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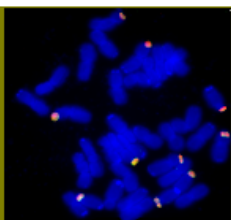
Biologia Vegetal i Edafologia

SECCIÓ DE BOTÀNICA

**UNA APROXIMACIÓ A L'ESTUDI DE
L'EVOLUCIÓ I LA SISTEMÀTICA
D'ARTEMISIA I GÈNERES AFINS
en els àmbits de la citogenètica
i filogènia moleculars**

**Sònia Marta Garcia Giménez
Barcelona, 2007**

ANNEXOS



1. Linkage of nuclear 45S and 5S rDNA in the sunflower family (Asteraceae) revealed by *in situ* hybridization, PCR and Southern blot

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Tandem arrays of 45S and 5S ribosomal RNA genes are usually located separately on chromosomes in most plants and animals. However, in some species of the family Asteraceae, fluorescent *in situ* hybridization has shown that these regions are colocalized (Fig. 1). To determine the genomic organization of the 5S and 45S repeats we surveyed several species from genus *Artemisia*, in which such colocalization had been previously detected. Using different primer combinations specific for 26S and 5S rRNA genes, a PCR product was obtained, indicating close linkage of these genes in some arrays at least. No products were obtained with DNA from species that do not show such colocalization. Although the data are preliminary, they support a putative location of the 5S rRNA gene(s) downstream from the 26S gene within the intergenic 26S-18S spacer (IGS), also confirmed by Southern blot hybridization. We examined related species from subtribe *Artemisiinae*, and tribes *Anthemideae*, *Astereae*, *Calenduleae*, *Gnaphalieae*, *Inuleae* and *Senecioneae* finding 5S and 45S linkage in *Anthemideae* and *Gnaphalieae*, isolated to a branch of subfamily *Asteroideae* (Fig. 2 and 3). On the basis of these observations, it is hypothesized that the 5S-45S units might have evolved together and in concert. Further studies are currently being conducted to reveal the organization, function and evolution of these unusual rDNA units (Fig. 4 and 5).

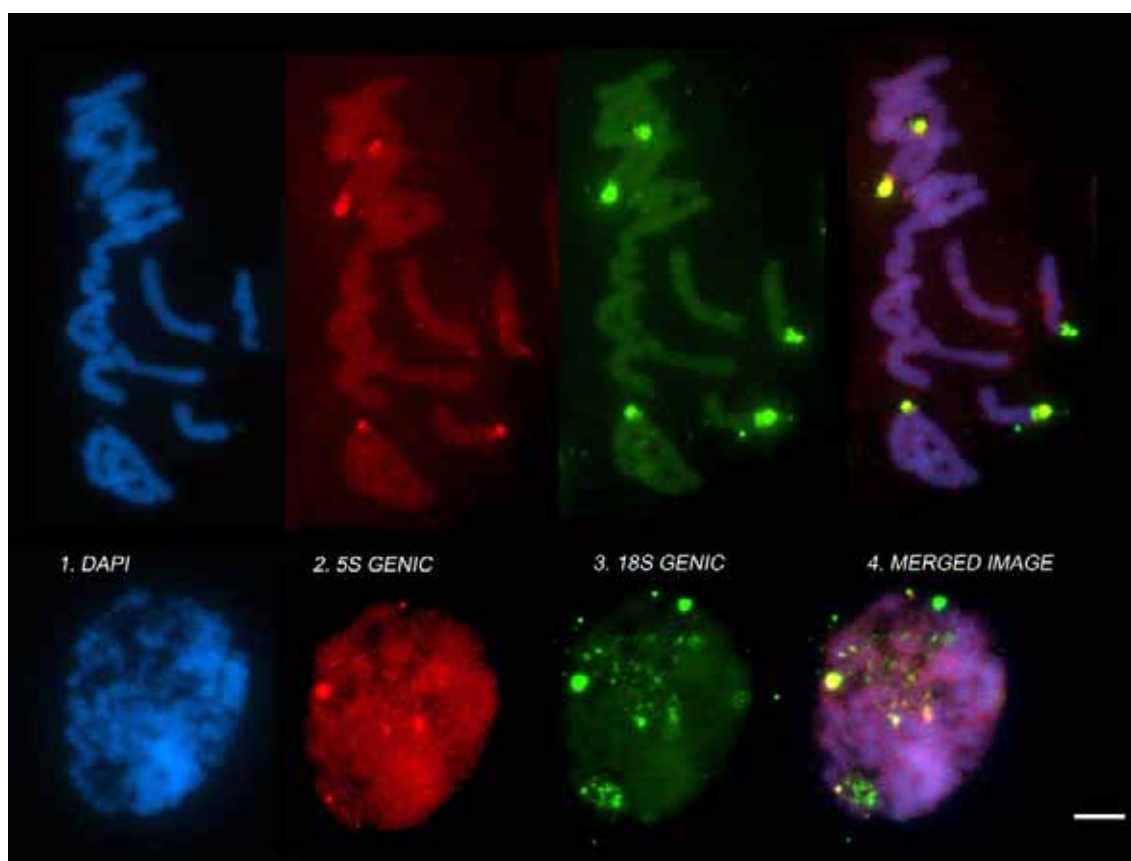


FIGURE 1. A species from subgenus *Tridentatae*, *Artemisia arbuscula* ssp. *arbuscula* $2n=2x=18$ showing the 5S genic probe (in red) and 18S genic probe (in green) in metaphase and interphase. Scale bar = 10 μ m.

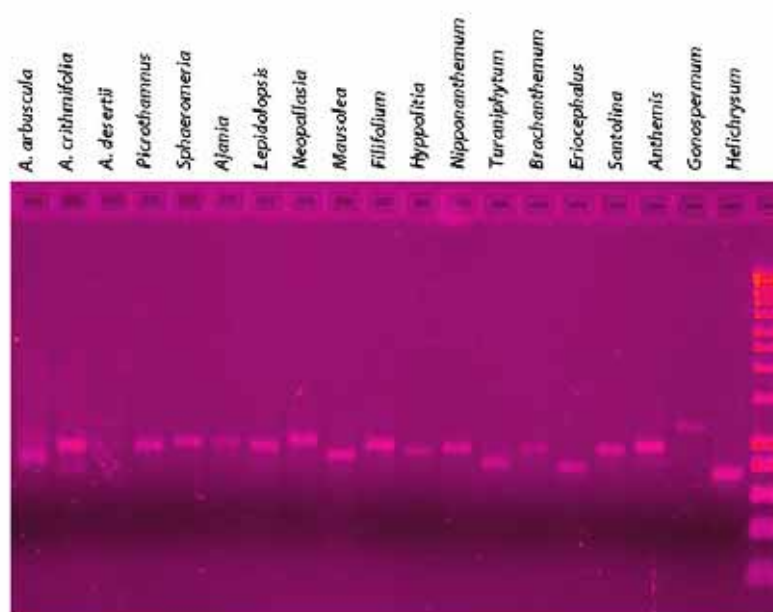


FIGURE 2. Gel indicating presence of a PCR product in different species of *Artemisia*, several genera of tribe *Anthemideae* and in one genus of tribe *Gnaphalieae* (*Helichrysum*). Different sizes (between 1000 and 600 bp) of product can be observed.

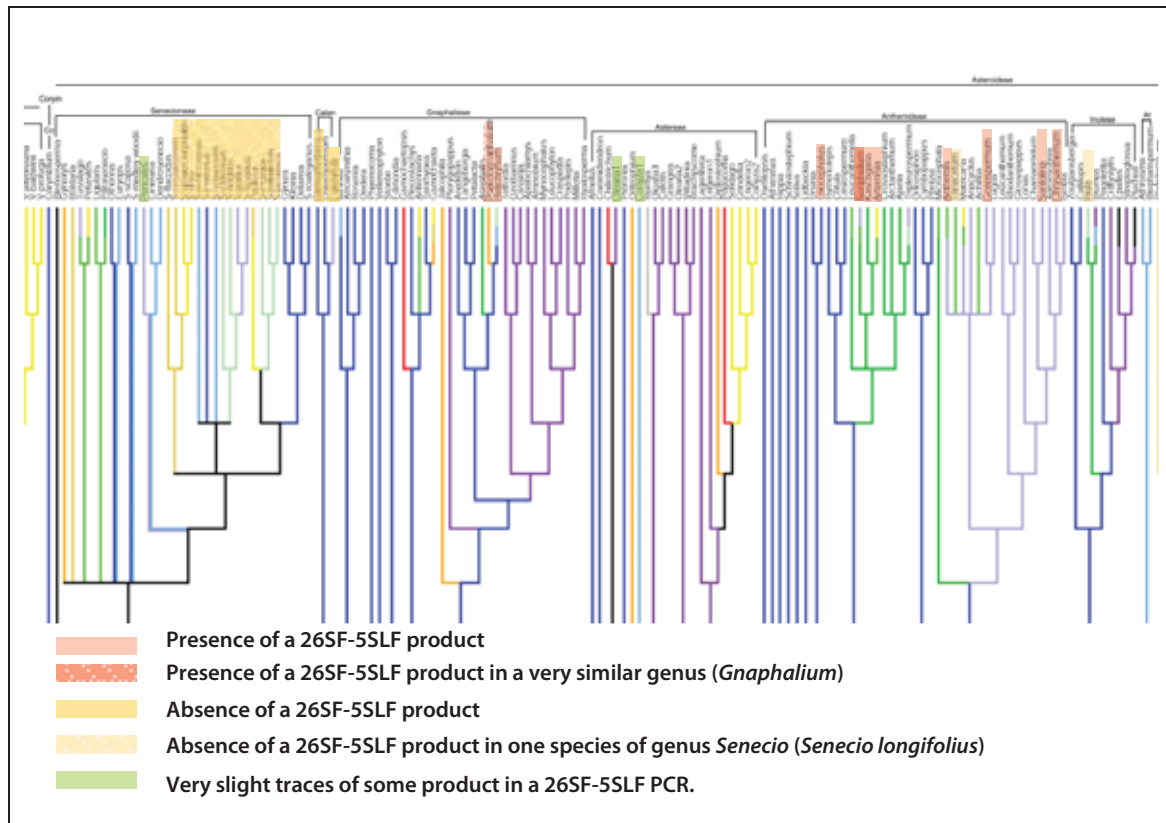


FIGURE 3. A fragment of the supertree of the Asteraceae (Funk et al., 2005) indicating absence/presence of a PCR product with the primer combinations 26SF-5SLF.

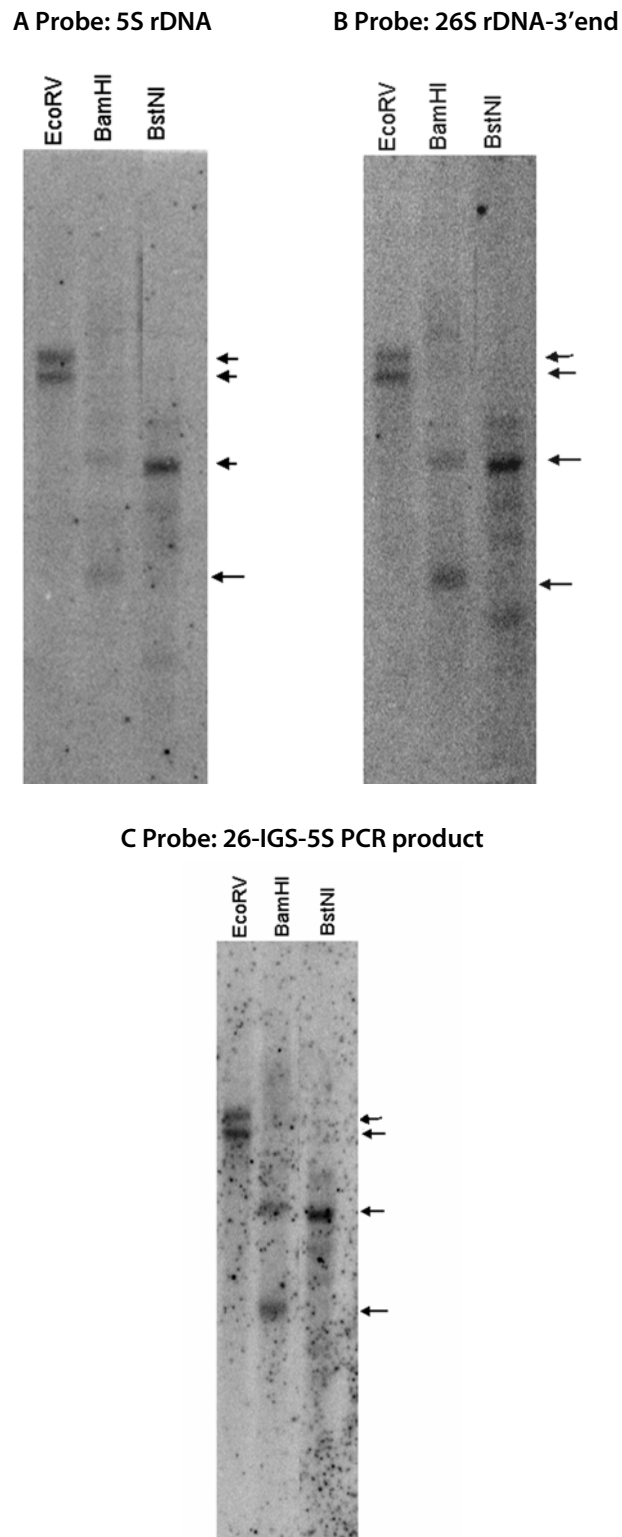


FIGURE 4. Southern blot hybridization showing common 26S and 5S ribosomal DNA hybridization bands, using different probes.

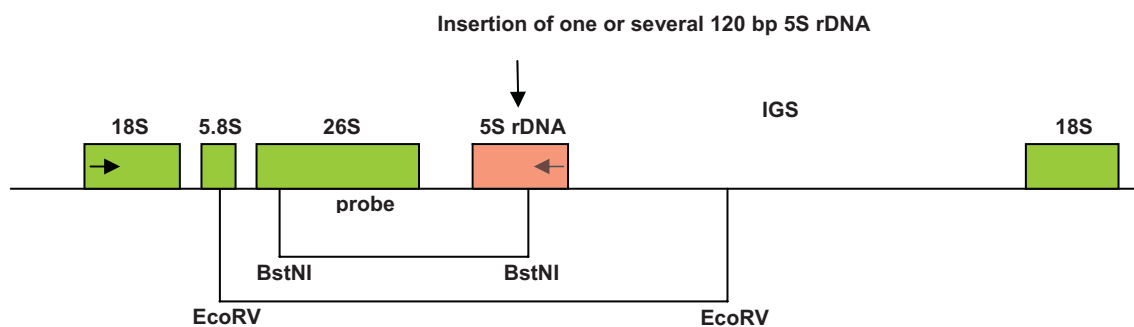


FIGURE 5. A proposed structure of the integral 45S-5S rDNA unit.

2. Genome size evolution and molecular cytogenetic studies in an ancient group of orophytic *Artemisia* (*Asteraceae*)

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The large genus *Artemisia* includes a group of European and South West Asiatic orophytic taxa, placed mainly within subgenus *Absinthium*, which inhabits high mountain areas from the Sierra Nevada, across the Pyrenees, the Alps and as far as the Caucasus. It is an interesting dysploid-polyploid complex, which has yet been studied from many points of view. However, questions regarding the origin of these species still remain unresolved. It is believed that alpine flora has a relictual character, and particularly in this group, the glaciations of the Quaternary might have played a major role in habitat fragmentation, with the creation of glacial refugia and subsequent speciation processes. This might explain the disjunct distribution of some of these taxa and the species richness and diversity of the group. On the light of new data on fluorochrome banding and *in situ* hybridization of rDNA (Fig. 1), genome size (Table 1), additional support is given to the hypothesis that polyploid species within this group (*A. nitida*, *A. splendens*, and *A. umbelliformis*) might come from independent or recurrent hybridization events between the extant diploid taxa (*A. alpina*, *A. eriantha*, *A. genipi*, *A. glacialis*, and *A. granatensis*), and/or with the participation of some extinct diploid precursors. Additionally, banding and FISH patterns of the analyzed taxa agree with those previously known for the same subgenus, and considerable genome size differences have been detected between disjunct populations of the same species, which could also be related with ongoing diversification processes linked to geographical isolation.

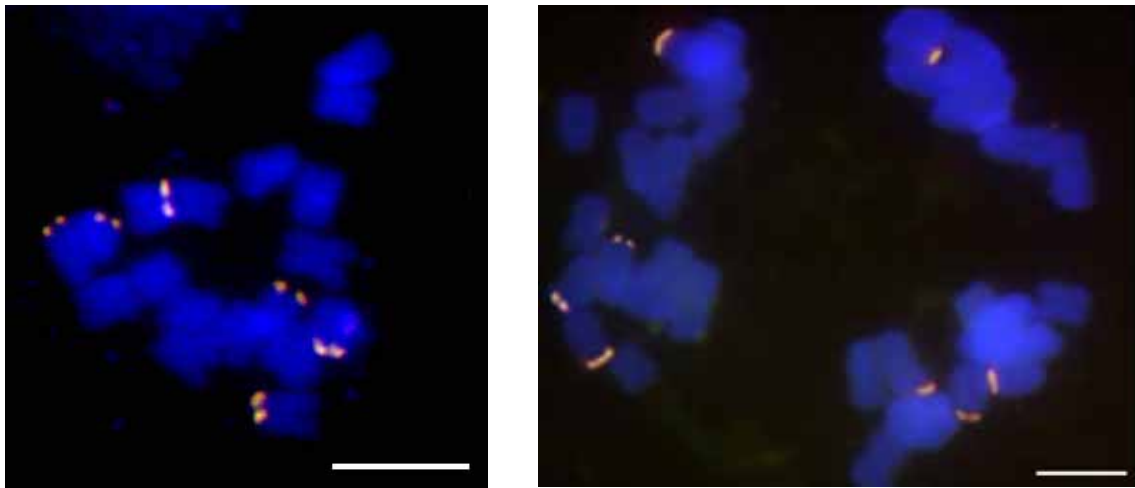


FIGURE 1. Fluorescent *in situ* hybridization metaphase plates of *A. eriantha* ($2n=18$, Urdiceto) and *A. umbelliformis* ($2n=34$, Noucreus), showing 6 and 8 rDNA sites, respectively. Scale bars = 10 μm .

TABLE 1. Genome size assessments of the populations studied. ¹2C nuclear DNA content (mean value and standard deviation of the samples). ²1 pg = 978 Mbp (Doležel et al., 2003). ³Somatic chromosome number. ⁴Monoploid genome size (2C value divided by ploidy level, Greilhuber et al., 2005). ⁵Internal standard used in each case.

Species	Provenance	2C ¹ (pg)(SD)	2C ² (Mbp)	2n ³	1Cx ⁴ (pg)	Standard ⁵
<i>A. eriantha</i>	Bulgary	6,24(0,08)	6102,72	18	3,12	<i>Petunia</i>
<i>A. eriantha</i>	Italy/Ischiator	6,11(0,15)	5975,58	18	3,06	<i>Petunia</i>
<i>A. eriantha</i>	Andorra/Casamanya	6,17(0,07)	6034,26	18	3,09	<i>Petunia</i>
<i>A. eriantha</i>	Spain/Urduceto	6,18(0,14)	6044,04	18	3,09	<i>Petunia</i>
<i>A. eriantha</i>	France/Galibier	5,72(0,05)	5594,16	18	2,86	<i>Petunia</i>
<i>A. eriantha</i>	France/Pas des Ladres	6,36(0,08)	6220,08	18	3,18	<i>Petunia</i>
<i>A. eriantha</i>	France/Col du Laurichard	6,33(0,09)	6152,76	18	3,17	<i>Petunia</i>
<i>A. eriantha</i>	Rumania/Fagaras	6,44(0,35)	6259,68	18	3,22	<i>Petunia</i>
<i>A. eriantha</i>	Rumania/Bucegi-Caraiman	6,26(0,21)	6122,28	18	3,13	<i>Petunia</i>
<i>A. eriantha</i>	Poland/Mount Tatra	5,94(0,22)	5809,32	18	2,97	<i>Petunia</i>
<i>A. eriantha</i>	Greece/Mount Olympus	5,83(0,24)	5701,74	18	2,92	<i>Petunia</i>
<i>A. eriantha</i>	Greece/Epiro Ploskos	5,93(0,22)	5799,54	18	2,97	<i>Petunia</i>
<i>A. genipi</i>	Italy/Scaletta	5,60(0,09)	5476,80	18	2,80	<i>Petunia</i>
<i>A. glacialis</i>	France/Col de Tortisse	8,92(0,41)	8723,76	16	4,46	<i>Petunia</i>
<i>A. granatensis</i>	Spain/Tajo del Goterón	7,61(0,15)	7442,58	16	3,81	<i>Petunia</i>
<i>A. granatensis</i>	Spain/Sierra Nevada	7,43(0,11)	7266,54	16	3,72	<i>Petunia</i>
<i>A. umbelliformis</i>	France/Canigó	13,10(0,18)	12811,80	34	3,28	<i>Pisum</i>
<i>A. umbelliformis</i>	France/Col de Tortisse	13,03(0,22)	12743,34	34	3,26	<i>Pisum</i>
<i>A. umbelliformis</i>	France/Col du Galibier	13,21(0,08)	12919,38	34	3,30	<i>Pisum</i>
<i>A. umbelliformis</i>	France/Col de la Bonette	13,18(0,19)	12899,82	34	3,30	<i>Pisum</i>
<i>A. umbelliformis</i>	Spain/Collaradeta	12,40(0,25)	12127,20	34	3,10	<i>Pisum</i>
<i>A. umbelliformis</i>	Switzerland/Riffelberg	13,44(0,05)	13144,32	34	3,36	<i>Pisum</i>
<i>A. umbelliformis</i>	Italy/Aosta valley	13,40(0,10)	13105,20	34	3,35	<i>Pisum</i>
<i>A. umbelliformis</i>	Spain/Tajo del Goterón	13,18(0,12)	12890,04	34	3,30	<i>Pisum</i>
<i>A. umbelliformis</i>	Spain/Palencia	13,27(0,06)	12978,06	34	3,32	<i>Pisum</i>

3. Genome size in the Asteraceae: first steps to a database

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The Asteraceae, one of the biggest and most recent plant families, comprise many economically relevant, useful and noxious plants, and have been profusely studied from many points of view, including genome size assessments. In this contribution we analyse the available data on nuclear DNA amounts of the Asteraceae to evaluate to what extent the family is well and regularly covered by such studies, and to identify gaps in this respect. With the purpose of making information from this family more accessible for both reference and analysis purposes, we have pooled all the available data from the Plant DNA C-values database (M.D. Bennet and I.J. Leitch) and the literature on this topic, and a preliminar database which gathers information exclusively in the Asteraceae has been built. This compilation has allowed addressing several questions such as the relationships between genome size and genome organization, ascending and descending dysploidy, systematics at different levels, phylogeny and evolution, life cycle, ecology and biogeography.

Genome size in the Asteraceae: first steps to a database

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Summary

The Asteraceae, one of the biggest and most recent plant families, comprise many economically relevant, useful and noxious plants, and have been profusely studied from many points of view, including genome size assessments. In this contribution we analyse the available data on nuclear DNA amounts of the Asteraceae to evaluate to what extent the family is well and regularly covered by such studies, and to identify gaps in this respect. With the purpose of making information from this family more accessible for both reference and analysis purposes, we have pooled all the available data from the Plant DNA C-values database (MD Benner and U Leitch) and the literature on this topic, and a preliminary database which gathers information exclusively in the Asteraceae has been built. This compilation will allow addressing several questions such as the relationships between genome size and genome organization, ascending and descending dysploidy, systematics at different levels, phylogeny and evolution, life cycle, ecology and biogeography.

Objectives

- Make data from Asteraceae more accessible for both reference and analysis purposes by pooling all the data from plant DNA C-value database research papers and unpublished data
- Mapping genome size data in the supertree of the Asteraceae
- Identify gaps in the knowledge of genome sizes in this large family
- Inference of systematic, evolutionary or ecological implications for the whole Asteraceae



Methods

- Database compiled with Microsoft Excel
- Publications consulted: 88 (where large discrepancies revealed, data were avoided for calculations)
- Years: 1967 – 2006
- Fields

Genus	Species	Tribe	Subtribe	2n	Ploidy level (x)	Life cycle	1C (Mbp)	1C (pg)	1Cx (pg)	2C (pg)	Method	Internal Standard	Reference
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First publication (*Helianthus annuus*): 1967, K P Bartsche, A H Sparrow, C H Nauman, and S S Schwemmer. The relationship of DNA content to nuclear and chromosome volumes and to radiosensitivity (LDSR). PNAS, 54: 533-540.



Asteraceae:
- 16 tribes
- 1353 genera
- 23000 species
(Bremer, 1994)

Number of genome size estimates collected: 1208
- Number of tribes: 7
- Number of genera: 99
- Number of species: 640

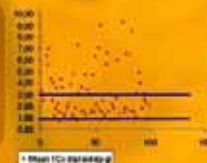


Genome size (2C) vs. number of measurements

- 1208 measurements, 640 species
- There is a 65-fold variation
- Most species show genome size between 1-9 pg (60%)
- Only 1.5% show genome size higher than 26 pg
- Highest value: *Senecio pendulus* (52.30 pg), *Leucanthemum subglaucum* (49.66 pg, 2n=90)
- Lowest value: *Leontodon longirostris* (0.80 pg), *Leontodon crispus* (1.05 pg, 2n=8)



Monoploid genome size (1Cx) vs. number of genera
There is a 14 fold variation among genera (only diploid species)
Most genera have 1 to 1 pg
Monoploid genome size differences are the real thing (ploidy levels)



Highest value: *Phalacroseris* (9.20 pg, 2n=18), *Siebera* (8.49 pg, 2n=20)
Lowest value: *Callicephalus* (0.64 pg, 2n=28), *Cheiranthus* (0.73 pg, 2n=30)

Genome size (1Cx) vs. tribe (only diploid species)

Anthemideae
Astereae
Carduaceae
Heliantheae
Inuleae
Lactuceae
Mutisieae
Senecioideae



Genome size in the supertree of Asteraceae

