

Nuclear DNA Amounts in Macaronesian Angiosperms

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Nuclear DNA contents for 104 Macaronesian angiosperms, with particular attention on Canary Islands endemics, were analysed using propidium iodide flow cytometry. Prime estimates for more than one-sixth of the whole Canarian endemic flora (including representatives of 11 endemic genera) were obtained. The resulting 1C DNA values ranged from 0.19 to 7.21 pg for *Descurainia bourgeauana* and *Argyranthemum frutescens*, respectively (about 38-fold difference). The majority of species, however, possessed (very) small genomes, with C-values <1.6 pg. The tendency towards small nuclear DNA contents and genome sizes was confirmed by comparing average values for Macaronesian and non-Macaronesian representatives of individual families, genera and major phylogenetic lineages. Our data support the hypothesis that the insular selection pressures in Macaronesia favour small C-values and genome sizes. Both positive and negative correlations between infragenetic nuclear DNA amount variation and environmental conditions on Tenerife were also found in several genera. © 2003 Annals of Botany Company

Key words: C-value, Canary Islands, endemic, flow cytometry, genome size, nuclear DNA content, Macaronesia.

INTRODUCTION

Oceanic islands play an important role in maintaining global biodiversity. It has been estimated that they harbour approximately one-sixth of vascular plant species, a large proportion of which is endangered (World Conservation Monitoring Centre, 1992). The Macaronesian phytogeographic region encompasses five assemblages of volcanic archipelagos in the eastern Atlantic Ocean (the Azores, Madeira, the Salvage Islands, the Canary Islands and the Cape Verde Islands), situated between 15 and 40°N. The flora of this region is diverse and complex. The strongest phytogeographic connections have been proposed to be with the Mediterranean basin and north-western Africa; apparent links with south-eastern Africa and neotropical regions can also be traced (Bramwell, 1986; Panero *et al.*, 1999). A distinct feature of indigenous plant species is a high proportion of woody life-forms in otherwise herbaceous groups, *Echium* and *Sonchus* being two prominent examples. Analysis of growth-form composition also reveals a high percentage of succulents and a low abundance of geophytes and annuals (Shmida and Werger, 1992). The Macaronesian flora has traditionally been suggested to represent the relictual fragment of a subtropical Tertiary plant biota once widespread in Europe and northern Africa (Bramwell, 1976). However, modern phylogenetic analyses of several Macaronesian plant groups (*Aeonium*, *Argyranthemum*, *Bencomia*, *Echium*) have not provided support for a relict origin, and indicated relatively recent insular diversification from continental ancestors (Emerson, 2002).

Oceanic islands typically show a high degree of endemism. Macaronesia harbours at least 30 endemic genera (Bramwell, 1976; Kunkel, 1993) and the proportion of Macaronesian endemic species for individual islands has been estimated at between 4 % (Graciosa in the Azores) and 27 % (La Palma in the Canary Islands) (Hobohm, 2000). The main archipelago, the Canary Islands, is by far the richest area, with about 570 endemic species (about 40 % of native plants) (Francisco-Ortega *et al.*, 2000). Such a high number of endemics could be attributed to the great diversity of habitats on the Canaries where six major ecological zones have developed (Fernandopullé, 1976). A broad range of geological ages (between 0.8 and 21 million years) (Carracedo, 1994), a varying degree of substrate erosion and the proximity of the African continent may also contribute to the species richness. Adaptive radiation (divergent evolution in response to different ecological pressures) and vicariance (divergence due to geographic isolation) seem to be the main processes favouring the diversification of ancestral plant types into several related taxa (Crawford *et al.*, 1987).

The flora of Macaronesia, and of the Canary Islands in particular, has been subjected to numerous karyological studies (e.g. Larsen, 1963; Borgen 1969; Bramwell *et al.*, 1976; Dalgaard, 1991). A very low incidence of polyploidy was repeatedly confirmed. On the basis of 453 species (304 Macaronesian endemics and 149 non-endemics) described cytologically, Borgen (1974) concluded that only 26.6 % of endemics and 27.8 % of the total flora are polyploids. These percentages may alter to a certain extent as some species traditionally regarded as diploids (e.g. *Canarina*, *Convolvulus*, *Crambe*, *Micromeria*) might actually be polyploid in their origin. Nevertheless, the frequency of

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TABLE 1. List of localities of Macaronesian endemics used in the present study

No.	Locality	Coordinates	Altitude (m)
1	Tenerife: Cañadas del Teide, rock crevices in Valle de Chiñoque, north of El Sanatorio	28°14' 20"N 16°36'10"W	2180
2	Tenerife: Cañadas del Teide, rock crevices and volcanic sands east-south-east of Parador Nacional de las Cañadas hotel	28°13'20"N 16°37'10"W	2180
3	Tenerife: Cañadas del Teide, rock crevices along hiking trail west of Colmenar hill	28°15'50"N 16°33'10"W	2060
4	Tenerife: Cañadas del Teide, El Portillo, volcanic sands along hiking trail near Montaña de las Arenas Negras hill	28°17'40"N 16°33'40"W	2000
5	Tenerife: Cañadas del Teide, Riscos de la Fortalesa, rock crevices at the top of the mountain	28°18'50"N 16°35'50"W	~2150
6	Tenerife: <i>Pinus canariensis</i> forest along the road approx. 1 km south of Miradores de la Cumbre, near Montaña Avosa hill	28°21'50"N 16°28'00"W	1970
7	Tenerife: Margarita de Piedra, rock crevices in Barranco de la Zarza	28°20'30"N 16°31'30"W	1420
8	Tenerife: Aquamansa, Fayal-brezal community along the road approx. 1 km south-west of the village	28°21'30"N 16°30'30"W	1160
9	Tenerife: Los Pinos, wall next to road in the village	28°22'50"N 16°30'50"W	520
10	Tenerife: Anaga, Casa Carlos, rock crevices in laurel forest along the road near Pico del Ingles hill	28°31'50"N 16°15'30"W	940
11	Tenerife: Anaga, Lomo de Las Bodegas village, laurel forest near Chinobre hill	28°33'20"N 16°10'50"W	770
12	Tenerife: Anaga, Chamorga village, rock crevices in Barranco de Roque Bermejo and laurel forest fragments north-east of the village, near Montaña Tafada hill	28°34'N 16°09'W	0–600
13	Tenerife: Anaga, Taganana village, rock crevices in Roque de Enmedio	28°34'00"N 16°12'40"W	330
14	Tenerife: Valle de Güímar, succulent community (cardonal) north of Puerto de Güímar	28°18'00"N 16°22'20"W	30
15	Tenerife: Güímar, Pájara village, rock crevices in Ladera de Güímar	28°17'30"N 16°25'30"W	840
16	Tenerife: Teno, Masca village, rock crevices in Barranco de Masca	28°17'30"N 16°51'W	0–600
17	Tenerife: Teno, Las Portelas village, rock crevices along the road near La Tabaiba	28°19'30"N 16°51'10"W	~650
18	Tenerife: Teno, rock crevices in Roque El Fraile	28°21'40"N 16°53'40"W	220
19	Tenerife: Teno, succulent community (cardonal) near Punta de Teno	28°20'40"N 16°55'00"W	40
20	Tenerife: Vilaflor, <i>Pinus canariensis</i> forest along the road approx. 5 km north of the town	28°10'40"N 16°38'40"W	1880
21	Tenerife: Frontón de San Miguel, succulent community (tabaibal) in valley near the village	28°06'40"N 16°36'50"W	730
22	Tenerife: Adeje, rock crevices in Barranco del Infierno	28°08'10"N 16°42'30"W	490
23	La Palma: Caldera de Taburiente, rock crevices near Mirador de los Andenes	28°45'40"N 17°52'00"W	2330
24	La Palma: forest along the road approx. 1 km north-west of Tagoja hill (north-west of Santa Cruz de la Palma city)	28°43'20"N 17°47'10"W	1110
25	La Palma: Caldera de Taburiente, sandy areas approx. 1.5 km north-north-west of the Centro de Visitantes	28°40'00"N 17°51'20"W	950
26	La Palma: Caldera de Taburiente, sandy areas along the road approx. 0.5 km south-east of the Mirador de la Cumbrecita	28°41'40"N 17°51'10"W	1210
27	La Palma: Jedey village, sandy areas along the road SSE of the village	28°34'50"N 17°52'40"W	700
28	La Palma: Caldera de Taburiente, leg. Hanneken (Bot. Garden Berlin)	–	–
29	Gran Canaria: Ayacata, basalt, leg. Roysl (Bot. Garden Berlin)	–	1330
30	Gran Canaria: Moya, Barranco del Laurel, leg. Roysl (Bot. Garden Berlin)	–	600
31	Gran Canaria: Montana de Arucas, north-coast, leg. Roysl (Bot. Garden Berlin)	–	400
32	Gran Canaria: Maspalomas, silicate, leg. Roysl (Bot. Garden Berlin)	–	–
33	Fuerteventura (Bot. Garden Berlin, orig. seeds from Bot. Garden Erlangen)	–	–
34	Lanzarote: Famara, basalt, leg. Roysl (Bot. Garden Berlin)	–	300

polyploid species, at least amongst the endemic elements, would remain strikingly low.

The extensive karyological literature contrasts with the almost complete lack of information about nuclear DNA amounts of Macaronesian plants. Hitherto, only 12 estimates of C-value (the DNA amount in the unrepliated haploid nucleus irrespective of the ploidy level of the taxon) ranging from 0.54 pg in *Aeonium haworthii* to 8.63 pg in *Ranunculus cortusifolius* have been completed (Cerbah *et al.*, 1999; Bennett and Leitch, 2001; Hanson *et al.*, 2001; Ellul *et al.*, 2002). Nuclear DNA amount is undoubtedly a key character, with many uses in various biological fields. It has been employed as an effective tool for distinguishing taxa in several groups of vascular plants, e.g. in *Petunia* (Mishiba *et al.*, 2000) and *Helleborus* (Zonneveld, 2001). Moreover, an analysis of genome size can even reveal new taxa that have so far been neglected (Greilhuber and Speta, 1985). There is increasing demand for C-values as a

phylogenetic markers in evolutionary studies (Leitch *et al.*, 1998). Correlations between nuclear DNA amount and plant phenology (Grime and Mowforth, 1982), life history (Bennett, 1972) or sensitivity to frost (MacGillivray and Grime, 1995) indicate the utilization of C-values as ecological indicators. Although such information was based on 3500+ estimates of angiosperm C-values, further targeted work is essential for better representation of taxonomic groups, geographic regions and plant life forms (Bennett *et al.*, 2000). The principal goal of current work was to improve our knowledge of nuclear DNA amount from a geographic point of view. Macaronesia was chosen as an appropriate region because of the high level of endemism that enabled the comparative study of patterns of nuclear DNA amount variation in a phytogeographically distinct area. Moreover, a conspicuous concentration of plant biota at risk is to be found there; it has been estimated that 41 % of Canarian endemic flora is endangered

(I.U.C.N., 1983). Therefore, there is an urgent need for various biological data applicable to conservation biology (Bennett *et al.*, 2000).

MATERIALS AND METHODS

Plant material

Seeds of most species were collected on the Canary Islands during 2000–2001 (Table 1). The Botanical Garden in Berlin provided seeds of an additional eight species, most of which were also originally sampled in the field. Seeds were germinated on wet filter papers in Petri dishes, and plants were cultivated under the same conditions in a glasshouse at the Experimental Garden of the Institute of Botany in Průhonice, Prague (50°00'N, 14°30'E). Vouchers are kept in the private herbarium of the first author. The great majority of species is currently being grown at the Botanical Garden of the Charles University, Prague.

In total, 104 Macaronesian angiosperms with various distribution patterns were analysed; seven of these were endemic to Macaronesia, 56 endemic to the Canary Islands, 32 endemic to Tenerife, six endemic to La Palma, and the endemics of Lanzarote, Fuerteventura and Gran Canaria were represented by one species. Seven genera (*Allagopappus*, *Dicheranthus*, *Gesnouiina*, *Lugoa*, *Plocama*, *Tinguarra* and *Todaroa*) were endemic to the Canary Islands, and *Argyranthemum*, *Isoplexis*, *Pericallis* and *Schizogyne* are the Macaronesian genera-endemics. The plants were classified into 20 families and 14 orders according to current phylogenetic views (Stevens, 2002), and all but three (*Asparagus umbellatus*, *Dactylis smithii*, *Dracunculus canariensis*) belong to the Eudicots. The vast majority of species was perennial; *Senecio teneriffae* and *Volutaria canariensis* being the only two exceptions. Chamaephytes clearly prevailed from a life-form point of view [the terminology of life-forms follows the categories adopted in the World Checklist and Bibliography Series database at Royal Botanic Gardens, Kew, UK (www.rbkew.org.uk/wcb/index.html)]. Considering the ecological profile of the present species set, representatives of five major zones covering an altitudinal range of more than 2300 m were included.

Flow cytometry

To reveal potential intrapopulation variability in nuclear DNA amount, three to six plants from each species were measured simultaneously in the first step. As one narrow peak with unimodal distribution was always obtained, one individual per species was randomly selected and analysed further. Each plant was measured at least three times on different days by the same operator, and a different leaf was used for each analysis.

Nuclear samples were prepared from a young fresh intact leaf. Approximately 50 mm² of leaf tissue was co-chopped with an appropriate volume of internal standard in 1 ml of ice-cold Otto I Buffer (0.1 M citric acid, 0.5 % Tween 20) using a new razor blade (Otto, 1990). The following internal standards were employed: *Raphanus sativus* L. 'Saxa' (2C

= 1.11 pg), *Lycopersicum esculentum* Mill. 'Stupnické polní tyčkové rané' (2C = 1.96 pg), *Glycine max* (L.) Merrill 'Polanka' (2C = 2.5 pg), *Zea mays* L. 'CE-777' (2C = 5.43 pg) and *Pisum sativum* L. 'Ctirad' (2C = 9.09 pg) (Doležel *et al.*, 1992, 1994; Lysák and Doležel, 1998). Selection of a suitable standard followed these criteria: (1) the smallest ratio between the 2C-values of an analysed plant and the internal standard to minimize the potential non-linearity of flow-cytometer measurements; (2) at least 12 % difference in 2C-values of the internal standard and sample to exclude bias due to very close or overlapping peaks; and (3) the same internal standard was used for all species belonging to one genus. The crude suspension was filtered through a 42-µm nylon filter and centrifuged at 150 g for 5 min. The pellet was resuspended in 100 µl fresh Otto I buffer and samples were incubated for 30 min at room temperature. Subsequently, 1 ml of staining solution Otto II (0.4 M Na₂HPO₄.12H₂O) supplemented with propidium iodide and RNase (both at 50 µg ml⁻¹) was added and, after incubation for 30–45 min at room temperature, fluorescence intensity of isolated nuclei was measured using a Partec PA II flow cytometer (Partec GmbH, Germany) equipped with an argon ion laser (488 nm). The flow rate did not exceed 50 fluorescent events per second in a huge majority of species, and the fluorescence of at least 5000 particles was recorded. Two histograms for each sample were recorded and only when both peaks were symmetrical and of approximately equal height were analyses taken into account when calculating the C-value of an analysed plant. When converting picogram values to base pairs, 980 megabase pairs (Mbp) were assumed to be equivalent to 1 picogram of DNA (Bennett *et al.*, 2000).

Chromosome counts

Observations were made on root tip cells of germinated seedlings. Samples were pre-treated with a saturated solution of p-dichlorobenzene or 1-monobromnaphthalene for 3 h at room temperature, fixed in 3 : 1 ethanol : acetic acid overnight at 4 °C and kept in 70 % ethanol at the same temperature. After maceration in 1 : 1 hydrochloric acid : ethanol for 60 s, the root tip cuttings were squashed in lacto-propionic orceine. As a rule, at least three mitoses per plant and two individuals per species were counted.

Statistical analyses

DNA amount data were analysed with the SAS 8.1 statistical package using ANOVA, CORR, GLM and UNIVARIATE procedures (SAS Institute, Cary, NC, USA). Differences in DNA content between species within a genus were tested by ANOVA, and Tukey's procedure was applied to compare mean values. Differences in C-values between major angiosperm lineages of Macaronesian plants were analysed using the GLM procedure because of an unbalanced design. Differences in C-values and genome sizes between Macaronesian vs. non-Macaronesian representatives were tested using the signed rank test on paired data to avoid problems of data non-linearity. The Spearman-rank correlation coefficient was employed in testing

TABLE 2. 2C nuclear DNA content with standard error, 1C nuclear DNA content in picograms and megabase pairs (1 pg = 980 Mbp), chromosome number (2n), ploidy level, unreplicated genome size, life-form, internal standard used, coefficients of variance of internal standard and the sample, species distribution patterns and original locality for 104 Macaronesian species from 20 families

Taxon	Family	2C DNA		1C DNA		IC DNA amount (pg) ^l	IC DNA amount (Mbp)	2n	Ploidy level (x) ^l /ploidy level	Genome size (2C DNA amount/ (pg) ^l)	Life form ^{**}	Internal standard [‡]	CV of internal standard (%)		Distribution pattern [§]	Locality
		amount ± s.e. (pg)	±	amount (pg)	±								internal standard (%)	CV of sample (%)		
<i>Allagappus dichotomus</i> (L. fil.) Cass.	Asteraceae	1.75 ± 0.01		0.88		862		20	2	0.88	C	G	2.60–3.573.71–4.88		C	14
<i>Argyranthemum adauctum</i> (Link) Humphr. ssp. <i>adauctum</i>	Asteraceae	13.69 ± 0.03		6.84 A		6703		18*	2	6.84	C	P	2.21–2.662.28–2.67		T	7
<i>Argyranthemum adauctum</i> (Link) Humphr. ssp. <i>diugourii</i> (Bolle) Humphr.	Asteraceae	13.77 ± 0.05		6.89 A		6752		18*	2	6.89	C	P	1.67–2.921.89–3.02		T	20
<i>Argyranthemum broussonetii</i> (Pers.) Humphr. ssp. <i>broussonetii</i>	Asteraceae	14.14 ± 0.06		7.07 B		6929		18	2	7.07	C	P	1.99–2.812.26–3.18		T	10
<i>Argyranthemum foeniculaceum</i> (Willd.) Webb ex Sch. Bip.	Asteraceae	14.28 ± 0.05		7.14 B		6997		18	2	7.14	C	P	1.54–2.481.84–2.60		T	17
<i>Argyranthemum frutescens</i> (L.) Sch. Bip. ssp. <i>frutescens</i>	Asteraceae	14.41 ± 0.04		7.21 C		7066		18	2	7.21	C	P	1.83–2.651.84–2.82		C	12
<i>Argyranthemum gracile</i> Sch. Bip.	Asteraceae	14.18 ± 0.06		7.09 B		6948		18	2	7.09	C	P	1.74–2.541.86–2.39		T	21
<i>Argyranthemum hoiaryifolium</i> Humphr. & Bramw.	Asteraceae	13.39 ± 0.08		6.69 D		6556		18*	2	6.69	C	P	1.27–2.231.54–2.64		P	26
<i>Argyranthemum teneriffae</i> Humphr.	Asteraceae	13.94 ± 0.05		6.97 E		6831		18*	2	6.97	H/C	P	1.65–2.251.82–2.39		T	2
<i>Artemisia thusculla</i> Cav.	Asteraceae	11.43 ± 0.06		5.71		5596		18	2	5.71	C	P	2.01–2.762.02–3.29		C	12
<i>Asparagus umbellatus</i> Link	Asparagaceae	2.56 ± 0.03		1.28		1254		20	2	1.28	Cl/NP	L	2.52–3.562.54–3.67		C	16
<i>Bupleurum salsicifolium</i> R. Br. in Buch ssp. <i>aciphyllum</i> (Webb ex Parl.) Sund. & Kunk.	Apiaceae	1.58 ± 0.004		0.79		774		32	4	0.40	C	L	1.84–2.242.50–2.97		C	12
<i>Canarina canariensis</i> (L.) Vatke	Campanulaceae	5.71 ± 0.02		2.85		2793		34	4	1.43	Cl/G	P	1.92–2.861.79–2.83		C	11
<i>Carlina xeranthemoides</i> L. fil.	Asteraceae	7.11 ± 0.06		3.56		3489		20*	2	3.56	C	Z	1.61–2.942.05–3.10		T	2
<i>Ceropegia dichotoma</i> Haw.	Apocynaceae (incl. Asclepiadaceae)	0.89 ± 0.01		0.44 A		431		22	2	0.44	S/H	R	2.79–3.564.06–4.53		C	12
<i>Ceropegia fusca</i> Bolle	Apocynaceae (incl. Asclepiadaceae)	0.86 ± 0.01		0.43 B		421		22	2	0.43	S/H	R	3.38–4.083.49–4.74		C	14
<i>Cheirolophus teydis</i> (Chr. Sm. in Buch) G. López	Asteraceae	1.43 ± 0.02		0.71		696		~30*	≥2	≤0.71	C	L	2.01–2.991.88–3.08		C	3
<i>Convolvulus floridus</i> L. fil.	Convolvulaceae	2.12 ± 0.01		1.06 A		1039		30	6	0.35	NP	G	2.82–3.383.94–4.41		C	19
<i>Convolvulus perraudieri</i> Coss.	Convolvulaceae	2.13 ± 0.01		1.06 A		1039		30*	6	0.35	Cl/NP	G	2.38–3.212.23–2.96		C	16
<i>Crambe arborea</i> Webb ex Christ var. <i>indivisa</i> Svent.	Brassicaceae	1.86 ± 0.01		0.93 A		911		30	6	0.31	C	G	2.90–3.434.11–4.49		T	15
<i>Crambe laevigata</i> DC. ex Christ	Brassicaceae	1.90 ± 0.01		0.95 B		931		~30*	6	0.32	C	G	2.55–3.703.57–3.85		T	16
<i>Crambe scaberrima</i> Webb ex Bramw.	Brassicaceae	1.88 ± 0.01		0.92 A		902		~30*	6	0.31	C	G	2.49–3.392.76–3.73		T	18
<i>Crambe strigosa</i> L. Hér.	Brassicaceae	1.95 ± 0.02		0.99 C		970		30	6	0.33	C	G	2.70–3.762.88–4.53		C	8
<i>Dactylis smithii</i> Link ssp. <i>smithii</i>	Poaceae	4.35 ± 0.02		2.18		2136		14	2	2.18	H	Z	1.95–2.522.08–3.37		C	12
<i>Descurainia bourgeauana</i> (Fourm.) O. E. Schulz	Brassicaceae	0.38 ± 0.01		0.19 A		186		14*	2	0.19	C	R	2.56–3.525.72–8.57		T	1
<i>Descurainia gilva</i> Svent.	Brassicaceae	0.45 ± 0.01		0.22 BC		216		14*	2	0.22	C	R	2.41–3.325.64–7.67		P	23
<i>Descurainia gonzalesii</i> Svent.	Brassicaceae	0.46 ± 0.01		0.23 B		225		14*	2	0.23	C	R	3.23–4.345.43–7.68		T	3
<i>Descurainia lemsii</i> Bramw.	Brassicaceae	0.45 ± 0.01		0.22 BC		216		14	2	0.22	C	R	2.63–2.994.99–6.56		T	6
<i>Descurainia millefolia</i> (Jacq.) Webb & Berth.	Brassicaceae	0.44 ± 0.01		0.22 C		216		14*	2	0.22	C	R	2.68–3.456.07–7.79		C	12
<i>Dichranthus plocamoides</i> Webb	Caryophyllaceae	1.49 ± 0.003		0.75		735		16	2	0.75	C	L	2.84–3.653.60–4.18		C	16
<i>Doryanum eriophthalmum</i> Webb & Berth.	Fabaceae	2.24 ± 0.01		1.12		1098		14	2	1.12	C	G	2.85–3.282.89–3.65		C	22
<i>Dracunculus canariensis</i> Kunth	Araceae	7.90 ± 0.09		3.95		3871		28	4	1.98	G	P	1.84–2.852.10–3.27		C	12
<i>Erigeron calderae</i> Hans.	Asteraceae	3.10 ± 0.01		1.55		1519		18*	2	1.55	H	G	3.27–4.123.49–4.44		T	4

TABLE 2 Continued

Taxon	Family	2C DNA		1C DNA		IC DNA amount (Mbp)	2n	Ploidy level (x) ¹ (ploidy level)	Genome size (2C DNA amount/ (pg) ²)		Life form ³ **standard ³	Internal standard ³ (%)	CV of internal standard (%)	CV of sample (%)	Distribution pattern ⁴	Locality
		amount ± s.e. (pg)	amount (pg) ¹	amount (pg) ¹	amount/ (pg) ²											
<i>Erysimum bicolor</i> (Hornem.) DC.	Brassicaceae	1.16 ± 0.01	0.58 A	568	28	4	0.29	C	L	2.11–3.013.55–4.87	M	29				
<i>Erysimum scoparium</i> (Brouss. ex Willd.) Wettst.	Brassicaceae	1.08 ± 0.02	0.54 B	529	28*	4	0.27	C	L	2.01–3.173.41–4.99	C	2				
<i>Forsydia angustifolia</i> Retz.	Urticaceae	0.64 ± 0.01	0.32	314	20	2	0.32	C	R	2.56–3.033.81–4.87	C	27				
<i>Gesnoinia arborea</i> (L. fil.) Gaud.	Urticaceae	1.02 ± 0.01	0.51	500	20	2	0.51	NP	L	2.70–3.455.54–6.91	C	11				
<i>Hypericum canariense</i> L.	Clusiaceae	1.01 ± 0.01	0.51 A	500	40	4	0.25	NP	R	2.62–3.383.24–4.08	M	30				
<i>Hypericum grandifolium</i> Choisy	Clusiaceae	0.81 ± 0.01	0.41 B	402	40	4	0.20	C	R	2.98–3.483.85–4.84	M	8				
<i>Hypochaeris oligocephala</i> (Svent. & Bramw.) Lack	Asteraceae	2.28 ± 0.02	1.14	1117	6	8	1.14	H	L	2.31–3.162.99–3.55	T	18				
<i>Isoplexis canariensis</i> (L.) Loud.	Plantaginaceae	1.99 ± 0.01	1.00	980	56	8	0.25	C	G	2.43–3.013.85–4.05	C	11				
<i>Lactuca palmensis</i> Bolle	Asteraceae	2.05 ± 0.01	1.03	1009	?	(2)	(1.03)	H	G	2.29–3.203.23–4.01	P	23				
<i>Lavandula buchii</i> Webb var. <i>buchii</i>	Lamiaceae	1.01 ± 0.01	0.51 A	500	22	2	0.51	C	L	2.12–3.543.81–6.76	T	12				
<i>Lavandula multifida</i> L. ssp. <i>canariensis</i> (Mill.) Pit. & Pt.	Lamiaceae	1.02 ± 0.01	0.51 A	500	22	2	0.51	C	L	2.28–3.085.08–5.42	M	31				
<i>Limonium macrophyllum</i> (Brouss.) O. Kuntze	Plumbaginaceae	10.90 ± 0.06	5.45 A	5341	14*	2	5.45	H	P	1.97–2.871.78–2.56	T	12				
<i>Limonium pectinatum</i> (Ait.) O. Kuntze var. <i>pectinatum</i>	Plumbaginaceae	5.23 ± 0.01	2.62 B	2568	12	2	2.62	H	P	1.59–2.272.01–3.02	C	12				
<i>Lobularia canariensis</i> (Webb) Borgen ssp. <i>palmensis</i> (Christ) Borgen	Brassicaceae	1.13 ± 0.01	0.56	549	22	2	0.56	C	L	2.30–3.092.78–4.16	C	26				
<i>Lotus dumetorum</i> Webb ex Murr.	Fabaceae	1.22 ± 0.003	0.61 A	598	14*	2	0.61	H	L	2.28–2.893.60–4.24	T	12				
<i>Lotus campylocladus</i> (Webb & Berth.)	Fabaceae	1.24 ± 0.01	0.62 B	608	14*	2	0.62	H	L	2.25–3.022.21–3.42	T	6				
<i>Lotus glaucus</i> Ait.	Fabaceae	2.48 ± 0.02	1.24 C	1215	28*	4	0.62	H	L	2.03–2.822.09–2.90	M	10				
<i>Lugoia revoluta</i> (Chr. Sm. in Buch) DC.	Asteraceae	11.94 ± 0.01	5.97	5851	18*	2	5.97	H/C	P	2.12–2.952.12–2.87	T	12				
<i>Micromeria glomerata</i> Pérez	Lamiaceae	0.88 ± 0.01	0.44 A	431	?	?	?	C	R	3.65–4.595.18–6.11	T	13				
<i>Micromeria herpptomorpha</i> Webb & Berth.	Lamiaceae	0.76 ± 0.01	0.38 B	372	?	(≥2)	(≤0.38)	C	R	3.29–3.995.11–6.35	P	25				
<i>Micromeria hyssopifolia</i> Webb & Berth. var. <i>hyssopifolia</i>	Lamiaceae	0.72 ± 0.01	0.36 C	353	?	(≥2)	(≤0.36)	C	R	3.19–4.124.32–5.77	C	8				
<i>Micromeria lachnophylla</i> Webb & Berth.	Lamiaceae	0.74 ± 0.01	0.37 CD	363	30*	≥2	≤0.37	C	R	3.20–4.084.48–5.84	T	5				
<i>Micromeria varia</i> Benth ssp. <i>varia</i>	Lamiaceae	0.75 ± 0.01	0.37 BD	363	30	≥2	≤0.37	C	R	3.20–4.214.43–5.90	C	12				
<i>Nauplius sericeus</i> (L. fil.) Cass.	Asteraceae	1.72 ± 0.01	0.86	843	14*	2	0.86	C	G	2.52–3.342.98–3.54	F	33				
<i>Nepeta teydea</i> Webb. & Berth.	Lamiaceae	0.55 ± 0.01	0.27	265	16	2	0.27	H	R	2.65–3.493.67–4.87	C	1				
<i>Paronychia canariensis</i> (L. fil.) Juss.	Caryophyllaceae	2.68 ± 0.02	1.34	1313	32	4	0.67	C	L	2.45–2.872.27–2.91	C	12				
<i>Pericallis appendiculata</i> (L. fil.) B. Nord.	Asteraceae	1.09 ± 0.01	0.55 A	539	60	6	0.18	C	L	2.82–4.414.89–7.13	C	11				
<i>Pericallis cruenta</i> (L. fil.) Bolle	Asteraceae	1.36 ± 0.01	0.68 B	666	60	6	0.23	H	L	2.40–3.254.12–4.94	C	7				
<i>Pericallis echinata</i> (L. fil.) B. Nord.	Asteraceae	1.46 ± 0.02	0.73 C	715	60	6	0.24	H	L	2.24–3.253.97–4.52	T	9				
<i>Pericallis lanata</i> (L. fil.) B. Nord.	Asteraceae	1.15 ± 0.004	0.57 D	559	60	6	0.19	C	L	2.32–2.673.00–4.85	T	16				
<i>Pericallis papyracea</i> (DC.) B. Nord.	Asteraceae	1.20 ± 0.01	0.60 E	588	60	6	0.20	H	L	2.46–3.003.93–5.36	P	24				
<i>Pericallis webbii</i> (Sch. Bip.) Bolle	Asteraceae	1.14 ± 0.01	0.57 D	559	60	6	0.19	H	L	2.42–3.134.53–5.51	G	31				
<i>Periploca laevigata</i> Ait.	Apocynaceae	1.00 ± 0.01	0.50	490	22	2	0.50	Cl NP	L	2.51–2.944.72–5.57	C	14				
<i>Phagnalon umbelliforme</i> DC.	(incl. Asclepiadaceae)	2.19 ± 0.03	1.09	1068	18	2	1.09	C	G	2.49–3.282.76–3.49	C	14				
<i>Pimpinella cumbrae</i> Link	Apiaceae	4.60 ± 0.05	2.30	2254	20	2	2.30	H	Z	1.87–3.132.45–3.51	C	3				
<i>Plantago arborescens</i> Poir. ssp. <i>arborescens</i> var. <i>arborescens</i>	Plantaginaceae	0.97 ± 0.01	0.48 A	470	12	2	0.48	C	L	2.18–2.853.73–4.96	C	10				
<i>Plantago famaræ</i> Svent.	Plantaginaceae	1.00 ± 0.01	0.50 B	490	12	2	0.50	C	L	2.29–2.993.86–4.94	L	34				
<i>Plantago webbii</i> Barn.	Plantaginaceae	1.11 ± 0.01	0.56 C	549	12*	2	0.56	C	L	2.26–2.993.32–5.00	C	23				
<i>Plocama pendula</i> Ait.	Rubiaceae	2.79 ± 0.01	1.39	1362	44	4	0.70	NP	G	2.53–2.912.38–3.39	C	19				
<i>Polycarpha aristata</i> (Ait.) DC.	Caryophyllaceae	0.93 ± 0.01	0.47 A	461	?	(2)	(0.47)	H/C	R	2.76–3.332.94–3.56	C	8				

TABLE 2 Continued

Taxon	Family	2C DNA amount \pm s.e. (pg)	1C DNA amount (pg) [†]	IC DNA amount (Mbp)	2n	Ploidy level (x) [†] /ploidy level)	Genome size (2C DNA amount/ (pg) [†])	Life form ^{**}	Internal standard [‡] (%)	CV of internal standard (%)	CV of sample (%)	Distribution pattern [§]	Locality
<i>Polycarpha latifolia</i> Willd.	Caryophyllaceae	0.89 \pm 0.003	0.44 B	431	18	2	0.44	H	R	2.77–3.192.68–3.03		C	10
<i>Polycarpha smithii</i> Link	Caryophyllaceae	1.09 \pm 0.01	0.54 C	529	?	?	?	H	L	2.55–3.683.03–5.11		C	28
<i>Pterocarpus tenuis</i> Webb ex Christ	Caryophyllaceae	0.91 \pm 0.003	0.45 D	441	18 ^{†*}	2	0.45	H/C	R	2.78–3.202.67–3.38		C	5
<i>Pterocarpus dumetorum</i> (Brouss. ex Willd.) Coult.	Dipsacaceae	3.56 \pm 0.01	1.78	1744	18 ^{†*}	2	1.78	C	G	2.34–3.322.44–2.68		C	15
<i>Reichardia ligulata</i> (Vent.) Kunk. & Sund.	Asteraceae	3.78 \pm 0.01	1.89	1852	16 ^{†*}	2	1.89	H	G	2.06–3.081.75–2.66		C	12
<i>Rumex lanaria</i> L.	Polygonaceae	12.47 \pm 0.09	6.23 A	6105	36	4	3.12	C	P	2.92–3.842.17–3.94		C	12
<i>Rumex maderensis</i> Lowe	Polygonaceae	1.38 \pm 0.01	0.69 B	676	20	2	0.69	H	L	2.49–3.053.45–4.69		M	7
<i>Salvia broussonetii</i> Benth	Lamiaceae	0.86 \pm 0.01	0.43	421	22	2	0.43	C	R	3.07–4.023.55–5.55		T	16
<i>Scrophularia glabrata</i> Ait.	Scrophulariaceae	2.06 \pm 0.01	1.03 A	1009	56	8	0.26	H	G	3.10–4.024.10–4.62		T	2
<i>Scrophularia smithii</i> Hornem. ssp. <i>smithii</i>	Scrophulariaceae	2.08 \pm 0.01	1.04 B	1019	58	8	0.26	H	G	2.80–3.422.91–3.69		T	10
<i>Senecio palmensis</i> (Chr. Sm. in Buch) Link	Asteraceae	1.96 \pm 0.01	0.98 A	960	20 ^{†*}	2	0.98	C	G	2.50–3.032.80–3.23		C	23
<i>Senecio teneriffae</i> Schultz Bip.	Asteraceae	5.26 \pm 0.02	2.63 B	2577	60	6	0.88	T	P	1.64–2.372.83–3.46		C	7
<i>Seseli webbit</i> Coss.	Apiaceae	3.79 \pm 0.02	1.89	1852	22	2	1.89	H	G	2.86–3.252.56–2.93		C	12
<i>Schizogyne glaberrima</i> DC.	Asteraceae	2.05 \pm 0.01	1.02 A	1000	18	2	1.02	C	G	2.54–3.002.98–3.69		C	32
<i>Schizogyne sericea</i> (L. fil.) DC.	Asteraceae	2.05 \pm 0.01	1.02 A	1000	18	2	1.02	C	G	2.52–3.233.09–3.74		M	12
<i>Sideritis canariensis</i> L.	Lamiaceae	3.56 \pm 0.01	1.78 A	1744	44	4	0.89	C	G	3.25–4.072.45–3.03		C	26
<i>Sideritis macrostachys</i> Poir.	Lamiaceae	4.03 \pm 0.03	2.01 B	1970	~36 ^{†*}	4	1.01	C	G	2.98–4.782.80–4.40		T	12
<i>Sideritis oroteneriffae</i> Negrin & Pérez var. <i>oroteneriffae</i>	Lamiaceae	3.65 \pm 0.01	1.82 C	1784	44	4	0.91	C	G	2.59–3.732.29–3.88		T	3
<i>Silene berthelotiana</i> Webb	Caryophyllaceae	5.11 \pm 0.04	2.55 A	2499	24 ^{†*}	2	2.55	H	P	1.77–2.842.04–3.44		C	6
<i>Silene lagunensis</i> Chr. Sm. ex Christ	Caryophyllaceae	5.19 \pm 0.01	2.59 BC	2538	24 ^{†*}	2	2.59	H	P	1.75–2.372.04–3.30		T	12
<i>Silene nocteolens</i> Webb & Berth.	Caryophyllaceae	5.16 \pm 0.01	2.58 C	2528	24 ^{†*}	2	2.58	H	P	1.86–2.802.11–3.40		T	5
<i>Silene pagonicalyx</i> (Svent.) Bramw.	Caryophyllaceae	5.23 \pm 0.01	2.61 B	2558	24 ^{†*}	2	2.61	H	P	2.15–3.122.73–3.84		P	23
<i>Sonchus acaulis</i> Dum.-Cours.	Asteraceae	2.86 \pm 0.03	1.43 A	1401	18	2	1.43	H	L	2.90–3.652.59–3.14		C	8
<i>Sonchus congestus</i> Willd.	Asteraceae	2.87 \pm 0.02	1.44 B	1411	18	2	1.44	C	L	2.80–3.092.57–3.11		C	9
<i>Sonchus radicans</i> Ait.	Asteraceae	2.62 \pm 0.02	1.31 C	1284	18 ^{†*}	2	1.31	H	L	2.24–3.042.33–3.06		T	12
<i>Teline canariensis</i> (L.) Webb. & Berth.	Fabaceae	3.00 \pm 0.03	1.50	1470	48 ^{†*}	4	0.75	NP	G	3.32–4.264.21–4.63		C	12
<i>Tinguarra montana</i> (Webb ex Christ) A. Hans. & Kunk.	Apiaceae	2.45 \pm 0.02	1.22	1196	22	2	1.22	H	L	2.88–3.812.54–3.89		C	6
<i>Todaroa aurea</i> Parl.	Apiaceae	2.87 \pm 0.01	1.44	1411	22	2	1.44	H	L	2.72–3.632.08–2.71		C	27
<i>Tolpis laciniata</i> (Sch. Bip. ex Webb & Berth.) Webb	Asteraceae	2.66 \pm 0.03	1.33 A	1303	18	2	1.33	H	L	2.50–3.081.86–2.46		C	25
<i>Tolpis webbit</i> Sch. Bip. ex Webb & Berth.	Asteraceae	2.75 \pm 0.01	1.37 B	1343	18 ^{†*}	2	1.37	H	L	2.55–3.072.42–3.11		T	1
<i>Volularia canariensis</i> Wagenitz	Asteraceae	1.60 \pm 0.01	0.80	784	32	4	0.40	T	L	2.81–3.132.62–3.62		C	14

* Chromosome numbers determined in the present work; †, new species record. All other counts were taken from literature.

† An assumed ploidy level and genome size given in parentheses.

‡ G, *Glycine max* 'Polanka' (2C = 2.5 pg); L, *Lycopersicon esculentum* 'Stupnické polní tyčkové rané' (2C = 1.96 pg); P, *Pisum sativum* 'Citrad' (2C = 9.09 pg); R, *Raphanus sativus* 'Saxa' (2C = 1.11 pg); Z, *Zea mays* 'CE-777' (2C = 5.43 pg).

§ C, Canary Islands; F, Fuerteventura; G, Gran Canaria; L, Lanzarote; M, Macaronesia; P, La Palma; T, Tenerife.

¶ Letters indicate group of taxa within the same genus that are not significantly different at $\alpha = 0.05$.

** C, Chamaephyte; CI G, climbing geophyte; CI NP, climbing nanophanerophyte; G, geophyte; H, hemicyptophyte; NP, nanophanerophyte; S H, succulent hemicyptophyte; T, therophyte.

TABLE 3. Selected descriptive statistics of IC-value sets for major phylogenetic lineages of Macaronesian (M; estimated here) and non-Macaronesian angiosperms (non-M; from Bennett and Leitch, 2001)

Phylogenetic lineage	Eudicots		Monocots		Basal eudicots		Eurosid I		Eurosid II		Euasterids I		Euasterids II	
	M (n = 101)	non-M (n = 1929)	M (n = 3)	non-M (n = 1487)	M (n = 14)	non-M (n = 207)	M (n = 9)	non-M (n = 721)	M (n = 12)	non-M (n = 152)	M (n = 24)	non-M (n = 280)	M (n = 42)	non-M (n = 357)
Mean	1.69	3.27	2.47	10.5	2.09	6.12	0.76	2.3	0.55	1.38	0.78	2.21	2.61	3.95
Minimum	0.19	0.05	1.28	0.15	0.44	0.25	0.32	0.1	0.19	0.05	0.27	0.33	0.55	0.4
25 % quartile	0.51	0.85	1.73	2.5	0.58	0.72	0.51	0.65	0.22	0.55	0.43	0.9	0.90	1.85
Median	0.99	1.65	2.18	5.8	1.95	2.1	0.61	1.15	0.55	1.12	0.51	1.4	1.4	3.05
75 % quartile	1.78	4.15	3.07	13.94	2.61	9.68	1.12	2.35	0.92	1.76	1.05	2.84	3.38	5.1
Maximum	7.21	79.33	3.95	127.4	6.23	79.33	1.5	27.4	0.99	8.7	2.01	15.3	7.21	24.83
Mode	0.51	0.7	—	0.95	—	0.48	0.51	0.55	0.22	0.58	0.37	0.85	0.57	2.9

All values in picograms.

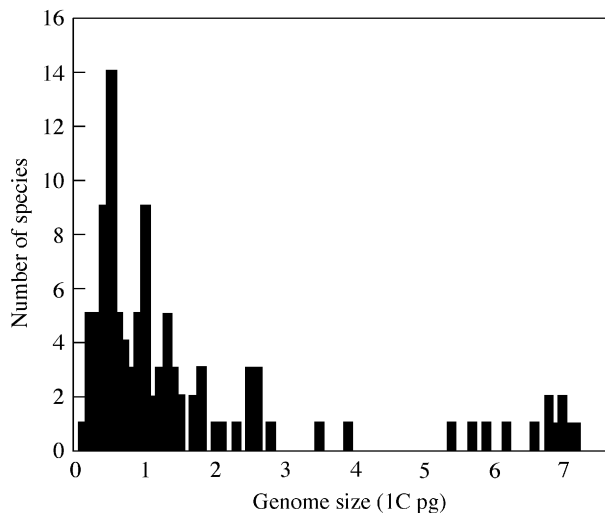


FIG. 1. 1C DNA amount distribution (pg means) of 104 Macaronesian species investigated.

whether infrageneric DNA amount variation (mean values for individual species) correlated with the altitude and environmental characteristics (climatic data were taken from Fernandopullé, 1976).

RESULTS

Chromosome counts

Table 2 gives a chromosome count or an estimated number for 35 Macaronesian angiosperms; eight of these represent new species records. Special attention was paid to taxa from the supracanarian zone (generally above 2000 m altitude). The chromosome counts for 63 other taxa were taken from the literature, and the number of chromosomes for six species is apparently unknown.

An analysis of ploidy level revealed the following: diploids, 63 taxa; tetraploids, 16 taxa; hexaploids, 13 taxa; and octoploids, three taxa. The proportion of polyploid plants was therefore 33.7%. Despite usually being regarded as diploids, *Crambe* species were included amongst the hexaploids on the basis of comparative genome mapping results (Leitch and Bennett, 1997). Three taxa with a basic chromosome number higher than 13 were excluded from the comparison as it was uncertain whether they were diploid or polyploid (Grant, 1971), and the omission also applied to six species which lacked an exact chromosome count.

Nuclear DNA amounts

Table 2 shows 2C DNA content (pg) and standard error, 1C DNA content expressed in picograms and megabase pairs, number of chromosomes and ploidy level, genome size (calculated as 2C DNA value/ploidy level), life-form, internal standard used, coefficients of variance (CV) of both the internal standard and plant analysed, species distribution pattern and original locality for 104 taxa from 20 families. Prime C-values represent 98% of the estimates in the

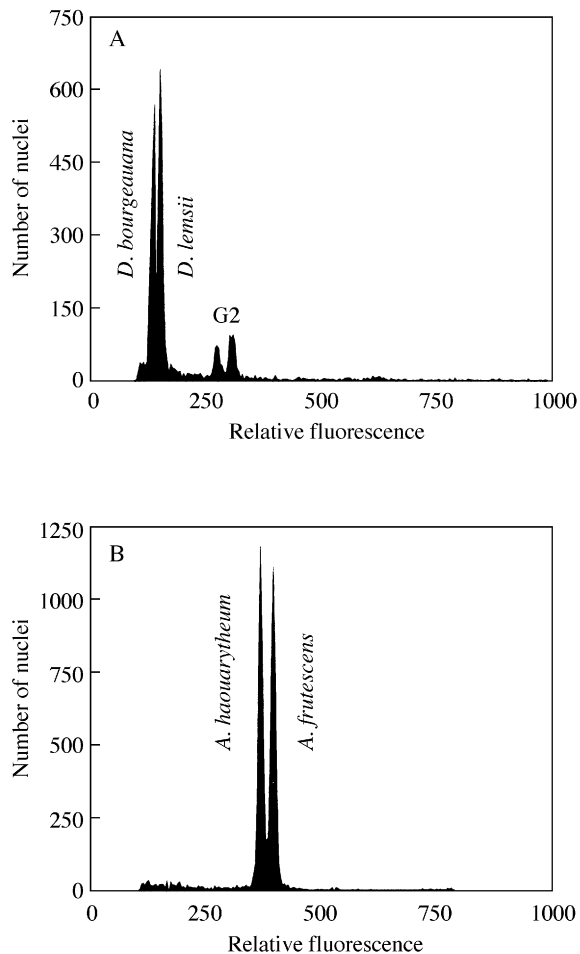


FIG. 2. Flow-cytometric histograms showing the difference in 2C nuclear DNA content for species with the smallest DNA amounts, *Descurainia bourgeauana* and *D. lemsii* (A), and species with the highest DNA amounts, *Argyranthemum haouarytheum* and *A. frutescens* (B). Nuclei of both species in the genus were isolated and stained with propidium iodide simultaneously. Small G2 peaks represent the nuclei of *Descurainia* with doubled (4C) nuclear DNA content.

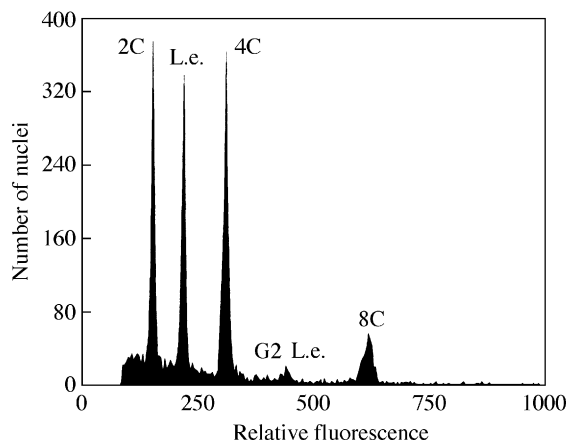


FIG. 3. Flow-cytometric histogram obtained after simultaneous analysis of propidium iodide-stained nuclei of *Lycopersicon esculentum* (L.e.; internal standard) and *Rumex maderensis*. Occurrence of nuclei with 2C, 4C and 8C DNA amounts indicates endoreduplication.

present species set; only *Hypochoeris oligocephala* and *Plocama pendula* have been analysed previously.

Flow-cytometric measurements yielded histograms with CV values ranging from 1.27 to 4.78 % (mean 2.85 %) for internal standards, and 1.54 to 8.57 % (mean 3.57 %) for analysed plants, depending on the taxon, DNA amount and the quality of sample preparation. A CV value not exceeding the arbitrary level of 3 % was achieved in 69 and 47 % of internal standard and analysed plant acquisitions, respectively. The standard error of the mean described the difference in 2C-values between individual runs of the same species caused by cytometer instability, non-identical sample preparation, etc. Values smaller than 1 % of the plant 2C DNA amount were attained in 82 % of taxa; the limit of 2 % was never been exceeded. Therefore, the data on nuclear DNA content can be regarded as reliable.

1C nuclear DNA contents obtained in the present study ranged from 0.19 pg in *Descurainia bourgeauana* to 7.21 pg in *Argyranthemum frutescens*, representing a difference of approx. 38-fold (Table 2; Fig. 1). However, the majority of species (71 %) fell into the lower part of the range, with C-values between 0.2 and 1.6 pg. Genome sizes had a range very similar to that of 1C DNA content, with hexaploid *Pericallis appendiculata* having the smallest genome (0.18 pg). The highest genome size was found in diploid *Argyranthemum frutescens*. Comparison of mean values for Macaronesian vs. non-Macaronesian (taken from Bennett and Leitch, 2001) representatives of individual genera and families affirmed the tendency towards small C-values and genome sizes in Macaronesian taxa (data not shown). At the rank of family, both C-values and genome sizes of endemic plants were significantly smaller ($P < 0.01$, $n = 20$) than those of non-Macaronesian taxa, and the comparisons at the generic level brought identical results ($P = 0.017$, $n = 22$). *Artemisia*, *Reichardia* and *Rumex* were the most prominent exceptions, with Macaronesian representatives having larger C-values. Although species from 56 genera were included in the study, only 22 genera were employed in the foregoing comparison as no previous C-value data were available for the remaining 34 genera. Small nuclear DNA amounts in Macaronesian native flora were also confirmed in data sets taking the phylogenetic position of the species into account. Selected descriptive statistics for major angiosperm lineages clearly indicate that non-Macaronesian plants possessed larger C-values than their Macaronesian counterparts (Table 3). Macaronesian species of particular interest with very low DNA amounts include *Nepeta teydea* (1C = 0.27 pg; the smallest C-value among Euasterids II and even the Asterids as a whole), *Micromeria* spp. (1C = 0.36–0.44 pg; very small C-values in Euasterids II) and *Pericallis appendiculata* (1C = 0.55 pg; the second smallest C-value in Euasterids I). Average 1C nuclear DNA amounts \pm s.d. for main phylogenetic lineages of Macaronesian plants were as follows: Eurosids II, 0.55 ± 0.33 pg; Eurosids I, 0.76 ± 0.42 pg; Euasterids I, 0.78 ± 0.52 pg; Basal Eudicots, 2.09 ± 1.85 pg; Monocots, 2.47 ± 1.36 pg; Euasterids II, 2.61 ± 2.44 pg. Apparently, there are some differences in mean C-values (one group with 1C < 1 pg, the other with 1C > 2 pg); however, only Eurosids II/Euasterids II and Euasterids I/Euasterids II

differed significantly. Somatic tissues of five taxa (*Ceropegia dichotoma*, *C. fusca*, *Dicheranthus plocamoides*, *Polycarpha smithii* and *Rumex maderensis*) underwent endoreduplication, and three peaks corresponding to nuclei with 2C, 4C and 8C DNA content were observed. Flow histograms of particular interest are shown in Figs 2 and 3.

Correlation of C-value with environmental conditions

Correlations between C-value and altitude, average annual temperature, humidity and rainfall were calculated in genera where at least three species from Tenerife were available. The nuclear DNA amount in *Argyranthemum* was negatively correlated with altitude ($r = -0.806$, $P < 0.0001$, $n = 8$) and annual rainfall ($r = -0.783$, $P < 0.0001$, $n = 8$), and positively correlated with mean annual temperature ($r = 0.704$, $P < 0.0001$, $n = 8$). The number of species in other genera was too small to permit meaningful comparisons so the results were treated only as tendencies. The DNA amount in *Silene* and *Micromeria* followed the same trends as in *Argyranthemum* (with the exception of a non-significant correlation with altitude). A completely different pattern of variation was observed in *Crambe* and *Sonchus* where nuclear DNA content increased with higher mean altitude and rainfall, and decreased with average annual temperature. The DNA amount in *Pericallis* was negatively correlated with relative humidity ($r = -0.616$, $P = 0.0005$, $n = 4$). However, this relationship may be biased to a certain extent as the genus comprised species of two growth-forms (chamaephytes *P. appendiculata* and *P. lanata* possessed significantly smaller genomes than hemicryptophytes *P. echinata* and *P. cruenta*).

DISCUSSION

Nuclear DNA amounts for 104 Macaronesian angiosperms from 56 genera and 20 families were estimated in this study. Species from this phytogeographic region have been under-represented in previous investigations of nuclear DNA amount, and only 12 estimates were available (Cerbah *et al.*, 1999; Bennett and Leitch, 2001; Hanson *et al.*, 2001; Ellul *et al.*, 2002). The present paper therefore increases knowledge of nuclear DNA content in Macaronesian angiosperms by almost nine times. Thirty-four of the 56 genera (60.7 %) were previously without C-values and the present estimates thus represent the first data on nuclear DNA content. A huge majority (93 %) of taxa analysed was restricted to the Canary Islands. A comparison with recent figures on Canarian flora (Francisco-Ortega *et al.*, 2000) indicates that information on C-value and genome size for more than one-sixth (approx. 17.9 %) of Canarian endemics was obtained. It should, however, be emphasized that there are significant differences concerning both the total number and the proportion of endemic plants given by various authors. Although Macaronesia lacks any endemic family, more than 30 endemic genera can be found there (Bramwell, 1976; Kunkel, 1993). This study encompassed species from 11 endemic genera, thus representing about one-third of generic coverage.

The overall 1C-values obtained here varied from 0.19 pg in *Descurainia bourgeauana* to 7.21 pg in *Argyranthemum frutescens* (about 38-fold difference). Nuclear DNA amounts for 12 Macaronesian angiosperms published previously differed approx. 16-fold, from 0.54 pg in the Tenerife-endemic *Aeonium haworthii* to 8.63 pg in the Macaronesian *Ranunculus cortusifolius*. By merging both lists, we can conclude that C-values of Macaronesian vascular plants currently differ at least 45-fold. This is a relatively narrow range compared with the approx. 1000-fold variation in angiosperms as a whole (Bennett *et al.*, 2000). However, it should be noted that very few Macaronesian monocots have been studied and there is a strong possibility that the inclusion of additional taxa, e.g. from *Liliaceae* s.l., would extend the C-value range. Two species (*Hypochoeris oligocephala*, *Plocama pendula*) from our collection have been analysed by previous authors (Bennett and Leitch, 1995; Cerbah *et al.*, 1999). Both values were similar and differed by only 3.5 % in the former and by 4.3 % in the latter taxon, with our estimates being negligibly smaller.

The majority of taxa analysed here possessed small genomes, with 1C nuclear DNA amounts less than 1.6 pg. They fall into the lowest third of the C-value database comprising about 3500 records of flowering plants (Bennett and Leitch, 2001). The estimates for *Descurainia* (0.19–0.23 pg) even approached the minimum known for the angiosperms. 1C DNA amount for *Nepeta teydea* represented the smallest value in the Asterids, and *Pericallis appendiculata* had the second smallest C-value in Euasterids II (only *Leontodon longirostris* possessed a smaller 1C nuclear DNA amount). Comparisons of average C-values and genome sizes for Macaronesian vs. non-Macaronesian representatives of individual genera and families confirmed the significantly smaller estimates for the former group. Obvious differences in C-values were also found when matching data for major phylogenetic lineages of Macaronesian and non-Macaronesian taxa (e.g. mean and median for the Macaronesian Eudicots were 1.93 and 1.67 times smaller, respectively).

Leitch *et al.* (1998) analysed angiosperm C-values in a phylogenetic context and concluded that ancestral taxa probably possessed small genomes with $1C \leq 3.5$ pg. A considerable proportion (86.5 %) of Macaronesian plants meet this criterion, and more than two-thirds of them had very small genomes (defined as $1C \leq 1.4$ pg). These findings would support the relictual nature of Macaronesian flora (Bramwell, 1976). However, recent phylogenetic investigations of several endemic groups (e.g. *Aeonium*, *Argyranthemum*, *Bencomia*, *Echium*) revealed the derived position of Macaronesian representatives indicating their recent origin (Emerson, 2002). In most cases, there has been only a single colonization of the archipelago (Francisco-Ortega *et al.*, 1997; Barber *et al.*, 2000; Helfgott *et al.*, 2000) followed by a rapid speciation. Two theories may explain the smaller C-values of Macaronesian taxa in comparison with their non-Macaronesian counterparts: (1) there has been a loss of DNA since the archipelago was colonized; (2) ancestral species possessed small genomes and only negligible changes have occurred during subsequent

speciation processes. Chromosomal surveys on both oceanic and more continental islands revealed chromosomal stasis even though speciation has often been accompanied by striking morphological divergence (Stuessy and Crawford, 1998). Data obtained in the present study support a working hypothesis that small C-values are an evolutionary advantage under insular selection pressures. However, further targeted work is essential to test this theory.

Although several molecular analyses of Macaronesian angiosperms have been published, meaningful comparison between the phylogenetic position and nuclear DNA content is possible only in a few cases. In the genus *Sideritis*, two major clades were identified (Barber *et al.*, 2000): one comprised *S. macrostachys* (1C = 2.01 pg) and *S. roteneriffae* (1C = 1.82 pg); the other *S. canariensis* from La Palma (1C = 1.78 pg). C-values rather reflected the sectional classification: *S. macrostachys* (sect. *Creticae*) had a substantially larger genome than the two remaining species belonging to sect. *Marrubiastrum*. Nuclear DNA content variation that parallels the taxonomic classification is also known in the genus *Pinus*, for example (Hall *et al.*, 2000). Six *Pericallis* species from the present study occupied four clades on the phylogenetic tree based on ITS sequences (Panero *et al.*, 1999). *P. cruenta* (Tenerife, 1C = 0.68 pg), *P. echinata* (Tenerife, 1C = 0.73 pg) and *P. papyraceae* (La Palma, 1C = 0.6 pg) were grouped together, and *P. webbii* (Gran Canaria, 1C = 0.57 pg), *P. lanata* (Tenerife, 1C = 0.57 pg) and *P. appendiculata* (Tenerife, 1C = 0.55 pg) were each situated on a different branch (the position of the latter two species with woody stems was closer). Along with higher C-values for hemicryptophytes, the present data also indicate that there could be an inter-island differentiation in 2C-values between species of the same life form. Similar traces of inter-island diversification can also be found in other genera (e.g. *Argyranthemum*), and this question merits further study.

An increasing number of papers have investigated correlations between DNA content and environmental conditions. Altitude, average annual temperature, humidity and rainfall on Tenerife were included as environmental variables in the present study. Positive correlation between nuclear DNA amount and mean annual temperature was found in *Argyranthemum*; C-values in this genus were negatively correlated with altitude and annual rainfall. The same trends were also observed in *Silene* and *Micromeria*. Presented results are in accordance with the pattern of DNA amount variation in *Berberis*, where diploids with lower DNA content grew in high-elevation habitats with greater rainfall, and species with higher DNA content preferred sites with higher temperatures (Bottini *et al.*, 2000). However, the nuclear DNA content divergence in *Crambe* and *Sonchus* followed a completely opposite trend and their C-values increased with altitude and rainfall and decreased with temperature. As concluded by Grime and Mowforth (1982), large genomes in British flora have probably evolved under low temperature conditions. In the last group of genera (*Descurainia*, *Polycarpaea*), no correlation with environmental traits was found, as previously noted e.g. in the genus *Lonchocarpus* (Palomino and Sousa, 2000).

Taken together, these data suggest that the nuclear DNA amount of Macaronesian endemics on Tenerife was negatively correlated with altitude in genera distributed over a large altitudinal range (from lowland to supracanarian zone); on the contrary, genera with a limited range in altitude (from lowland to laurel forests) showed a positive correlation between DNA content and altitude. However, more data are required for reliable conclusions.

The chromosome number in somatic cells was counted in about one-third of the species; eight counts represented new species records. Almost all the present counts agreed with one or more previously published values for the same taxon. The only exception was *Descurainia gonzalesii* where a diploid number with 14 chromosomes was ascertained. Borgen (1969) and Bramwell (1977) observed 21 (= 3x) and 28 (= 4x) chromosomes in somatic cells, respectively. All records might be correct since the species could comprise diploid, triploid and tetraploid individuals. Seeds from two different localities were sown in the present study but only one batch of seeds germinated successfully (non-viable seeds from the other site might have come from triploid plants). Borgen (1969) also reported the tetraploid number (2n = 44) for *Ceropegia fusca*. Flow-cytometric histograms revealed endoreduplication in somatic tissues of this species, and it is likely that cells with doubled chromosome number were counted in Borgen's study. Endopolyploidization also occurred in four other Macaronesian taxa; the (sub)succulent leaves were a common feature in all of them. Their low nuclear DNA amounts support the hypothesis that somatic polyploidization is a general property of succulents that have small genomes (De Rocher *et al.*, 1990).

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