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Evolutionary and ecological implications of genome size in the North American endemic sagebrushes and allies (*Artemisia*, Asteraceae)

SÒNIA GARCIA[1]*, MIGUEL Á. CANELA[2], TERESA GARNATJE[3], E. DURANT MCARTHUR[4], JAUME PELLICER[1], STEWART C. SANDERSON[4] and JOAN VALLÈS[1]

[1]*Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona. Avinguda Joan XXIII s. n., 08028 Barcelona, Catalonia, Spain*
[2]*Departament de Matemàtica aplicada i Anàlisi, Facultat de Matemàtiques, Universitat de Barcelona. Gran Via de les Corts Catalanes, 585, 08007 Barcelona, Catalonia, Spain*
[3]*Institut Botànic de Barcelona (CSIC-ICUB), Passeig del Migdia s. n., Parc de Montjuïc, 08038. Barcelona, Catalonia, Spain*
[4]*Shrub Sciences Laboratory, Rocky Mountain Research Station, Forest Service, United States Department of Agriculture. Provo, UT 84606, USA*

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The genome size of 51 populations of 20 species of the North American endemic sagebrushes (subgenus *Tridentatae*), related species, and some hybrid taxa were assessed by flow cytometry, and were analysed in a phylogenetic framework. Results were similar for most *Tridentatae* species, with the exception of three taxonomically conflictive species: *Artemisia bigelovii* Gray, *Artemisia pygmaea* Gray, and *Artemisia rigida* Gray. Genome size homogeneity (together with the high morphological, chemical, and karyological affinities, as well as low DNA sequence divergence) could support a recent diversification process in this geographically restricted group, thought to be built upon a reticulate evolutionary framework. The *Tridentatae* and the other North American endemic *Artemisia* show a significantly higher genome size compared with the other subgenera. Our comparative analyses including genome size results, together with different kinds of ecological and morphological traits, suggest an evolutionary change in lifestyle strategy linked to genome expansion, in which junk or selfish DNA accumulation might be involved. Conversely, weed or invasive behaviour in *Artemisia* is coupled with lower genome sizes. Data for both homoploid and polyploid hybrids were also assessed. Genome sizes are close to the expected mean of parental species for homoploid hybrids, but are lower than expected in the allopolyploids, a phenomenon previously documented to be related with polyploidy. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 631–649.

ADDITIONAL KEYWORDS: Compositae – C value – hybridization – polyploidy – reticulate evolution – r/K selection – speciation – *Tridentatae* – weed.

INTRODUCTION

The sagebrushes (subgenus *Tridentatae*, *Artemisia*, Asteraceae) are probably the most common woody plants in terms of area occupied and number of individual plants in the western USA, and are profusely distributed from Canada to Mexico (McArthur & Sanderson, 1999). They comprise about a dozen species (and 20 taxa altogether, including subspecific entities; Shultz, 2005) of landscape-dominant, xerophytic shrubs, endemic to North America. The base chromosome number is exclusively $x = 9$ (there are other *Artemisia* based on $x = 8$, although $x = 9$ is the most widespread in the genus), and ploidy levels range from $2x$ to $8x$ (but are mostly $2x$ and $4x$; McArthur & Sanderson, 1999). Based on evidence from different sources, the North American endemic sagebrushes can be considered to be a fairly

*Corresponding author. E-mail: soniagarcia@ub.edu

homogeneous group, with its systematic relationships poorly resolved (Kornkven, Watson & Estes, 1998, 1999; Vallès *et al*., 2003). The *Tridentatae* had been previously considered as a section of *Artemisia* subgenus *Seriphidium* (Rydberg, 1916), and were raised to subgeneric status by McArthur & Plummer (1978); the separation between both subgenera was confirmed afterwards in studies of the molecular phylogeny (Torrell *et al*., 1999). Interspecific relationships within this subgenus are unclear: different lineages had been proposed on the basis of leaf morphology, habitat preference, and ability to root sprout after fire (reviewed in Kornkven *et al*., 1998), but subsequent molecular data did not support their recognition. Moreover, several taxa have been included and excluded from the subgenus in different studies, particularly some species considered at present as *Tridentatae* members (*sensu* Shultz, 2006), such as *A. bigelovii* Gray, *A. pygmaea* Gray, and *A. rigida* Gray. Other works also suggested the inclusion of species such as *A. californica* Lessing, *A. filifolia* Torrey, or *A. palmeri* Gray, which typically belong to other *Artemisia* subgenera (Kornkven *et al*., 1998, 1999; Shultz, 2005).

The subgenus can be considered to be a large species complex centred upon *A. tridentata*, the most abundant and widespread species (McArthur *et al*., 1979; McArthur, Welch & Sanderson, 1988). Some other species are also ecologically important and landscape-dominant, i.e. *A. arbuscula* Nutt., *A. cana* Pursh, and *A. nova* Nelson. The remaining *Tridentatae* [*A. argillosa* Beetle, *A. bigelovii*, *A. longiloba* (Osterh.) Beetle, *A. pygmaea*, *A. rigida*, *A. rothrockii* Gray, *A. spiciformis* Osterh., and *A. tripartita* Rydb.] are more restricted in their distribution. Some *Artemisia* species from other subgenera are also endemic to western North America (*A. californica*, *A. filifolia*, *A. ludoviciana* Nutt., *A. nesiotica* Raven, *A. palmeri*, *A. papposa* Blake & Cronquist, *A. pedatifida* Nutt., and *A. porteri* Cronquist); other species, also present in North America, are distributed almost worldwide (*A. absinthium* L., *A. campestris* L., *A. frigida* Willd., and *A. vulgaris* L.).

The study of genome size has applications in many plant research fields, e.g. ecology, evolutionary biology, systematics, taxonomy, and biogeography (Bennett & Leitch, 2005a, 2005b, 2005c). The relationships between the nuclear DNA level and cytological traits, reproductive biology, ecology, environmental features, distribution, biomass production, and many other plant characteristics have been widely investigated and established in many plant groups. Additionally, the possibility of genome size variation, at specific or subspecific levels, has been studied in depth, being an object of controversy (Greilhuber, 2005; Murray, 2005).

We undertook genome size analysis on the *Tridentatae* and allies to: (1) exploit the nuclear DNA level information for taxonomic purposes, i.e. to identify evolutionary relationships between these plants, by analysing genome size variation in a phylogenetic framework; (2) detect any relationship between the nuclear DNA levels and morphological traits of these plants, their surrounding environmental features, their geographical distribution, and weed characteristics, among other features; (3) study the scope of genome size variation at the species/population level; (4) observe genome size changes linked to hybridization processes; and (5) increase general knowledge in *Artemisia* C values, particularly to complete the survey of genome sizes in the *Tridentatae* and in other North American endemics of this genus.

MATERIAL AND METHODS

Plant material

Table 1 lists the 51 populations studied, along with their site of origin and collection information. Twelve *Tridentatae* species, with 13 subspecific entities (which constitute a complete representation of the North American endemic sagebrushes), four populations of hybrids, and eight closely related *Artemisia* species from subgenera *Artemisia* and *Dracunculus*, were included.

Flow cytometry measurements

The DNA 2C values of the tested species were estimated using flow cytometry. *Pisum sativum* L. 'Express long' (2C = 8.37 pg), and *Petunia hybrida* Vilm. 'PxPc6' (2C = 2.85 pg) were used as internal standards (Marie & Brown, 1993) to cover the range of 2C values found (HPCV = 2.54% and 1.87%, respectively: mean half-peak coefficient of variation corresponding to ten samples from five different individuals). Leaf tissue of five individuals for each studied population was chopped in Galbraith's isolation buffer (Galbraith *et al*., 1983) with a razor blade, together with the chosen internal standard; two samples per individual were independently extracted. Samples were subsequently stained with propidium iodide (Sigma-Aldrich) and were then measured in an Epics XL flow cytometer (Coulter Corporation). To ensure that the instrument shows a linear response across the range of genome sizes studied, we performed several assays that included both internal standards and one of the populations with the highest genome size (*A. cana* ssp. *cana* n. 2128) at the same time. The difference between the obtained results with respect to each standard was negligible (less than 2% of deviation), and hence we can ascertain the linearity of the flow cytometer in this interval, and

Table 1. Provenance of the populations of *Artemisia* studied

Taxa	Origin of materials	Coll n.*
Subgenus *Tridentatae*		
A. arbuscula subsp. *arbuscula*	Corn Creek Canyon, Millard Co., Utah. 1830 m	2877
A. arbuscula subsp. *arbuscula*	South of Jordanelle Reservoir, Wasatch Co., Utah. 1890 m	3027
A. arbuscula subsp. *arbuscula*	Sage Junction, Lincoln Co., Wyoming. 1930 m	3028
A. arbuscula subsp. *longicaulis*	Toulon, Pershing Co., Nevada. 1335 m	2860
A. arbuscula subsp. *longicaulis*	Bruneau, Owyhee Co., Idaho. 1012 m	2855
A. arbuscula subsp. *thermopola*	East bank of Snake River, South Boundry Yellowstone National Park, Teton Co., Wyoming. 2130 m	3032
A. argillosa	Coalmont, Jackson Co., Colorado. 2497 m	3034
A. bigelovii	Emery Co., Utah. 1801 m	2869
A. bigelovii	15 km east of Fremont Junction. Emery Co., Utah. 1777 m	3050
A. bigelovii	Padre Canyon, Cocconino Co., Arizona. 1799 m	3051
A. cana subsp. *bolanderi*	17 km north-west of Bridgeport, Mono Co., California. 2270 m	3047
A. cana subsp. *cana*	Sheridan, Sheridan Co., Wyoming. 1140 m	2128
A. cana subsp. *viscidula*	Strawberry Valley, Wasatch Co., Utah. 2374 m	2844
A. cana subsp. *viscidula*	Soldier Summit, Wasatch. Co., Utah. 2255 m	2875
A. cana subsp. *viscidula*	Fossil Butte National Monument, Lincoln Co., Wyoming, 1650 m	2851
A. longiloba	Evanston, Uinta Co., Wyoming. 2067 m	3025
A. nova	Tunnel Spring, Desert Experimental Range, Millard Co., Utah. 2174 m	2876
A. nova	Pine Valley Pass, Millard Co., Utah. 1820 m	2873
A. nova	Birch Springs Road, Mount Borah, Custer Co., Idaho. 2120 m	3053
A. nova var. *duchesnicola*	Tridell Road, Uintah Co., Utah. 1702 m	3029/3030
A. pygmaea	Yuba Dam Road, Juab Co., Utah. 1535 m	2870
A. pygmaea	San Rafael Swell, Emery Co., Utah. 2195 m	2836
A. rigida	Malheur Reservoir, Malheur Co., Oregon. 1035 m	2859
A. rothrockii	Reed Flats, White Mountains, Inyo Co., California. 3072 m	19803†
A. spiciformis	Ford Ridge, Bristle Cone Scout Camp, Carbon Co., Utah. 2856 m	2839
A. tridentata subsp. *parishii*‡	West of Rosamond, Kern Co., California. 722 m	3037/3038
A. tridentata subsp. *tridentata*	Salt Cave Hollow, Salt Creek Canyon, Juab Co., Utah. 1870 m	2871
A. tridentata subsp. *tridentata*	Beaver, Beaver Co., Utah. 1780 m	s. n.
A. tridentata subsp. *vaseyana*	Salt Cave Hollow, Salt Creek Canyon, Juab Co., Utah. 1878 m	2872
A. tridentata subsp. *vaseyana*	Hobble Creek Canyon, Utah Co., Utah. 1555 m	2874
A. tridentata subsp. *vaseyana*	Spring City, Sanpete Co., Utah. 1950 m	2879
A. tridentata subsp. *wyomingensis*	Gordon Creek, Carbon Co., Utah. 1980 m	2880
A. tridentata subsp. *xericensis*	Mann Creek Reservoir, Washington Co., Idaho. 929 m	2858
A. tripartita subsp. *rupicola*	Pole Mountain, Albany Co., Wyoming. 2647 m	3033
A. tripartita subsp. *tripartita*	Dubois Sheep Station, Clark Co., Idaho. 1650 m	2845
A. tripartita subsp. *tripartita*	Birch Springs Road, Mount Borah, Custer Co., Idaho. 2191 m	3054
Hybrid taxa§		
A. cana subsp. *cana* × *A. tridentata* subsp. *wyomingensis*	Pleasant Grove Plots, Uinta National Forest, Utah Co., Utah. 1734 m	2759 2760
A. tridentata subsp. *tridentata* × *A. tridentata* subsp. *vaseyana*	Orem, Utah Co., Utah. 1474 m	3049
A. tridentata subsp. *tridentata* × *A. tridentata* subsp. *vaseyana*	Shrub Sciences Laboratory. Provo, Utah. 1374 m	3048
Other *Artemisia*		
Subgenus *Artemisia*		
A. californica	Santa Clarita, Los Angeles Co., California. 487 m	3039
A. californica	Los Peñasquitos Canyon Preserve, San Diego, San Diego Co., California. 70 m	3043
A. ludoviciana	Salt Cave Hollow Road, Uinta National Forest, Salt Creek Canyon, Juab Co., Utah. 2084 m	3087

Table 1. *Continued*

Taxa	Origin of materials	Coll n.*
A. nesiotica	San Clemente Island, Los Angeles Co., California. 100 m	3090
A. palmeri	Los Peñasquitos Canyon Preserve, San Diego, San Diego Co., California. 70 m	3044
A. papposa	Milepost 130, U. S. Highway 20, 16 km west of Hill City. Elmore Co., Idaho. 1679 m	3077
Subgenus *Dracunculus*		
A. filifolia	Moccasin, Mohave Co., Arizona. 1530 m	2868
A. pedatifida	North of Point of Rocks, Sweetwater Co., Wyoming. 1675 m	1138
A. spinescens	Winton Road, Sweetwater Co., Wyoming. 1600 m	2403

*E. Durant McArthur collection numbers; vouchers are deposited in the herbarium of the Rocky Mountain Research Station, Provo, Utah (SSLP).
†Leila M. Shultz collection number.
‡Separate floral morphologies (see McArthur, 2005).
§Synthetic hybrids (see McArthur *et al*., 1998; McArthur & Sanderson, 1999).

the convenience of the use of the chosen internal standards. Additional details about the method used are described in Garcia *et al*. (2004).

Chromosome counts

As chromosome number was unknown for some of the populations studied, we performed chromosome counts following the classical karyological technique: pretreatment of healthy root-tip meristems with 0.05% aqueous colchicine, fixation in Carnoy's solution, acid hydrolysis (1 N HCl at 60 °C), and staining in 1% aqueous aceto-orcein; for the details of methodology see Pellicer *et al*. (2007a).

DNA amplification and sequencing strategies

With the purpose of analysing genome size variation in a phylogenetic framework, a phylogenetic tree was generated, which included all of the *Tridentatae* (12 species), the other North American endemic *Artemisia* of this study, and a representation of each *Artemisia* subgenus. The analysis was based on the sequences of the internal transcribed spacer 1 (ITS1) and ITS2 regions of the nuclear ribosomal DNA. Most sequences have been published previously (Kornkven *et al*., 1998; Vallès *et al*., 2003; Sanz *et al*., 2007) and are available from GenBank; to complete the representation, however, sequences for ten taxa were newly generated. The double-stranded DNA ITS region was amplified with primers 1406f (Nickrent, Schuette & Starr, 1994) and ITS4 (White *et al*., 1990). The profile used for amplification was the same as that used in Vallès *et al*. (2003). PCR products were purified with the QIAquick PCR purification kit (Qiagen). ITS4 was used as sequencing primer, and direct sequencing of the amplified DNA segment was performed using the Big Dye Terminator Cycle sequencing v3.1 (PE Biosystems). Nucleotide sequencing was carried out at the Serveis Cientificotècnics at the Universitat de Barcelona, on an ABI PRISM 3700 DNA analyser (PE Biosystems). DNA sequences were edited by Chromas 1.56 (Technelysium PTy) and were aligned visually. We were not able to amplify DNA of *A. frigida*, and this species is therefore not present in the phylogenetic analysis. The sequence alignment matrix is available from the corresponding author.

Model selection and Bayesian inference analysis

For the phylogenetic analyses, we chose the Bayesian inference (BI) method, because previous work with these species had been carried out using maximum parsimony, and BI has shown higher resolution. To determine models under the Akaike information criterion (AIC) (Posada & Buckley, 2004), the data set was analysed using MrModeltest 2.2 (Nylander, 2004). The model SYM + G +I fitted our data best, and was used to perform a Bayesian analysis with MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001). Four Markov chains were run simultaneously for 1 000 000 generations, and these were sampled every 100 generations. Data from the first 1000 generations were discarded as the burn-in period, after confirming that likelihood values had stabilized prior to the 1000th generation. Posterior probabilities were estimated

through the construction of a 50% majority rule consensus tree. The outgroup species, *Kaschgaria brachanthemoides* (Winkler) Poljakov and *Nipponanthemum nipponicum* (Franchet ex Maximowicz) Kitamura, were chosen on the basis of previous work (Vallès *et al*., 2003; Sanz *et al*., 2007).

Statistical analyses

The ecological, environmental, and morphological data used for statistical analyses have been extracted from the abundant literature existing for the *Tridentatae* and other *Artemisia* species (McArthur *et al*., 1979; Cronquist, 1994; McArthur & Stevens, 2004; Shultz, 2006; Plants database of the United States Department of Agriculture, http://plants.usda.gov/, accessed in December 2006). Some cautions/premises were established to develop these analyses: (1) only diploid taxa have been used, so as to avoid biased results to monoploid genome downsizing in polyploids (except for *A. argillosa* and *A. rothrockii*, which are only known at the tetraploid level); (2) when there are several subspecific entities for a species, only one has been chosen, for consistency, in the analysis and to avoid uneven representation; (3) as the taxonomic nomenclature of the *Tridentatae* is often confusing, we have considered taxa to be at the species level if they have been formally treated at this level at least once previously; (4) the analyses of the differences between the mean DNA level and all of the other parameters were performed using both the phylogenetically based generalized least squares (PGLS) algorithm, as implemented in the PHYLOGR R package (R Project, 2005), to analyse genome size variation in a phylogenetic context, and the one-way ANOVA for comparative purposes. Genome size data from previous work (Torrell & Vallès, 2001; Garcia *et al*., 2004) was also employed to calculate the mean values used for these analyses.

RESULTS

Table 2 presents the 2C DNA levels estimated for the sampled taxa, together with other karyological data. The range of variation was 3.72-fold for 2C values and 1.65-fold for monoploid genome size (Fig. 1). The analyses were of good quality (global mean HPCV = 1.96%). The first estimates given for the species are marked with an asterisk in Table 2, and a fluorescence histogram exemplifying one of the most common results is presented in Figure 2. We made a complete subgeneric analysis in order to assess interspecific, intraspecific, and interpopulation genome size differences. With the data presented here, genome sizes are known for all the species and

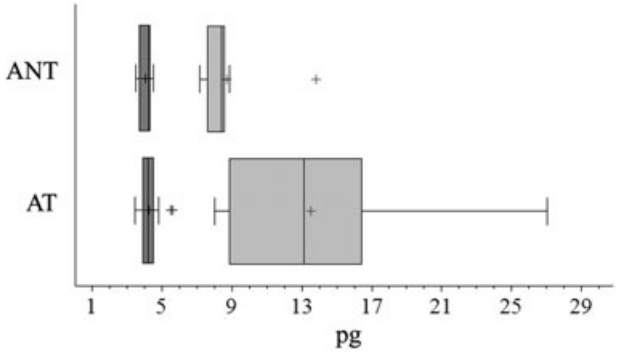

Figure 1. Range of variation in monoploid (1Cx, dark-grey shading) and holoploid (2C, light-grey shading) genome sizes for both the *Tridentatae* (AT) and non-*Tridentatae* (ANT) species.

subspecies of the subgenus *Tridentatae*, as well as most of the North American *Artemisia* endemic species from other subgenera, completing previous research in this particular group (see Torrell & Vallès, 2001; Garcia *et al*., 2004). As for chromosome counts, the results obtained are consistent with previous data concerning these species (McArthur & Sanderson, 1999; references therein), and ploidy levels range from diploid ($2n = 18$), the most common, to octoploid ($2n = 72$), which represent all the ploidy levels found in the subgenus until now.

Except for three taxa (*A. bigelovii*, *A. pygmaea*, and *A. rigida*) discussed later in more detail, the diploid *Tridentatae* show similar nuclear DNA levels, with a mean value of 8.98 pg, ranging between 8.24 and 9.47 pg. Values for the other diploid *Artemisia* range between 7.14 and 8.86 pg, with a mean value of 7.48 pg. Scarce intraspecific genome size differences have been found in cases where different populations of the same species were assessed, most ranging from 1 to 2%. In the case of two populations of *A. tridentata* ssp. *parishii* with clearly segregating flowering phenotypes (one upright and the other drooping; McArthur, 2005; collections 3037 and 3038, respectively, of Table 2), nuclear DNA level differences between the phenotypes are also negligible. Exceptionally, considerable differences were found in *A. pygmaea* (5.98%) and in *A. tridentata* ssp. *spiciformis* (10.02%), even though the populations of these species show few morphological differences. The synthetic hybrid taxa studied present genome size values (2C) close to the expected mean in both diploid and polyploid populations, although the polyploid offspring show lower 1Cx values (Table 2).

The results of the statistical analyses, obtained with the data shown in Table 3, are presented in Table 4. The mean genome sizes of the *Tridentatae*

Table 2. Nuclear DNA content (2C and 1Cx) and other karyological characters of the populations studied

Taxa	2C (s.d.)†	2C (Mbp)‡	2n§	P.L.	1Cx¶	Standard††
Subgenus *Tridentatae*						
A. arbuscula subsp. *arbuscula* (2877)	9.21 (0.06)	9007.38	18	2	4.61	*Petunia*
	9.22 (0.11)‡‡					
A. arbuscula subsp. *arbuscula* (3027)	9.04 (0.13)	8841.12	18	2	4.52	*Petunia*
A. arbuscula subsp. *arbuscula* (3028)	15.55 (0.35)	15207.9	36	4	3.89	*Pisum*
A. arbuscula subsp. *longicaulis* (2855)*	22.85 (0.18)	22347.3	54	6	3.81	*Pisum*
A. arbuscula subsp. *longicaulis* (2860)*	23.10 (0.39)	22591.8	54	6	3.85	*Petunia*§§
A. arbuscula subsp. *thermopola* (3032)*	9.47 (0.13)	9261.66	18	2	4.73	*Pisum*
A. argillosa (3034)*	15.77 (0.65)	15423.06	36	4	3.94	*Petunia*§§
A. bigelovii (3051)	8.00 (0.10)	7824.00	18	2	4.00	Petunia
A. bigelovii (3050)	15.06 (0.13)	14728.68	36	4	3.76	*Pisum*
	15.49 (0.10)‡‡					
A. bigelovii (2869)	15.32 (0.09)	14982.96	36	4	3.83	*Pisum*
A. cana subsp. *bolanderi* (3047)*	9.01 (0.09)	8811.78	18	2	4.50	*Petunia*
A. cana subsp. *cana* (2128)	27.04 (0.42)	26445.12	72	8	3.38	*Pisum*
	25.65 (0.61)‡‡					
A. cana subsp. *viscidula* (2844)	8.73 (0.24)	8537.94	18	2	4.37	*Petunia*
	8.54 (0.09)‡‡					
A. cana subsp. *viscidula* (2851)	8.51 (0.13)	8322.78	18	2	4.26	*Petunia*
A. cana subsp. *viscidula* (2875)	8.58 (0.19)	8391.24	18	2	4.29	*Petunia*
A. longiloba (3025)*	16.62 (0.45)	16254.36	36	4	4.15	*Pisum*
A. nova (3053)	9.09 (0.06)	8890.02	18	2	4.51	*Petunia*
	6.37 (0.14)‡‡					
A. nova (2873)	17.25 (0.15)	16870.5	36	4	4.31	*Pisum*
A. nova (2876)	17.10 (0.11)	16723.8	36	4	4.28	*Pisum*
A. nova var. *duchesnicola* (3029)*	22.90 (0.39)	22396.2	54	6	3.82	*Pisum*
A. nova var. *duchesnicola* (3030)*	22.43 (0.24)	21936.54	54	6	3.74	*Pisum*
A. pygmaea (2836)	10.89 (0.24)	10650.42	18	2	5.45	*Petunia*
	11.54 (0.18)‡‡					
A. pygmaea (2870)	11.14 (0.19)	10894.92	18	2	5.57	*Petunia*
A. rigida (2859)*	8.23 (0.13)	8048.94	18	2	4.12	*Petunia*
A. rothrockii (19803)*	16.41 (0.25)	16048.98	36	4	4.10	*Pisum*
A. spiciformis (2839)	9.00 (0.19)	8802	18	2	4.50	*Petunia*
	8.18 (0.30)‡‡					
A. tridentata subsp. *parishii* (3037)*	16.61 (0.27)	16244.58	36	4	4.15	*Pisum*
A. tridentata subsp. *parishii* (3038)*	16.32 (0.17)	15960.96	36	4	4.08	*Pisum*
A. tridentata subsp. *tridentata* (1996)	8.42 (0.27)	8234.76	18	2	4.21	*Petunia*
	8.17 (0.08)‡‡					
A. tridentata subsp. *tridentata* (2871)	8.24 (0.25)	8058.72	18	2	4.12	*Petunia*
A. tridentata subsp. *vaseyana* (2879)	15.12 (0.37)	14787.36	36	4	3.78	*Petunia*§§
A. tridentata subsp. *vaseyana* (2872)	8.89 (0.20)	8694.42	18	2	4.45	*Petunia*
	8.66 (0.07)‡‡					
A. tridentata subsp. *vaseyana* (2874)	8.85 (0.22)	8655.3	18	2	4.43	*Petunia*
A. tridentata subsp. *wyomingensis* (2880)*	15.07 (0.19)	14738.46	36	4	3.77	*Petunia*
A. tridentata subsp. *xericensis* (2858)*	16.24 (0.13)	15882.72	36	4	4.06	*Pisum*
A. tripartita subsp. *rupicola* (3033)*	8.68 (0.19)	8489.04	18	2	4.34	*Petunia*
A. tripartita subsp. *tripartita* (3054)	8.85 (0.08)	8655.30	18	2	4.42	*Petunia*
A. tripartita subsp. *tripartita* (2845)*	15.32 (0.18)	14982.96	36	4	3.83	*Petunia*§§
Hybrids						
A. cana subsp. *cana* × *A. tridentata* subsp. *wyomingensis* (2759)	19.15 (0.68)	18728.70	54	6	3.19	*Pisum*
A. cana subsp. *cana* × A. tridentata subsp. *wyomingensis* (2760)	18.72 (0.35)	18308.16	54	6	3.12	*Pisum*

Table 2. *Continued*

Taxa	2C (s.d.)†	2C (Mbp)‡	2n§	P.L.	1Cx¶	Standard††
A. tridentata subsp. *tridentata* × *A. tridentata* subsp. *vaseyana* (3048)	15.71 (0.14)	15364.38	36	4	3.93	*Pisum*
A. tridentata subsp. *tridentata* × *A. tridentata* subsp. *vaseyana* (3049)	8.52 (0.25)	8332.56	18	2	4.26	*Petunia*
Other *Artemisia*						
Subgenus *Artemisia*						
A. californica (3039)*	8.38 (0.22)	8195.64	18	2	4.19	*Petunia*
A. californica (3043)*	8.57 (0.12)	8381.46	18	2	4.28	*Petunia*
A. ludoviciana (3087)*	13.82 (0.17)	13515.95	36	4	3.45	*Pisum*
A. nesiotica (3090)	8.38 (0.15)	8195.64	18	2	4.19	*Petunia*
A. palmeri (3044)*	7.14 (0.07)	6982.92	18	2	3.57	*Pisum*
A. papposa (3077)*	8.44 (0.17)	8254.32	18	2	4.22	*Petunia*
Subgenus *Dracunculus*						
A. filifolia (2868)	7.26 (0.06)	7100.28	18	2	3.63	*Petunia*
A. pedatifida (1138)*¶¶	8.86 (0.09)	8665.08	18	2	4.43	*Petunia*
A. spinescens (2403)*¶¶	7.58 (0.20)	7413.24	18	2	3.79	Petunia

*Taxa for which the genome size has been estimated for the first time.
†2C nuclear DNA content (mean value and standard deviation of the samples) in pg.
‡1 pg = 978 Mbp (Doležel *et al*., 2003).
§Somatic chromosome number.
¶Monoploid genome size.
††Internal standard used in each case (see text for details about *Pisum* and *Petunia*). (* Taxa for which genome size has been estimated for the first time).
‡‡Data belonging to previous studies (Torrell & Vallès, 2001; Garcia *et al*., 2004); genome size (2C) from the previously studied *A. nova* population might have been a confusion, as it is not consistent with the five populations of *A. nova* analysed in the present paper.
§§It was not possible to use a internal standard with a genome size closer to the value of these populations; however, the linearity of the flow cytometer has been assessed and guarantees a fluctuation threshold lower than 2% in this range of data (see Materials and Methods).
¶¶Only two individuals have been measured.

are significantly different from the other *Artemisia*, with the *Tridentatae* showing larger values. Differences in monoploid genome size (Cx) between ploidy levels are also statistically significant ($P = 0.0058$, Table 4), with monoploid genome sizes decreasing with increasing ploidy levels (Fig. 3). As seen in Table 4, most comparisons between nuclear DNA levels and ecological, morphological, or environmental traits give nonsignificant differences, both in the ordinary (ANOVA) and in the PGLS tests, although meaningful results are obtained with plant height, growth rate, and distribution.

As for phylogenetic analysis, performed with the purpose of analysing genome size variation in a phylogenetic context, Figure 4 shows the phylogram from the BI analysis for 28 *Artemisia* taxa, together with 1Cx values. The tree was rooted using *K. brachanthemoides* and *N. nipponicum*. The species used for this analysis, together with GenBank accession numbers and other data, are shown in Table 3. This reconstruction, based only on the analysis of ITS1 and ITS2 nrDNA (nuclear ribosomal DNA) regions, does not resolve the interspecific relationships among most taxa. However, two well-supported clades are clearly seen: one of which contains two species from the subgenus *Dracunculus*, which appears as the sister group of the remaining *Artemisia*, and some North American *Dracunculus* species (*A. filifolia*, *A. pedatifida*, and *A. spinescens*). According to this analysis, all subgenera are paraphyletic, with the exception of *Seriphidium* and *Absinthium* (however, the analysis is biased because of the scarce sampling of the non-*Tridentatae* subgenera). Most *Tridentatae* species appear together in a supported clade, of which *A. pygmaea* would be the sister group (although the p.p. value is not significant enough); this reconstruction places *A. argillosa*, *A. bigelovii*, and *A. rigida* apart from the other *Tridentatae*.

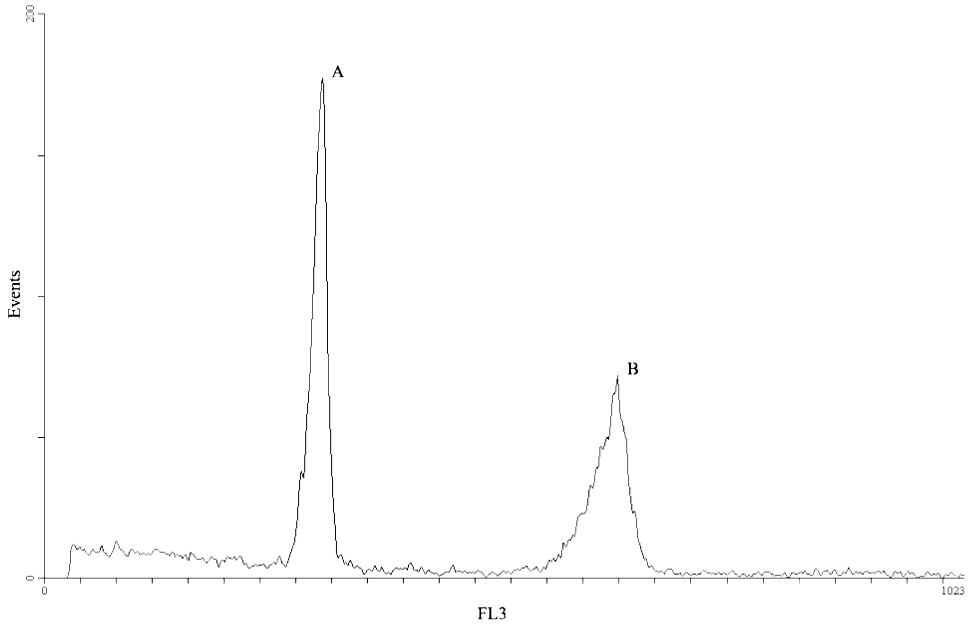

Figure 2. Fluorescence histogram of the genome size assessment of *A. nova* (2876). A, peak of the standard, *Pisum sativum* (2C = 8.37 pg); B, peak of *A. nova* (2C = 17.10 pg).

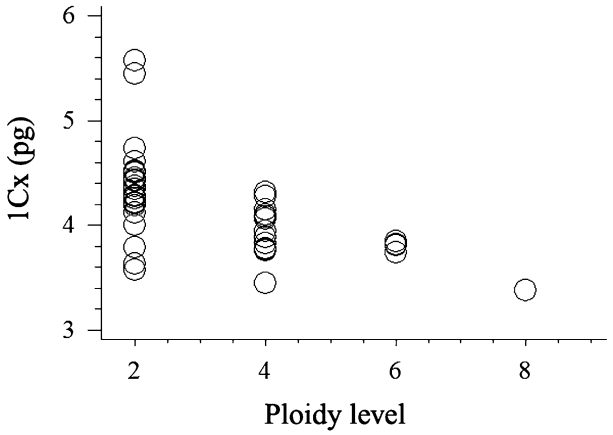

Figure 3. Decreasing monoploid genome sizes (1Cx) with increasing ploidy levels in the species studied.

DISCUSSION

Inter- and intraspecific genome size differences

As previously stated, results for the *Tridentatae* species at the same ploidy level are fairly similar, with the exception of three taxa that are particularly conflictive (*A. bigelovii*, *A. pygmaea*, and *A. rigida*). Such homogeneity in genome size data might be a reflection of the limited genetic differences that characterize the group, such as their fairly homogeneous karyotype morphology (McArthur, Pope & Freeman, 1981; Garcia *et al*., in press) and the low levels of sequence divergence detected in this study, and in previous ones (Kornkven *et al.*, 1998, 1999; Stanton *et al*., 2002), which could also explain the habitual hybridization and backcrossing among many *Tridentatae* taxa.

Together with the low interspecific differences found, low intraspecific ones have been obtained for most taxa at the same ploidy level. In a general sense, the extent of intraspecific genome size variation is controversial, as the C value is considered to be constant for a given species, and some authors have successfully attributed meaningful intraspecific differences to methodological errors or taxa misidentification in some cases (Greilhuber, 1998; Ohri, 1998; Greilhuber, 2005). Adaptive changes in genome size as a response to stressful environments (Cullis, 2005) have also been found, and factors like changes in repetitive DNA (Rabinowicz, 2000) or retrotransposon activity (Bennetzen, Ma & Devos, 2005) can be a true source of variation within a taxon, among others. Doležel & Bartoš (2005) stated that differences of 5% should be considered acceptable in some groups. Among the different populations of the same taxon that have been assessed in this study, and in the previous ones (Torrell & Vallès, 2001; Garcia *et al*., 2004; Garcia *et al*., 2006), low differences have been detected for the majority of species (most ranging from 1 to 2%), except for the case of *A. pygmaea* (5.98%) and *A. tridentata* ssp. *spiciformis* (10.02%), in which similar circumstances or mechanisms as those previoulsy mentioned could explain these values.

Table 3. Environmental, ecological, and morphological characteristics, together with the mean values of holoploid genome size data (2C) and ploidy levels of the taxa included in the analyses. GenBank accession numbers of the taxa used for phylogenetic analyses are shown in the first column

Taxa	Gembank acc. n. ITS1/ITS2	Group[1]	2C[2]	P. L.[3]	Dist.[4]	E. R.[5]	M. A. P.[6]	D. T.[7]	P. H.[8]	S. P.[9]	F. E.[10]	G. R.[11]	S. T.[12]
A. arbuscula subsp. *arbuscula*	AF060464/AF061380	AT	9.24	2	3	3	3	1	1	1	Y	S	1
A. bigelovii	AF060469/AF061385	AT	8.00	2	2	2	2	3	2	3	N	S	2
A. cana subsp. *viscidula*	EU111664/EU111665*	AT	8.61	2	3	3	3	1	3	3	Y	F	1
A. nova	AF045412/AF079964	AT	9.09	2	3	3	1	3	2	3	N	S	2
A. pygmaea	AF060468/AF061384	AT	11.19	2	2	1	1	3	1	2	N	S	2
A. rigida	AF060465/AF061382	AT	8.23	2	2	2	2	3	1	1	Y	S	2
A. spiciformis	EU111666/EU111667*	AT	8.59	2	3	3	3	1	3	3	Y	F	1
A. tridentata subsp. *vaseyana*	AF045411/AF079963	AT	8.80	2	3	3	1	2	3	3	N	S	1
A. tripartita subsp. *tripartita*	AF060463/AF061379	AT	8.85	2	3	2	3	2	3	3	Y	S	2
A. longiloba	EU124796/EU124799*	AT	9.21	2	2	1	2	3	1	2	–	S	3
A. argillosa	EU111676/EU111677*	AT	15.77	4	2	2	3	2	2	3	–	–	3
A. rothrockii	EU124794/EU124797*	AT	16.41	4	1	1	2	3	2	1	N	S	2
A. californica	AF060474/AF061388	ANT	8.48	2	2	2	2	2	3	3	N	S	1
A. filifolia	AF060477/AF061393	ANT	7.20	2	3	–	3	2	3	3	Y	F	3
A. palmeri	AF060470/AF061386	ANT	7.14	2	1	–	2	2	3	3	Y	F	1
A. papposa	EU111668/EU111669*	ANT	8.44	2	1	–	2	2	1	2	N	S	2
A. spinescens	EU111670/EU111671*	ANT	7.58	2	3	3	1	3	1	3	–	S	3
A. pedatifida	EU111672/EU111673*	ANT	8.86	2	2	2	1	3	1	–	–	S	3
A. nesiotica	EU111674/EU111675*	ANT	8.38	2	1	1	1	2	2	3	N	S	1
A. ludoviciana	EU124795/EU124798*	ANT	13.82	4	3	3	3	2	3	3	Y	F	2
A. vulgaris†*	AF045385/AF079937	ANT	6.08	2	3	1	2	2	3	2	N	F	2
A. fragrans†*	AF045406/AF079957	ANT	5.35	2	–	1	1	3	2	2	–	–	3
A. herba-alba†*	AF045403/AF079954	ANT	6.57	2	–	1	1	3	2	2	–	–	3

Table 3. *Continued*

Taxa	Gembank acc. n. ITS1/ITS2	Group[1]	2C[2]	P. L.[3]	Dist.[4]	E. R.[5]	M. A. P.[6]	D. T.[7]	P. H.[8]	S. P.[9]	F. E.[10]	G. R.[11]	S. T.[12]
A. arborescens†*	AF045393/AF079945	ANT	11.61	2	–	1	2	2	3	3	–	–	2
A. absinthium†*	AF045394/AF079946	ANT	8.64	2	3	1	3	2	2	3	Y	F	2
A. campestris†*	AF045398/AF079950	ANT	5.87	2	3	3	1	3	2	2	Y	F	2
A. chamaemelifolia†*	AF045388/AF079940	ANT	6.04	2	–	1	3	1	2	2	–	–	1
A. dracunculus†*	AF045401/AF079952	ANT	5.94	2	3	1	2	2	2	2	–	–	2
N. nipponicum†*	L77772/DQ028913	O	11.87	2	1	–	–	–	2	2	–	–	–
K. brachanthemoides†*	AF504189/AF504162	O	14.09	2	1	1	1	2	1	1	–	–	2

*Newly generated sequences.
†Previously published genome size data for these species (Torrell & Vallès (2001; Garcia *et al*., 2004).
[1]Group. AT, *Artemisia* subgenus *Tridentatae*; ANT, Non *Tridentatae Artemisia*; O, Outgroup (other subgenera).
[2]Mean 2C values of the populations measured in the present work and in the previously published work.
[3]Ploidy level.
[4]Distribution. Values: 1 = restricted (1–5 states in the USA); 2 = medium (> 5–10 states); 3 = wide (> 10 states).
[5]Elevational range. Values: 1 = narrow (< 1500 m); 2 = medium (2000–2500 m); 3 = wide (> 2500 m).
[6]Mean annual precipitation. Values: 1 = low (< 300 mm); 2 = medium (300–400 mm); 3 = high (500–1000 mm).
[7]Drought tolerance. Values range from 1 = poorly drought tolerant to 3 = strongly drought tolerant.
[8]Plant height. Values: 1 = dwarf (< 0.5 m); 2 = subshrubs (0.5–1.5 m); 3 = shrubs (> 1.5 m).
[9]Seed production. Values: 1 = low (< 2000 seeds/ g^{-1}); 2 = medium (2000–5000 seeds/ g^{-1}); 3 = high (> 5000 seeds/ g^{-1}).
[10]Fire ecology. Y = layers/stumps sprout after fire; N = killed by fire.
[11]Growth rate. F = fast; S = slow.
[12]Salinity tolerance. Values range from 1 = poorly salinity tolerant to 3 = strongly salinity tolerant.

Table 4. Mean holoploid genome size (2C) and results of the comparisons, using the ordinary test (ANOVA) and the phylogenetically based generalized least squares (PGLS) algorithm. Significances belong to the group AT + ANT

	AT*	ANT*	AT + ANT	Ordinary test	PGLS test
Group	8.98	7.48	8.08	$P = 0.014$	$P = 0.092$
Elevation range					
1	10.2	7.33	7.90	Nonsignificant	Nonsignificant
2	8.36	8.67	8.49		
3	8.87	8.72	8.25		
Mean annual precipitation					
1	9.69	7.1	7.97	Nonsignificant	Nonsignificant
2	8.48	7.95	8.13		
3	8.82	7.29	8.17		
Drought tolerance					
1	8.81	6.04	8.12	Nonsignificant	Nonsignificant
2	8.82	7.99	8.14		
3	9.14	6.85	7.99		
Plant heigth					
1	9.47	8.29	8.96	$P = 0.038$	$P = 0.087$ (1–2)
2	8.55	6.68	7.09		
3	8.71	8.19	8.37		
Seed production					
1	8.73		8.73	Nonsignificant	Nonsignificant
2	10.2	6.33	6.93		
3	8.66	8.43	8.54		
Fire ecology					
Y	8.70	7.21	8.04	Nonsignificant	Nonsignificant
N	9.27	7.85	8.56		
Growth rate					
F	8.60	6.99	7.45	$P = 0.009$	$P = 0.054$
S	9.08	8.35	8.80		
Salinity tolerance					
1	8.81	7.51	8.16	Nonsignificant	Nonsignificant
2	9.07	7.76	8.36		
3	9.21	7.11	7.46		
Distribution					
1	9.71	8.57	9.02	Nonsignificant	$P = 0.055$ (1–2)
2	8.79	7.58	8.62		$P = 0.011$ (1–3)
3	6.75		6.75		

*AT, *Artemisia* subgenus *Tridentatae*; ANT, non-*Tridentatae Artemisia*. The codification of each category is the same as in Table 3.

CAN GENOME SIZE DISCRIMINATE THE SAGEBRUSHES AMIDST *ARTEMISIA*?

The taxonomic limits of the subgenus *Tridentatae* are subject to discussion, although most sagebrushes are clearly distinct from the other subgenera, forming a natural group of species based on habit, morphology, anatomy, chemistry, and cytology (McArthur, 1979). Our genome size research reported herein supports the separation of the *Tridentatae* from the other subgenera. Statistical analyses show a significant difference between the mean genome sizes of the *Tridentatae* with respect to those of the non-*Tridentatae Artemisia* (Fig. 1). The *Tridentatae* genome size is larger than those of the other subgenera of this study (*Tridentatae* mean 1Cx = 4.49 pg, vs. non *Tridentatae* mean 1Cx = 3.74 pg), as was previously reported by Garcia *et al*. (2004) on a limited data set (1Cx mean values for each subgenera: *Dracunculus* 1Cx = 2.67 pg, *Artemisia* 1Cx = 3.05 pg, *Absinthium* 1Cx = 3.56 pg, *Seriphidium* 1Cx = 3.89 pg, *Tridentatae* 1Cx = 4.08 pg). Differences in monoploid genome size between ploidy levels are also statistically significant (see Fig. 3), as previous studies had stated for other taxa (Leitch & Bennett, 2004), and hence a decreasing monoploid genome size

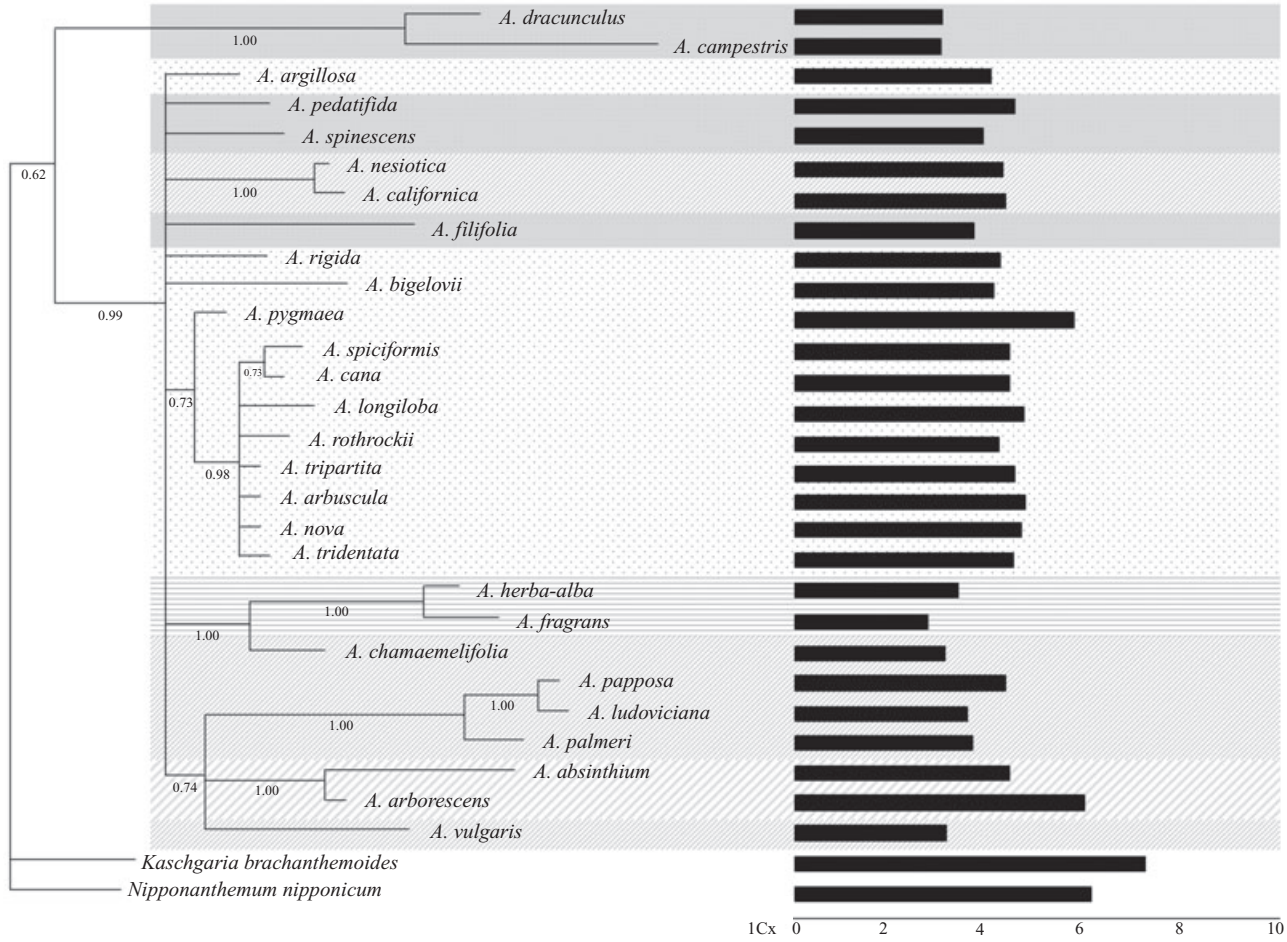

Figure 4. Phylogram from Bayesian inference phylogenetic analysis of internal transcribed spacer 1 (ITS1) and ITS2 sequence data for 28 *Artemisia* and two outgroup species. The Bayesian clade-credibility values (posterior probability > 0.5) are given below the branches. Monoploid genome sizes (1Cx) are indicated next to each species (pg). Subgenus *Dracunculus* subgenus *Tridentatae* subgenus *Artemisia* subgenus *Seriphidium* subgenus *Absinthium*.

is detected with increasing ploidy levels, confirming the general phenomenon of nuclear DNA loss (1Cx) with polyploidy in many cases.

Species with large genomes are restricted to the more derived families, and phylogenetic reconstructions indicate that a very small genome size represents the ancestral condition for most major angiosperm clades (Leitch, Chase & Bennett, 1998; Soltis *et al*., 2003). Although evolution of genome size in the Angiosperms is dynamic, with both increases and decreases (Bennett & Leitch, 2005c), we believe that the larger genome size of the *Tridentatae* in respect to the other *Artemisia* subgenera is evidence of a derived phylogenetic position. The subgenus *Tridentatae* is thought to have evolved from the subgenera *Artemisia* or *Dracunculus* (on the basis of distribution, flower morphology, and secondary woodiness; McArthur, 1983), which most likely bridged the Bering Strait from Central Asia (the centre of origin and diversification of *Artemisia*) to North America (McArthur & Plummer, 1978; McArthur *et al*., 1981). Some of their species, such as *A. dracunculus* and *A. frigida*, naturally occur in both areas, and their low genome sizes (5.94 and 5.25 pg, respectively, Garcia *et al*., 2004; Pellicer *et al*., 2007b) could be related to a possible role as ancestral stock for the *Tridentatae*. In this sense, the tree topology of Figure 4 suggests that at least some species from subgenus *Dracunculus* are basal to all the other *Artemisia* species.

Genome size and colonizing ability

The ability of the *Tridentatae* to colonize extensive areas reflects competitive success, suggesting that the larger genomes characterizing this subgenus has not

been a constraint, at least at this level, and in that region. Such a genome expansion without increasing ploidy level could be explained by activation of transposable elements (Kellogg & Bennetzen, 2004), by the presence of B chromosomes (these have already been detected in the *Tridentatae* and, particularly, they could explain an increased genome size in *A. pygmaea*; Garcia *et al*., in press), or by any other mechanism. The other process that best explains a global genome size (2C) increase is polyploidy, which is very prevalent in the *Tridentatae*, with some species only known as polyploids, such as *A. rothrockii* and *A. argillosa* (Mahalovich & McArthur, 2004). We hypothesize that the reduced competition pressure in the *Tridentatae* habitats allows expansion of genome size (2C), and probably polyploidy, whereas in environments subject to competitive constraints, the pattern followed is the decrease in total nuclear DNA level. Hence, at the other extreme of the genome size variation spectrum we could cite the case of many island -colonizing species, where a significant reduction in holoploid genome size, presumably in response to insular selection pressures, has been detected (Suda, Kyncl, & Freiová, 2003; Garcia *et al*., 2006; Garnatje, Garcia & Canela, 2007). Indeed, molecular mechanisms are known that can lead to genome size increase or decrease (Petrov *et al*., 2000; Bennetzen, 2005). This hypothesis fits well with renewed theories about selfish and junk DNA, which postulate that the C value of a species is merely a by-product of the persistent accumulation of phenotypically neutral DNA (driven by genetic drift, by mutation pressure, or by the maintenance of extinct genes), which is excised only when it becomes too costly (Gregory & Hebert, 1999). Recent studies, however, confer more importance to junk DNA, which should be regarded as a major player in many of the processes that shape the genome and control the activity of its genes (Biémont & Vieira, 2006).

Fitness, adaptation, and genome size

The r/K selection theory (MacArthur & Wilson, 1967) posits that evolutionary systems must choose whether they invest more resources in reproduction or development, a choice that is dependent on the selective environment. In a changing or disturbed context, selection for abundant offspring will prevail (r selection), whereas selection for development is suitable in predictable conditions, with an adequate supply of resources (K selection). In the light of previous data and our present results, we suggest that the genus *Artemisia* in North America displays a continuum from one evolutionary strategy to the other, during its speciation and diversification processes, which is coupled with a considerable genome size increase (although some authors support that both strategies are exclusive; Flegr, 1997). In the proposed scenario, the *Tridentatae* (together with the other North American endemics) arose *in situ* in North America from an ancestor coming from the subgenera *Artemisia* or *Dracunculus*, when alternating moist and dry climates during the Pleistocene provided the opportunity to fill large new niches (McArthur, 1983). Given the abundance and present distribution of *A. s.l.* in North America, they might have spread profusely at initial stages of the colonization. Most likely, species with more r-selection traits (profuse seed production, more herbaceous habit, shorter generation times, smaller sizes, etc.) might have pioneered this colonization. This role could have been played by species such as *A. dracunculus* or *A. frigida*, by other counterparts in their subgenera (*A. campestris* or *A. vulgaris*, for example), or by some ancestral taxa with similar features. All of them show reduced holoploid genome sizes, as compared with the *Tridentatae*, and are present in Eurasia, and also cover large areas of North America (in particular, these four species are listed as weeds or invasive plants in the USA; see below for a further discussion on this topic). The *Tridentatae* could have arisen from subsequent evolutionary processes in any of those species, resulting in an optimal adaptation to their environment, and thus acquiring traits that would class them more as K strategists, together with a holoploid genome size increase.

To test the hypothesis that this change in lifestyle strategy is linked with a significant genome size increase, we collected environmental and morphological data on different characteristics of these species (Table 3) that could have a bearing on r or K selection, and study the relationship between these traits. A discussion of every feature for all of these groups follows next (see Table 4 for statistical analyses and comparisons between groups).

Elevation range

This variable was included as it may reflect an ability to colonize different environments and habitats. No significant correlations or meaningful differences between groups were observed. However, we note that *A. frigida* exhibits an altitudinal gradient from 900 to 3500 m, and presents one of the lowest genome sizes of perennial *Artemisia* inhabiting North America. Its small genome size may well be involved in this broad adaptability. In contrast, *A. pygmaea*, with the largest genome size of the species studied, only inhabites a narrow elevation range.

Mean annual precipitation and drought tolerance

Differences between groups are nonsignificant in all cases. However, the largest genome sizes in the *Tri-*

dentatae are found in the species group inhabiting areas with lowest mean annual precipitation, which also coincides with the highest genome size found in the most drought tolerant species, confirming previous research (Garcia *et al*., 2004).

Mean plant height

In all groups, but particularly in the *Tridentatae*, lower statured species tend to show larger genome sizes. The r/K theory asserts that K strategists should be larger in size. These lower statured species, however, tend to show a woody habit (which implies more biomass, i.e. selection for development). This trait is particularly outstanding in *A. pygmaea*, the smallest of all sagebrushes, but which exhibits a dwarf shrub habit, and the largest genome size. Indeed, seeds and seedlings of the pygmy sagebrush are the largest of the whole subgenus; this is probably another sign of selection for development. In this sense, the *Tridentatae* polyploids tend to show lower sizes than the diploids of the same species (Barker & McKell, 1986; Sanderson, McArthur & Stutz, 1989; McArthur & Sanderson, 1999).

Seed production

The hypothesis states that plants with r-selection traits tend to produce more seeds than K selectors. Hence, according to our prediction, species with profuse seed set should show lower genome sizes than less-profuse seed producers. However, we were unable to find either statistical support or meaningful differences between the genome sizes of the different groups to support this premise. Nevertheless, one of the highest seed producers is again the low-genome-sized *A. frigida*: each 2.5-cm length of inflorescence contains approximately 1000 seeds (Harvey, 1981), with about 10 000 000 cleaned seeds per kg (Plummer, Christensen & Monsen, 1968).

Fire ecology

Species that layer or stump sprout after fire show smaller genome sizes than those that are entirely killed by fire. The differences are not significant, but the trend is consistent in the three groups. The ability to colonize disturbed environments linked to r strategists could also be related to this lower genome size in these species.

Growth rate

The differences between slow- and fast-growing species are significant, and in all groups the slow-growing ones have increased genome sizes. Smaller genomes are usually correlated with shorter life cycles (which imply fast growth), and usually slow growth is linked with long-lived plants, a trait that better fits the K-strategy growth.

Salinity tolerance

Differences are again nonsignificant in all groups, so we cannot set a link between genome size and salinity tolerance from these data. However, a particularly halofilous species, *A. filifolia*, which exclusively inhabits dunes or sandhills, presents one of the lowest genome sizes of the North American endemics. Most traits of this species (profuse seed set, quick growth and maturation, ability to resprout vigorously after fire, relatively tall stature, but less woody than common sagebrushes) would class this *Artemisia* as an r strategist.

Distribution

In all groups studied, the plants showing a more extensive distribution have lower genome sizes. The differences are statistically significant when all species are included in the analysis. Species with wider distribution are usually r strategists (and hence have lower genome sizes, according to our hypothesis), whereas more restricted species tend to be K strategists, with higher genome sizes. Again, the case of *A. pygmaea* ($2C = 11.19$ pg, $2n = 18$), with a restricted, scattered distribution on the cold desert of the Great Basin, and with the highest genome size of all the sagebrushes, might represent a model of this hypothesis. *Artemisia frigida* ($2C = 5.25$ pg, $2n = 18$) would be placed at the other end of the r/K gradient: this is probably the most widely distributed and abundant species of the whole genus (USDA, 1937; Harvey, 1981).

From all these data and statistical analyses it is clear that neither one group (the *Tridentatae*) nor the other (the remaining *Artemisia*) meet exactly all of the conditions that would shape an r or a K strategist, although many species can be safely included in one or the other category (as previously noted, it is known that a given species will mainly adopt one strategy, even though traits of the other can be present). However, from the trend outlined from these relationships, it seems that fast-growing, less drought tolerant, taller (but less woody), and more widely distributed species tend to show lower nuclear DNA levels, and would be more easily classified as r strategists, whereas slow-growing, more drought tolerant, smaller, or more woody species, and more restricted in distribution tend to have higher DNA levels (and would tend towards being K strategists). Apart from the species included in this study, we note that the uncommon annual *Artemisia* species (*A. annua* and *A. scoparia*, for instance) are also better fitted by the r-selection category, showing smaller genome sizes

(see Torrell & Vallès, 2001; Garcia *et al*., 2004), although exceptions can be found.

Weed behaviour and genome size

Studies have shown that weeds and invasive species (the model of r strategists) tend to show lower genome sizes compared with their counterparts of their genera (Bennett & Leitch, 2005a). In contrast, species appearing in the red list of endangered species mostly show high nuclear DNA levels (Vinogradov, 2004). From the US Invasive Plants List (http://plants.usda.gov, accessed in /December 2006), 11 *Artemisia* species are cited (*A. absinthium*, *A. annua*, *A. biennis*, *A. campestris*, *A. cana*, *A. dracunculus*, *A. filifolia*, *A. frigida*, *A. ludoviciana*, *A. tridentata*, and *A. vulgaris*). Except from *A. ludoviciana*, which is only known as a tetraploid, and *A. cana* ssp. *cana*, which is an octaploid (although other subspecies of *A. cana* are known at the diploid level, but the list does not mention which of these behaves as a weed), all of the other species never exceed 9.01 pg. This finding also supports the hypothesis that high genome size might inhibit such weedy (hence, r strategist) behaviour.

Hybrid formation

Owing to their widespread and sympatric or tightly parapatric distribution, to their wind pollination, and to their genetic similarity, *Tridentatae* taxa tend to hybridize. The data set in this study includes genome size data for polyploid and homoploid synthetic hybrids (Table 2; McArthur *et al*., 1998; McArthur & Sanderson, 1999). Nuclear DNA levels of both sets of the hybrids are consistent with the expected levels predicted from their parents' genome sizes, although the tetraploid and hexaploid offspring show a little less DNA than the mean levels, most likely because of their polyploid nature (Fig. 5). In both these polyploid hybrids, a similar genome size decrease is detected. This could also reflect rapid genome reorganization after hybridization (which is coupled with ribosomal DNA loss in some cases, Garcia *et al.*, unpubl. data).

Taxa of questionable taxonomic position and genome size

Genome size variation at species level has been considered as a predictor of taxonomic heterogeneity, and as an indicator of incipient speciation in process (Murray, 2005). Hence, a critical study of genome size can contribute to the clarification of taxonomic placement between closely related species. The *Tridentatae* form a natural, homogeneous group of taxa

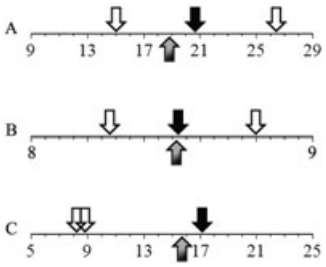

Figure 5. Genome sizes (2C) of the hybrid taxa. A, parents, *A. cana* ssp. *cana* (mean 2C = 26.35 pg), *A. tridentata* ssp. *wyomingensis* (mean 2C = 15.07 pg); hybrid, expected (2C = 20.71 pg), observed (mean 2C = 18.94 pg). B, parents, *A. tridentata* ssp. *tridentata* (mean 2C = 8.28 pg), *A. tridentata* ssp. *vaseyana* (2C = 8.80 pg); hybrid, expected (2C = 8.54 pg), observed (2C = 8.52 pg). C, parents, *A. tridentata* ssp. *tridentata* (mean 2C = 8.28 pg), *A. tridentata* ssp. *vaseyana* (2C = 8.80 pg); hybrid, expected (2C = 17.08 pg), observed (2C = 15.71 pg). The polyploid hybrids show lower genome sizes than are expected from their parents' values (A, C), whereas the 2C value of the homoploid hybrid is very close to the expected mean. ▭ Parental 2C ■ Expected 2C ▬ Observed 2C.

(Kornkven *et al*., 1998; Torrell *et al*., 1999; Vallès *et al*., 2003); however, *A. bigelovii*, *A. pygmaea*, and *A. rigida*, classically included in this group, have been the subject of controversy, with countless studies proposing either their inclusion or exclusion.

If mean genome sizes of the traditional *Tridentatae (sensu* Shultz, 2006) are aligned from the lowest to the highest (at diploid level, Table 3), *A. bigelovii* and *A. rigida* appear at the lowermost end (8.00 and 8.23 pg, respectively), and *A. pygmaea* appears at the uppermost end (11.19 pg), whereas the remaining species converge in the narrow range between 8.54 and 9.24 pg. This exercise may be revealing about the potential use of genome size in this field, but some other data about these three species question their placement within the *Tridentatae*. Additionally, our phylogenetic reconstruction places these three species, together with *A. argillosa*, outside the clade embracing all of the *Tridentatae* (Fig. 4).

The first is the case of *A. bigelovii*, the floral morphology of which (it is the only *Tridentatae* with heterogamous capitula), molecular phylogenetic data (Kornkven *et al*., 1998), essential oil composition (Holbo & Mozingo, 1965; Geissman & Irwin, 1974), and our own results on molecular cytogenetics by FISH and molecular phylogenetics (Garcia *et al.* unpubl. data) do not support its inclusion in *Tridentatae*. *Artemisia bigelovii* has been considered to occupy an unclear position between the true sage-

brushes (*Tridentatae*) the and subgenus *Artemisia*; however, it has been generally treated as a *Tridentatae* on the basis of many characters, such as wood anatomy, leaf form, karyotype morphology, RAPD genetic markers and cpDNA restriction site analyses (McArthur *et al*., 1981, 1998; Kornkven *et al*., 1999). The second case is that of *A. pygmaea*. This is a dwarf, depressed shrub, with different leaf morphology and larger seeds, compared with the other *Tridentatae* (Cronquist, 1994; McArthur & Stevens, 2004). It is a relatively uncommon species, which occurs on dry alkaline sites, probably because of the numerous morphological adaptations that it incorporates for the extremely xeric sites that it inhabits, where few other species occur (in the deserts of Nevada, Utah, and Arizona). Based on these specialized features, Rydberg (1916) placed *A. pygmaea* in a separate section (sect. *Pygmaea* Rydb.) in subgenus *Seriphidium*. The overall karyotype morphology of *A. pygmaea* is shared with the traditional *Tridentatae*, although it does have bigger chromosomes and the habitual presence of a B chromosome (Garcia *et al*., in press). The essential oil composition also supports its exclusion from the core of the true sagebrushes (Holbo & Mozingo, 1965; Geissman & Irwin, 1974). Additionally, molecular biology studies have placed this species as sister to the other *Tridentatae* (Kornkven *et al*., 1998; Watson *et al*., 2002; Garcia *et al*., unpubl. data). The third case is that of *A. rigida*. This species also displays specialized morphological and anatomical modifications to the arid conditions of western North America (Hall & Clements, 1923; Shultz, 1993). Similar to the pygmy sagebrush, *A. rigida* was also placed alone in another section within *Seriphidium*, sect. *Rigidae* Rydb. (Rydberg, 1916). Holbo & Mozingo's (1965) chromatographic characterization also pointed to its exclusion from the true sagebrushes. Although many studies have claimed for the retention of these three species within the *Tridentatae* (Hall & Clements, 1923; Ward, 1953; Beetle, 1960; McArthur *et al*., 1981; Bremer & Humphries, 1993; Kornkven *et al*., 1998, 1999; Shultz, 2006), our present findings both from molecular phylogeny and genome size again place a question mark about their taxonomic placement (Fig. 4).

Other north american endemic *Artemisia*

This study also reports on genome size data for some other non-*Tridentatae*. Some studies have placed some of these very close or even within the *Tridentatae* (McArthur & Pope, 1979; Kornkven *et al*., 1998, 1999). These species, assigned to other *Artemisia* subgenera (Shultz, 2006), are also endemic to North America and share some morphological traits, as well as overlapping distribution. We have studied several species from the subgenus *Artemisia*.

(1) *A. palmeri*, a large woody plant endemic to the coastal area near San Diego (California). It has been treated as a member of the subgenus *Seriphidium* (Ward, 1953), and was also considered in an independent genus, *Artemisiastrum* (Rydberg, 1916). However, it is best placed in subgenus *Artemisia*, as it displays growth, leaf form, and floral characters that typically characterize this subgenus, being especially reminiscent of (2) *A. ludoviciana* (Shultz, 1993; McArthur, 2005), which is only known at tetraploid level; both species present lower genome sizes than the mean of the *Tridentatae* at each ploidy level. Other species, (3) *A. californica* and (4) *A. nesiotica*, which is sometimes considered as a subspecies of the later, are woody, unlike most members of this subgenus; their genome sizes are also very similar (indeed, population 3039 of *A. californica* presents the same value as population 3090 of *A. nesiotica*), and would fall within the range of the *Tridentatae* values; finally (5) *A. papposa*, a very particular species, with entire, villous leaves, and the unusual character of having pappus in its seeds, the holoploid genome size of which is also close to the mean of the *Tridentatae*. From subgenus *Dracunculus* we have assessed genome size data for (6) *A. filifolia*, (7) *A. pedatifida*, and (8) *A. spinescens* (the latter has also been considered a separate monotypic genus, *Picrothamnus desertorum*). *Artemisia filifolia* has affinities with the *Tridentatae* (karyotype morphology, McArthur & Pope, 1979; and similarities in secondary chemistry, Kelsey & Shafizadeh, 1979), but its genome size is significantly lower than the *Tridentatae* mean. *Artemisia spinescens* also presents a lower value, whereas *A. pedatifida* presents a genome size near the mean for sagebrushes'. Interestingly, each of these North American endemics show substantially increased genome sizes with respect to the mean of their subgenera at the same ploidy level (see the second section of the discussion, and Garcia *et al*., 2004). This would also support our hypothesis of genome size expansion linked to the absence of competitive constraints and diversification when colonizing North America, not only in the emergence of a new subgenus with larger genome sizes than the rest, the *Tridentatae*, but also in the increased values of the other North American endemic *Artemisia*.

CONCLUSION

The higher genome size of the *Tridentatae*, and of the other North American *Artemisia* endemics, together with other shared traits (particularly woodiness) characterize what we could call in a wide sense the 'North American *Artemisia*' group, which is consistent

with a recent molecular phylogeny of the whole genus (Sanz *et al*., 2007). Apart from the exceptions previously discussed, the core of North American sagebrushes forms a homogeneous group of species (also visible in their similar genome sizes), which may be undergoing diversification and speciation processes. Reticulate evolution is probably a strong evolutionary mechanism acting on this species: a hypothesis reinforced by the difficulty experienced by many authors in establishing a clear phylogenetic framework for the *Tridentatae*, and the incongruences that appear therein. Finally, a change in lifestyle strategy linked to genome size gain in the North American *Artemisia* is suggested, on the basis of morphological and ecological traits, and geographical distribution. The developmental–reproduction trade-off (r/K selection) that these species might face in the struggle for life appears coupled with significant changes in nuclear DNA levels, in which presumed selfish and junk DNA (transposable elements, for instance) may probably be involved. As Gregory & Hebert (1999) stated, it will now be critical to ascertain whether these changes arose via the gradual accumulation or deletion of small segments of DNA, or whether a more punctuated pattern of change predominates.

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