweizer & Ehrendorfer, 1983).

The largely predominating base number in *Artemisia* (lacking the postulated ancestral $x = 10$) is $x = 9$, which is present in all *Artemisia* subgenera and is the only base number in the closely related genera forming subtribe Artemisiinae as well as in close subtribes, such as Handeliinae Bremer & Humphries and Tanacetinae Bremer & Humphries. The minority number $x = 8$ is restricted to subgenera *Absinthium* DC., *Artemisia* and *Dracunculus*. So, in contrast to polyploidy, dysploidy is neither present in all *Artemisia* subgenera nor in any of its closest relatives.

Kawatani & Ohno (1964) postulated descending dysploidy in *Artemisia* based only on the observation that a much larger number of taxa has the highest base number ($x = 9$) in the genus, which is in agreement with the fact that the most common direction of dysploidy in plants is descending (Siljak-Yakovlev, 1996). Vallès & Siljak-Yakovlev (1997) provided experimental evidence for this descending dysploidy. *Artemisia vulgaris*, a $2n = 16$ species ($x = 8$-based), has a chromosome pair clearly larger than all others, with pericentromeric heterochromatin (telomeric heterochromatin is the most common situation in the genus) and with centromeric fragility, as both chromosome arms appeared quite separated in metaphase plates, probably recalling their earlier condition as independent chromosomes. These chromosomal features support the idea of descending dysploidy as a result of a centric or Robertsonian fusion, from $2n = 18$ to $2n = 16$ chromosomes, meaning a change in base number from $x = 9$ to $x = 8$. Guerra (2008) reported Robertsonian exchanges (either fission or fusion) as important causes of dysploid variation in plants. Robertsonian exchanges are common in the $x = 8$-based *Artemisia* species, both at diploid and tetraploid levels. Taxa such as *A. granatensis* Boiss., *A. judaica* L., *A. lucentica* O. Bolòs & al., *A. reptans* C. Smith ex Link., and *A. splendens* Willd. also show them (Vallès & Garnatje, 2005; Matoba & al., 2007; Vallès & al., 2011, and references therein). The restriction of this dysploid base number to *Artemisia* and closely related genera may even be useful for taxonomic decisions. Recently, *Tanacetum paradoxum* Bornm. has been transferred to *Artemisia* (as *A. paradoxa* (Bornm.) Sonboli) on the basis of karyological data together with morphological and molecular evidence (Sonboli & al., 2011). Concerning the chromosomal aspects, this is a $2n = 16$ taxon with a larger pair of chromosomes, clearly indicating descending dysploidy. The fact that dysploidy does not occur in *Tanacetum* L., a strictly $x = 9$-based genus, supports the taxonomic rearrangement consistent with other data. The same applies to another *Tanacetum* species (*T. incanum* L.) that had already been transferred to *Artemisia* (*A. incana* (L.) Druce) and also has $2n = 16$ chromosomes, two of which are larger and show centromeric fragility (Torrell & al., 2001).

In addition to the two main base numbers, two more have been reported to occur in *Artemisia*. One, $x = 7$, could be a continuation of the descending dysploid series. However, this base number has only been counted in a single species, although in eight populations (*A. pattersonii* A. Gray; Wiens & Richter, 1966). The other base number, $x = 17$, is secondary, of polyploid origin, and occurs in a few taxa with $2n = 34$ chromosomes such as *A. umbelliformis* Lam. Autopolyploidisation of a $2n = 18$ ancestor (most likely *A. eriantha* Ten.) followed by the loss of two chromosomes by hypoaneuploidy, or allopolyploidy involving the mentioned $2n = 18$ species and a $2n = 16$ parent (probably *A. glacialis* L.) have been proposed as potential ways to have given rise to this cytotype (Gutermann, 1979; Ehrendorfer, 1980; Vallès & Oliva, 1990; Oliva & Vallès, 1994). The count of $2n = 34$ has also been reported for populations of *A. vulgaris*, *A. dubia* Wall. ex Besser and *A. momiyamae* Kitam. and an English taxon described as a hybrid (*A.* ×*wurzellii* C.M. James & Stace). Besides, cases of aneusomaty/mixoploidy are not rare in *Artemisia* and other Artemisiinae, and can be found in *Chrysanthemum* and *Dendranthema*. These phenomena can be linked to vegetative reproduction (Vallès & al., 2011, and references therein), which is particularly common in genera with many cultivated species.

Dysploidy is a very frequent phenomenon in Cardueae at all taxonomic levels, and Centaureinae are especially noteworthy (Fig. 2). Dysploidy accompanies the diversification of *Echinops* and the *Rhaponticum* group, but it remains unclear whether divergent counts within *Cheirolophus* (Watanabe, 2002, 2004) are due to dysploidy or represent counting errors. In any case, the diversity in chromosome numbers among and within Cardueae tribes, genera, and even species makes it hard to disentangle the effects of polyploidy and dysploidy. For example, in *Echinops*, the difficulties in locating putative polyploidisation event(s) hamper drawing a clear picture of the contribution of dysploidy in the annual clade, which contains chromosome number variation from $x = 7$ to $x = 14$ (Sánchez-Jiménez & al., 2010). In the remaining Echinopsinae, $x = 14$, 15 and 16 are the most frequent base numbers (Fig. 2), and $x = 17$ and 18 are sporadically found (Sánchez-Jiménez & al., 2010). Dysploidy is particularly apparent in Centaureinae, and no chromosome base number(s) emerge(s) as obviously dominant (Fig. 2). Within Centaureinae, *Rhaponticum* and related genera display a descending dysploid series with three base chromosome numbers, $x = 14$, 13 and 12. These may be derived from a plesiomorphic condition of $x = 15$ based on

outgroup comparison with likely sister groups from Centaureinae (Hidalgo, 2006; Hidalgo & al., 2007). Among chromosomal restructuring, there is evidence of chromosomal fusions similar to those described in *Artemisia*. *Oligochaeta divaricata* and *Rhaponticum carthamoides* (Willd.) Iljin are the only species with $2n = 24$ chromosomes, the lowest chromosome number in the group. In both, one chromosome pair is longer than the others, and displays centromeric heterochromatin and centromeric fragility implying recent chromosomal fusion (Hidalgo & al., 2007, 2008).

■ CHANGES AND EVOLUTION IN CHROMATIN TYPES AND rDNA REGIONS

Karyological variations involving changes in chromosome structure rather than in chromosome number are also relevant to plant speciation and evolution (Fukuda, 1984). Different types of heterochromatic regions, chromosome areas rich in GC or AT bases, and hybridisation of DNA probes containing repetitive elements, ribosomal DNA and other specific regions revealed by so-called chromosome painting (Sharma & Sharma, 2001), are useful for comparative genomics, providing data for detecting evolutionary mechanisms and phylogenetic affinities (Matsuda & al., 2008; Raskina & al., 2008; Liehr, 2009).

Physical mapping of certain heterochromatic regions, different base-rich fragments and rDNA loci, has been performed for many species of *Artemisia* (Vallès & al., 2011, and references therein). Most species show a common banding pattern with mostly telomeric and subtelomeric bands. This pattern is typical across tribe Anthemideae (Schweizer & Ehrendorfer, 1983; Kondo & al., 2003; Abd El-Twab & Kondo, 2006; Hoshi & al., 2006, and references therein). As expected since they are GC-rich, rRNA genes co-localize with CMA positive regions (CMA stains preferably GC-rich heterochromatin), although not all CMA-positive regions contain rRNA genes. Despite the general constancy of the physical map of Anthemideae chromosomes, there are some characters of taxonomic and phylogenetic value. The North American endemics of subgenus *Tridentatae* (Rydb.) McArthur emend. S. Garcia & al. show a basic CMA banding and FISH pattern with six bands at the diploid level, whereas those of the Old World subgenus *Seriphidium* Besser show mostly four bands (Torrell & al., 2003; Garcia & al., 2009a). This is consistent with the results of molecular phylogenetic analyses (McArthur & al., 1998a, b; Sanz & al., 2008; Garcia & al., 2011) which considered the two subgenera as independent, in contrast to evidence (Watson & al., 2002) from molecular phylogenetic data as well. Furthermore, the banding/FISH pattern supported exclusion of some North American taxa from the core of subgenus *Tridentatae* (Garcia & al., 2007), which was later confirmed by molecular data (Garcia & al., 2011).

Changes in heterochromatin and rDNA loci number with polyploidy are also interesting and may have value in evolutionary studies. To predict the evolution of the number of rDNA signals, similar to genome downsizing—a frequent finding in plants (Lim & al., 2000; Leitch & Bennett, 2004)—an "rDNA loci downsizing" with polyploidy could be expected. Weak rDNA loci signals and proportionally less sites in *A. lagocephala* (Fischer ex Besser) DC. suggested a loss of both rDNA loci and gene copies in hexaploid populations of this taxon (Pellicer & al., 2010b). A similar situation would explain the 16*x A. medioxima* karyotype, containing 20 rDNA loci instead

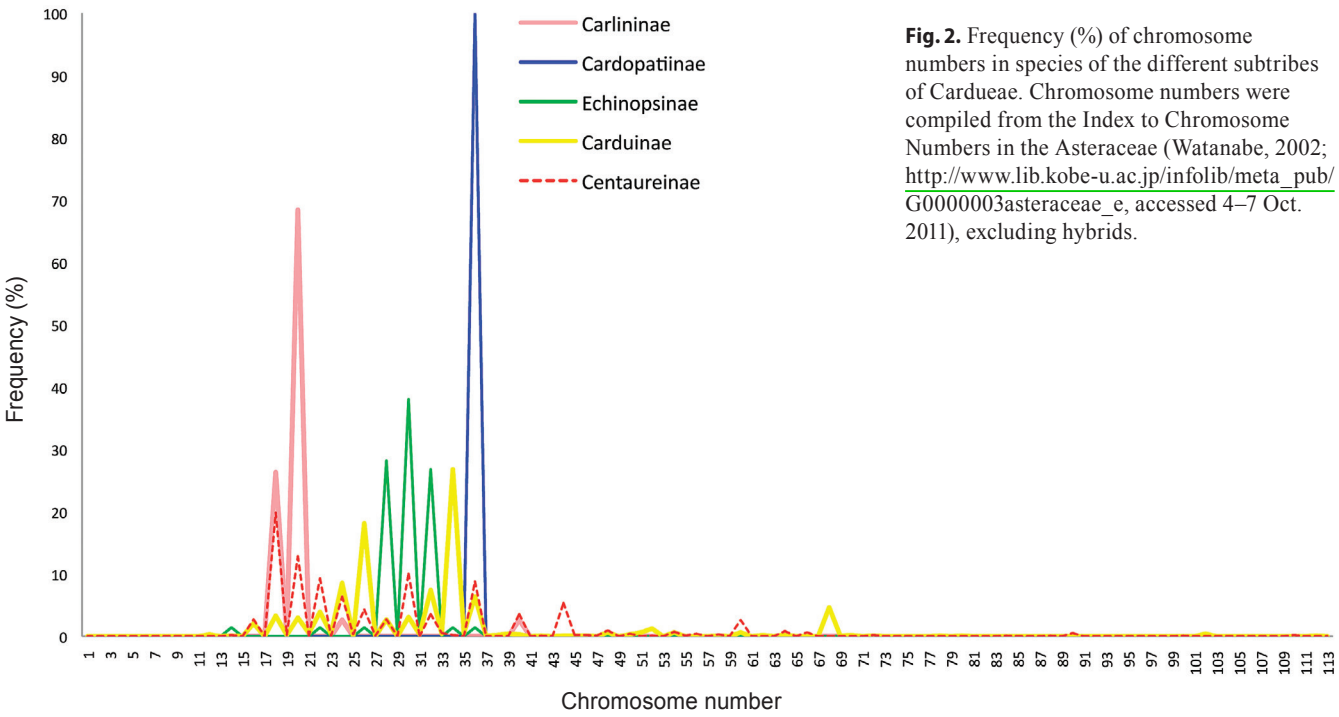

Fig. 2. Frequency (%) of chromosome numbers in species of the different subtribes of Cardueae. Chromosome numbers were compiled from the Index to Chromosome Numbers in the Asteraceae (Watanabe, 2002; http://www.lib.kobe-u.ac.jp/infolib/meta_pub/G0000003asteraceae_e, accessed 4–7 Oct. 2011), excluding hybrids.

of the 32 expected by a proportional loci increase in polyploids (Pellicer & al., 2010b). *Artemisia tridentata* Nutt. and *A. tripartita* Rydb. also show loci loss with increasing polyploidy; nevertheless, no rDNA loci loss, but an exactly additive pattern with respect to diploid progenitors was described for *Artemisia bigelovii* A. Gray and *A. nova* A. Nelson polyploids, and almost an exact addition in *A. argilosa* Beetle (Garcia & al., 2009a). The same situation was found among the South American polyploid endemic complex of *Artemisia* (Pellicer & al., 2010c). A precise multiplication of the diploid karyotype has also been found, within the Asteraceae, in *Tragopogon* L. (Pires & al., 2004). Concerning heterochromatin only, the third possible scenario has also been reported, i.e., an increase of heterochromatic sites in polyploids with respect to diploids, as detected in *A. argilosa* (Garcia & al., 2007) and more abundantly in *A. lagocephala*, from two DAPI sites at 2*x* to 78 in the 6*x* cytotype (Pellicer & al., 2010b).

Changes in chromosome structure also provide valuable information on the evolution at different taxonomic levels within the Cardueae. Some of the evolutionary patterns of differentiation of chromatin and DNA regions in Anthemideae discussed above also appear in Cardueae. This is the case in *Xeranthemum*, where Garnatje & al. (2004b) reported the loss of rDNA loci in polyploid congeners. In *X. longipapposum* Fish. & C.A. Mey. and *X. inapertum* (L.) Mill. the authors suggested a mechanism of amphyplasty by which the activity of the missing rDNA locus was silenced, lost or suppressed during the genome duplication process.

Four patterns of rDNA loci evolution are found in *Rhaponticum* and allied genera with a descending dysploid series with three basic chromosome numbers (Hidalgo, 2006). *Myopordon* Boiss. species have a characteristic CMA and DAPI telomeric banding pattern differing strongly from that of other representatives of the *Rhaponticum* group (Hidalgo & al., 2008). This unusual pattern is correlated with extreme, high-mountain environmental conditions where the species occur. The FISH pattern of *Oligochaeta divaricata* contrasts with that of *Callicephalus* C.A. Mey, *Myopordon* and *Rhaponticum* species by a much higher number of 18S-5.8S-26S (hereafter abbreviated as 35S) sites. In *Oligochaeta*, a switch in life cycle, breeding system, and environmental conditions probably induced a series of chromosomal rearrangements in order to reduce its genome size, promoting the fragmentation of 35S regions and their scattering in the genome (Hidalgo & al., 2008). In *Rhaponticum* species, two main events involving changes in genome structure explain rDNA locus diversity. Instead of four 35S sites—the typical number in the *Rhaponticum* group—some species of the genus present six sites (Hidalgo, 2006). Chromosome restructuring likely explains the two additional 35S sites. They may result from hybridisation and introgression in *R. heleniifolium* Godr. & Gren. and *R. scariosum* Lam., whereas they are coupled with dysploidy in *R. carthamoides*, the only member of the genus with 24 chromosomes (Hidalgo & al., 2008).

In tribe Cardueae, *Cheirolophus* shows a remarkable pattern of rDNA loci evolution since an unusual high number of 35S sites—constantly in terminal position—has been found in continental and Canary Islands species (Garnatje & al., in press). As *Cheirolophus* is the only member of Centaureinae to have radiated in the Canary Islands, a possible relationship between this island radiation and the increase in 35S sites could be envisaged. Interestingly, insular species have the lowest genome sizes (Garnatje & al., 2007) and the highest numbers of 35S sites.

Intraspecific variation in chromatin type and rDNA regions has also been observed in Cardueae. In a cytogenetic study of polyploid *Centaurea jacea* L. populations (Dydak & al., 2009), FISH revealed polymorphism in the number of 35S rDNA loci, with five to six pairs observed. The deletion of one rDNA locus in some populations as a result of polyploid genome reorganisation might explain intraspecific variation, showing that the number of rDNA copies can evolve very fast (Dydak & al., 2009).

■ TWO MODELS OF rDNA LOCI STRUCTURE AND DISTRIBUTION

The organisation of rRNA genes in the Asteraceae is very diverse and worth reporting. Although most groups in the family show the typical separate 5S and 35S rDNA arrays, an alternative rDNA organisation has recently been described, first in species of *Artemisia* (Garcia & al., 2009a, b) and lately in some other groups of the family (Garcia & al., 2010). It consists in a new kind of rDNA operon, which integrates all rRNA genes in a single unit in the order: 18S-5.8S-26S-5S; here, the 5S rRNA gene is inserted in inverted orientation in the long IGS region of rDNA proximal to the 26S gene (Garcia & al., 2009a, b). This feature is directly visible by FISH of rRNA genes in metaphase plates, where probes co-localise at the same loci (Torrell & al., 2003; Garcia & al., 2007, 2009b). This assembly of rRNA genes is similar to that of some other eukaryotes including yeast (Rubin & Sulston, 1973) or the liverwort *Marchantia polymorpha* L. (Sone & al., 1999) although in the latter case the 5S insertion apparently occurred in the forward orientation. Recently, Galián & al. (2012) described a comparable linkage in the rDNA of *Ginkgo biloba* L. There are scattered (although not rare) cases reported in the literature in which rDNA-FISH also suggests linkage between these genes (Garcia & al., 2009a, and references therein), although this has only been proven, to date (i.e., the genic regions have been sequenced), in the cases discussed above. These findings disproved the common assumption that rRNA genes in eukaryotes always occur in separated arrays, and exceptions continue to be found in different plant and animal groups.

It has been calculated that around 25% of species of the Asteraceae might display the linked rDNA configuration, including most members of tribes Anthemideae and Gnaphalieae (Cass.) Lecoq & Juillet as well as some groups of the Heliantheae alliance, all of them belonging to subfamily Asteroideae, which accounts for approximatly 70% of the species of the family (Garcia & al., 2010). Nevertheless, there exist exceptions: for example, *Elachanthemum* Y. Ling & Y.R. Ling, very closely related to *Artemisia* and clearly within the Artemisiinae clade (Sanz & al., 2008), has the "typical" eukaryotic arrangement, as do species of *Dendranthema*, another Anthemideae

genus; in both cases, however, there might be minor linked rDNA arrays as shown by PCR and RT-PCR (Garcia & al., 2010), although these are imperceptible by in situ hybridisation. This suggests that concerted evolution has been effective in homogenising one or the other kind of rRNA gene arrangement in this group. Species showing both configurations in more or less equal proportions have not been found (although a 5S solo locus was detected in *Coreopsis* L. of the Heliantheae alliance; Garcia & al., 2010). With regard to the rest of the family, and in particular to tribe Cardueae, the only rDNA configuration is the classical one with 5S and 35S rDNA in separate arrays (Garcia & al., 2012). The mobility in the genome of a retroelement which contains the 5S gene, named *Cassandra* (Kalendar & al., 2008), has been related to the alternative positions of 5S outside or inside the major rDNA array, although the available evidence is not conclusive in this respect.

■ GENOME SIZE CHANGES AND EVOLUTION

Most chromosomal changes discussed above imply genome size variation. Additional causes are minor, such as the occurrence of B-chromosomes (Levin & al., 2005), and there is size variation not always detectable at the cytogenetic level, such as the presence or absence of repetitive DNA and transposable elements (Kidwell, 2002, 2005). Nuclear DNA amount variation has strong relationships with many biological factors and implications in plant differentiation and evolution (Bennett & Leitch, 2011, and references therein).

Asteraceae are relatively well known regarding genome size, with 3% of the species investigated (Garnatje & al., 2010, 2011) as compared to 1.8% for the angiosperms (Bennett & Leitch, 2011). *Artemisia* is one of the genera particularly well covered in this respect, with data for 24.3% of the taxa investigated (Vallès & al., 2011, and references therein). Nuclear DNA content in this genus varies 9-fold, from 2C = 3.5 (*A. annua* L., Torrell & Vallès, 2001) to 31.51 pg (*A. copa* Philippi, Pellicer & al., 2010c). The two lowest 2C values are found in two annual taxa: *A. annua* and *A. scoparia* Waldst. & Kit. (2C = 3.54 pg, Garcia & al., 2004). However, the equally annual *A. leucodes* Schrenk (2C = 15.39 pg; Garcia & al., 2004) shows the largest genome size of diploid taxa in the genus. Interestingly, together with its outlier genome size, the species also has an unexpected placement in molecular phylogenies. This situation is not restricted to *A. leucodes* but also found in *A. judaica* (Torrell & Vallès, 2001; Vallès & al., 2003; Garcia & al., 2004), where both phylogenetic placement and genome size clearly differ from those of its subgeneric (*Artemisia*) counterparts.

Examples of agreement between genomes, molecular phylogenetic and other systematic data are abundant. At the suprageneric level, *Artemisia* and closely related genera constitute a monophyletic lineage characterised by the so-called *Artemisia* pollen type. The remaining genera that clearly separate from this *Artemisia* clade have the so-called *Anthemis* pollen type. Both pollen types differ in their exine ornamentation (Martín & al., 2001, 2003; Sanz & al., 2008; Pellicer & al., 2009b). Genome size is also significantly different between the two clades, in agreement with their separation based on both pollen type and DNA sequences (Garcia & al., 2004). At the infrageneric level, the three subgenera of *Artemisia* with the clearest delimitation in molecular analyses, *Dracunculus*, *Seriphidium* and *Tridentatae*, show more homogeneous genome sizes than subgenera *Absinthium* and *Artemisia* which are still not clearly structured and differentiated in traditional classifications and molecular phylogenetic analyses. Evolutionary changes resulting from artificial selection and domestication may also be found in genome size. This might be the case in the *A. arborescens* L. group, in which wild, cultivated and domesticated populations show significantly different nuclear DNA contents (Garcia & al., 2006b).

Genome size can be evaluated in relation to geographical expansion processes, which may be particularly interesting in island colonisations. At the specific level, this has been studied in the above-mentioned *A. arborescens*, where the insular populations of this Mediterranean taxon have significantly higher nuclear DNA amounts than the continental ones (Garcia & al., 2006b), and in *A. crithmifolia* L., very abundant along the European Atlantic coast from the southern Iberian peninsula to the Netherlands, with only two populations in the British Isles. These insular populations also have higher genome sizes than continental ones (Pellicer & al., 2009a). When considering variation between different species from the continent and oceanic islands, the opposite pattern is found. This is the case in another genus of Asteraceae, *Cheirolophus* (Garnatje & al., 2007) and is common in a large group of Macaronesian angiosperms (Suda & al., 2003, 2005). Thus, genome size data can help to elucidate how oceanic islands were colonised; in *Cheirolophus* the DNA content of *Ch. massonianus* (Lowe) A. Hansen & Sunding (1.44 pg, the highest value of all insular *Cheirolophus* species DNA) suggests that the colonisation of Madeira preceded that of the Canary Islands (where mean nuclear DNA content is 1.38 pg, Garnatje & al., 2007). In this case, the insular populations have lower nuclear DNA amounts than the continental ones, indicating a reduction in genome size during island colonisation.

Genome size may be also influenced by area fragmentation, as is the case in the above-mentioned *A. crithmifolia*, where continental populations are structured in two groups with significantly different genome sizes, separated by a discontinuity in its distribution area in the northern Iberian Peninsula (Pellicer & al., 2009a). In this respect, *Cheirolophus intybaceus* (Lam.) Dostál shows a different behaviour. This species, occupying a 50 km belt along the French and Iberian coasts, shows a significantly positive correlation between DNA content and latitude, so that its genome size decreases in drier and warmer habitats (lower latitudes); however, there are no significant differences between insular and continental populations, and the overall amount of DNA variation is so small in this taxon that no differentiated geographical groups can be established (Garnatje & al., 2009).

Of the causes inducing genome size variation, polyploidy deserves special attention. As discussed before, genome downsizing with polyploidy is very frequent (Leitch & Bennett, 2004). Pellicer & al. (2010a) established that genome size increase with ploidy level in *Artemisia* followed an asymptotic

model with a saturation behaviour. However, it seems clear that other genera of Asteraceae with polyploid series do not follow this pattern (Vallès & al., unpub. res.), and even in *Artemisia* some groups behave differently. For example, the South American endemic polyploid *A. mendozana* DC. does not show DNA loss, possibly due to the recent formation of this taxon (Pellicer & al., 2010c). Examples of both genome size relative decrease and proportional increase have been reported in subgenus *Tridentatae* as well (Garcia & al., 2009a).

In *Echinops*, the comparison between the genome size of *E. acantholepis* ($2n$ = 14, 2C = 6.52 pg, Garnatje & al., 2004a) and its sister species *E. gmelinii* ($2n$ = 26, 2C = 9.73 pg, Sánchez-Jiménez & al., 2009), does not help to explain the evolutionary process that resulted in different ploidy levels, with dysploidy and polyploidy being equally plausible, but does indicate genome downsizing (Garnatje & al., 2004a; Sánchez-Jiménez & al., 2009). Genome downsizing is also well documented in other Cardueae, associated both with polyploidy and dysploidy (in *Carthamus* L., Garnatje & al., 2006 and in the *Rhaponticum* group, Hidalgo & al., 2008, respectively).

Genome size variation in annual *Echinops* species is high: apart from the above *E. acantholepis* and *E. gmelinii*, the annual *E. nanus* Bunge ($2n$ = 28, 2C = 3.86 pg) has a particularly low value. This variability between closely related species in chromosome number and DNA amount has also been documented in other Irano-Turanian species with an annual life cycle, such as the *Xeranthemum* (Garnatje & al., 2004a) and *Rhaponticum* (Hidalgo & al., 2008) complexes. The Mediterranean species of the *Rhaponticum* group, although more numerous, were much more homogeneous in karyological-cytogenetic characters. Especially noteworthy is the huge genome size expansion at the base of and within the *Myopordon* lineage, that does involve neither polyploidy, nor chromosome number change nor divergent FISH pattern, but which is coupled with an increased number of telomeric CMA (in all studied species) and DAPI (*M. pulchellum* (C. Winkl. & Barbey) Wagenitz) banding signals (Hidalgo & al., 2008). Further studies are needed to describe and compare relationships between genome size and polyploidy.

■ CONCLUDING REMARKS

Polyploidy and dysploidy are key processes in the evolution of genera considered in this review, as in general in Asteraceae and other flowering plants. These mechanisms, coupled with variation in genome size and the organisation of different chromosomal regions (types of chromatin, rDNA loci), are major contributors to plant diversification. The high number of chromosome counts available for some of the taxa discussed in this paper (Watanabe, 2002) allows us to state that while some plant groups have many different chromosome numbers and more or less long polyploid series (such as *Artemisia*, *Chrysanthemum* and *Dendranthema* in the Artemisiinae and *Carduus* L., *Carthamus*, *Centaurea* and *Cirsium* in the Cardueae), in others chromosome numbers are quite constant (such as *Ajania* of the Artemisiinae and *Cheirolophus* of the Cardueae). Although a considerable part of the evolutionary history of the taxa considered is well-known and clear, further studies dealing with karyological and cytogenetic (including genome size assessments) aspects are necessary. Although two out of 13 genera with more than 1000 chromosome counts reported in the Asteraceae, *Artemisia* and *Centaurea* (Semple & Watanabe, 2009), have been included here, many genera in the subtribes considered still lack such data completely, and others are only poorly covered at the species level. Counting chromosome numbers in these genera and performing further karyological and cytogenetic research in the less known taxa will allow a deeper knowledge of the systematics, phylogeny and evolution of these groups.

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