Annals of Botany **92**: 21–29, 2003
doi:10.1093/aob/mcg105, available online at www.aob.oupjournals.org

Analysis of Nuclear DNA Content in *Capsicum* (Solanaceae) by Flow Cytometry and Feulgen Densitometry

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Received: 11 September 2002 Returned for revision: 6 January 2003 Accepted: 4 March 2003

Flow cytometric measurements of nuclear DNA content were performed using ethidium bromide as the DNA stain (internal standard, *Hordeum vulgare* 'Ditta', 1C = 5·063 pg) in 25 samples belonging to nine diploid species and four varieties of *Capsicum*: *C. chacoense*, *C. parvifolium*, *C. frutescens*, *C. chinense*, *C. annuum* var. *annuum*, *C. baccatum* var. *baccatum*, *C. baccatum* var. *pendulum*, *C. baccatum* var. *umbilicatum*, *C. eximium* and *C. pubescens*, all with $2n = 24$, and *C. campylopodium* with $2n = 26$. In addition, one sample each of *C. annuum* var. *annuum* and *C. pubescens* were also analysed using Feulgen densitometry (standard, *Allium cepa* 'Stuttgarter Riesen', 1C = 16·75 pg). Both staining methods resulted in very similar relative values. Genome size displays significant variation between but not within species (except in *C. campylopodium*), and contributes to their taxonomic grouping. 1C-values range from 3·34–3·43 pg (3273–3361 Mbp) in *C. chacoense* and the *C. annuum* complex to 4·53–5·77 pg (4439–5655 Mbp) in *C. campylopodium* and *C. parvifolium*. The data obtained support conclusions on phylogenetic relationships in the genus derived from karyotype analyses using chromosome banding approaches. In *Capsicum*, constitutive heterochromatin amount is correlated with genome size, except in *C. parvifolium*, and is regarded as an additive genomic component.

Key words: Solanaceae, *Capsicum*, genome size variation, flow cytometry, Feulgen densitometry, karyosystematics, karyotype evolution.

INTRODUCTION

Capsicum is an economically important genus in the Solanaceae, encompassing around 25 species native to tropical and temperate regions of the Americas. Five of its members (*C. annuum* L., *C. frutescens* L., *C. chinense* Jacq., *C. baccatum* L., and *C. pubescens* R. et P.) were domesticated by American Indians and were exploited on a global scale after Columbus owing to their valuable fruits, with pungent fruits being used as spices ('ajies', 'chillies', 'peppers') and sweet ones as vegetables ('sweet pepper'). Furthermore, the genus has medical and ornamental uses (cf. Hunziker, 1979; Pickersgill, 1991; Eshbaugh, 1993; Heiser, 1995).

Although the taxonomy of *Capsicum* has been studied by Hunziker (1950, 1956, 1998) and Heiser and Smith (1953) and, more recently, by Eshbaugh (1979) and Pickersgill *et al.* (1979), a complete treatment is still lacking. The classic taxonomical studies rely mainly on flower rather than fruit traits, as the latter are highly variable below the species level owing to human selective pressure (Pickersgill, 1988). In particular, corolla colour has been utilized to characterize in a practical way the cultivated species and their wild relatives, which are subdivided into a 'white-' and a 'purple-flowered group'. However, this grouping becomes inadequate to describe the flower colour variation found in more distant wild species and the genus as a whole.

The cytogenetics of domesticated taxa of *Capsicum* and their possible wild ancestors has received attention from plant breeders (cf. Lippert *et al.*, 1966; Pickersgill, 1991). The success of interspecific crosses, which is related to the genomic homology of the parental species, could allow genetic improvement of cultivars by introducing valuable alleles, such as those responsible for pathogen resistance, from the wild entities (cf. Boukema, 1980, 1983; Boiteux *et al.*, 1993). In this sense, a broad karyological knowledge of the genus will be useful for hybridization and biotechnological approaches, including transformation.

Chromosome data recorded for more than half of the species highlight the universal presence of diploid complements in the genus and two basic chromosome numbers, $x = 12$ and $x = 13$, the latter being restricted to few wild species (Moscone *et al.*, 1995, and references therein). Karyotype studies using classical staining procedures were hampered by chromosome uniformity. Nevertheless, application of Giemsa C- and fluorochrome-banding techniques to reveal constitutive heterochromatic regions, and silver staining to detect active nucleolus organizing regions (AgNORs), has brought useful markers for chromosome identification and allowed more refined karyosystematic analyses in *Capsicum* (Moscone *et al.*, 1993*a*, 1995, 1996).

Nuclear DNA content is a specific karyological feature that is very useful for systematic purposes and evolutionary

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considerations (Bennett and Leitch, 1995). However, there are no extensive DNA amount measurements for *Capsicum*. With the exception of *C. annuum*, which has received more attention, just one or two samples of each cultivated species and *C. eximium* Hunz. have been analysed by Feulgen densitometry using *Allium cepa* L. as the internal standard (Bennett and Smith, 1976; Mukherjee and Sharma, 1990). One to several accessions belonging to the cultivated species and three wild ones were examined by flow cytometry using mithramycin (MI) (Galbraith *et al*., 1983) and propidium iodide (PI) (Arumuganathan and Earle, 1991; Belletti *et al*., 1998) as DNA stains. The internal standards of choice in the flow cytometric studies were chicken red blood cells (Galbraith *et al*., 1983; Arumuganathan and Earle, 1991) and cells from young leaves of *Pisum sativum* L. or *Capsicum annuum* (Belletti *et al*., 1998). It should be noted that none of these reports included any karyotype information besides chromosome number.

Therefore, in an ongoing series of karyosystematic studies in *Capsicum* (cf. Moscone *et al*., 1996; Moscone, 1999), measurements of nuclear DNA content were performed using flow cytometry, and Feulgen densitometry as a control, with the objectives of: (1) cytologically characterizing species, varieties and cultivars together with chromosome banding approaches (Giemsa C-, fluorochrome-, and $AgNO_3$-banding); (2) relating DNA C-values to karyotype length and heterochromatin amount; and (3) exploring probable trends of genome evolution in the genus.

In this paper we report on the nuclear DNA content of 11 *Capsicum* taxa, six cultivated: *C. annuum* var. *annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* var. *pendulum* (Willd.) Eshbaugh and var. *umbilicatum* (Vellozo) Hunz. et Barboza and *C. pubescens*; and five wild ones: *C. chacoense* Hunz., *C. parvifolium* Sendtn., *C. baccatum* var. *baccatum*, *C. eximium* and *C. campylopodium* Sendtn. Life form and geographic distribution (or probable region of original domestication, where applicable) for most of these taxa have been described previously (Moscone *et al*., 1993*a*, 1995). In particular, *C. baccatum* var. *baccatum* and *C. eximium* are wild woody species, the former having a wide distribution in South America (Colombia, Peru, Bolivia, Paraguay, south-east Brazil and north Argentina), and the latter being endemic to south-western Bolivia and north-western Argentina (Hunziker, 1998). *Capsicum baccatum* var. *umbilicatum* is a herb cultivated in America whose varietal rank has recently been proposed (Hunziker and Barboza, 1998). Its place of domestication is still unknown.

MATERIALS AND METHODS

Provenance of the plant material studied is presented in Table 1. Voucher specimens have been identified by A. T. Hunziker and are deposited in the herbarium of Museo Botánico de Córdoba, Argentina (CORD). In Table 1, taxa are listed according to their likely affinities. Below species level, some samples are arranged in cytotypes defined by distinctive karyotype features, i.e. chromosome morphology and size, heterochromatin amount, type and distribution, and number of active nucleolus organizing regions (AgNORs) (see Moscone *et al*., 1995, 1996).

Seeds of the *Capsicum* accessions were soaked in 500–1000 ppm gibberellic acid (GA_3) in Petri dishes (Ellis *et al*., 1985) to break dormancy before transferring seedlings to pots of soil.

Flow cytometry

For comparative DNA measurements, every nuclear isolation was jointly carried out for one individual of the test material and one of the internal standard, *Hordeum vulgare* L. 'Ditta', thereby ensuring identical conditions for both during isolation and further processing. The nuclear suspension from young leaves (not the cotyledons) was obtained according to Galbraith *et al*. (1983) and De Laat *et al*. (1987), with modifications, and ethidium bromide staining was applied following Baranyi and Greilhuber (1996). Ethidium bromide is a DNA-binding fluorochrome which, like PI, has no base preference. The nuclear suspension was examined on a Partec CA II flow cytometer equipped with a 100 W high pressure mercury lamp and a photomultiplier for ethidium bromide (KG 1, BG 38 and BG 12 filters, TK 500 dichroic mirror, 40 × 1·25 glycerin immersion objective, RG 570 barrier filter). Preparation of the nuclear suspension, staining, and conditions of measurement are described in Baranyi and Greilhuber (1995, 1996). Three runs of measurements per seedling pair were performed. From 2000 to 5250 nuclei were counted per run at a flow speed of 220–240 min. The coefficient of variation in the G1 phase was, on average, 2·62 % for all *Capsicum* samples and 2·12 % for *H. vulgare*. The 1C-value of *H. vulgare* (5·063 pg; calculated from Baranyi and Greilhuber, 1996) was used to calculate genome size in absolute units. 1C DNA amounts are expressed in megabase pairs (Mbp) by converting picograms to Mbp according to the equivalence 1 pg = 980 Mbp [Bennett *et al*., 2000*b*; see Doležel *et al*. (2003) for the calculation]. In Table 1, the data obtained for each sample of a species are presented and a mean value is also given for all samples of a species, with the exception of *C. campylopodium* where the difference between accessions was significant.

Feulgen densitometry

Feulgen densitometry was performed following Greilhuber and Ebert (1994) to corroborate the DNA contents obtained by flow cytometry in *C. annuum* var. *annuum* EAM 204 and *C. pubescens* GB 79. Root tip meristems from primary roots 0·5–1 cm long were fixed in methanol : glacial acetic acid (3 : 1). In addition, neutral 4 % formaldehyde in Sörensen's phosphate buffer (pH 7) was utilized in half of the individuals of *C. annuum* var. *annuum* EAM 204 analysed to test possible interference of phenolic compounds with DNA stainability (cf. Greilhuber, 1988). *Allium cepa* L. 'Stuttgarter Riesen', with 1C = 16·75 pg (cf. Bennett and Smith, 1976), was used as internal standard for calculation of absolute genome size. Specimen and standard were processed strictly in parallel. Feulgen densitometric DNA measurements were carried out on a Leitz MPV II

microscope photometer on ten early telophase nuclei per plant and slide of the specimen and standard. Details of staining after cold hydrolysis of root tips, scanning at 1 μm steps, and calculations of mean and standard deviation of absolute 1C-values are given in Greilhuber (1988) and Greilhuber and Ebert (1994).

A single classification ANOVA, the Scheffé test, and a correlation analysis were performed using SPSS for Windows 6.0 (SPSS Inc., Chicago, IL, USA); a nested ANOVA was performed using the NESTAN routine of the BIOM-pc 2.1 package (Rohlf, 1992). The amount of heterochromatin was calculated by adding the individual band lengths along the chromosomes, and was expressed as percentage of the haploid karyotype length. Correlations between DNA content values and karyotype length or heterochromatin amount were evaluated using the non-parametric Spearman test (Sokal and Rohlf, 1995).

RESULTS

Ethidium bromide (EB) flow cytometry in 25 accessions belonging to nine diploid species and four varieties of *Capsicum* resulted in a maximum difference of 1·72-fold between species (Table 1). The absolute 1C nuclear DNA amount (unreplicated haploid genome) ranged from 3·35 pg (3283 Mbp) in *C. chacoense* ($2n = 24$) to 5·77 pg (5655 Mbp) in *C. parvifolium* ($2n = 24$), the mean 1C-value for the genus being 3·94 pg (3862 Mbp). Simultaneous flow histograms of each of the two *Capsicum* species with extreme DNA content values and the internal standard, *Hordeum vulgare* 'Ditta', are shown in Fig. 1. The Scheffé test revealed an apparently discontinuous distribution of 1C DNA amounts in *Capsicum*, with regular intervals of about 0·35 pg (343 Mbp) between groups. Feulgen densitometry in *C. annuum* var. *annuum* EAM 204 and *C. pubescens* GB 79 resulted in 1C-values of 3·16 and 4·56 pg, respectively (Table 1), while EB-flow cytometry gave values of 3·32 and 4·45 pg for the respective samples. The difference in C-values between both methodological approaches was very small, i.e. 4·8 % for EAM 204 and 2·4 % for GB 79. In EAM 204, one-way ANOVA showed a non-significant difference ($P > 0·05$) between Feulgen densitometric data obtained from formaldehyde and 3 : 1-fixed material.

Analysis of variance for all the EB-flow cytometric data showed a highly significant difference between species ($P < 0·001$) and samples ($P < 0·001$). Nevertheless, differences between populations of the same species were non-significant according to the Scheffé test ($P > 0·05$), except in the case of *C. campylopodium* ($2n = 26$) where cytotype 1 (5·74 pg) was 1·27-fold larger than cytotype 2 (4·53 pg) (see Scheffé grouping in Table 1; Fig. 2). Nested ANOVA demonstrated significant differences at the sample level ($F_{7, 20} = 5·69$, $P < 0·001$) but not at the species ($F_{3, 7} = 4·35$, $P > 0·05$) or individual level ($F_{20, 60} = 1·75$, $P > 0·05$) in *C. chacoense*, *C. frutescens*, *C. chinense* and *C. annuum* var. *annuum*, which are all included in the same Scheffé group (3·32–3·43 pg).

Almost identical 1C estimates were found in *C. baccatum* var. *baccatum* and two cytotypes of var. *pendulum* (3·68–3·71 pg), and a slightly higher value was found in var. *umbilicatum* (3·76 pg), with all varieties belonging to the same Scheffé group (Table 1). In *C. eximium* the 1C estimate was 4·06 pg and in *C. pubescens* 4·47 pg was the mean value; these species were placed in different Scheffé groups.

DNA content was positively and significantly correlated with karyotype length ($r = 0·780$; $P = 0·001$) and constitutive heterochromatin amount ($r = 0·728$, $P = 0·003$) (Table 1; Fig. 3), although *C. parvifolium* in particular has a comparatively large genome and little heterochromatin. The variation in genome size between *Capsicum* cytotypes is within the range 1 : 1·8, while heterochromatin amount varies within the range 1 : 15·3.

DISCUSSION

Comparison with literature data

Capsicum parvifolium, *C. baccatum* var. *umbilicatum* and *C. campylopodium* are examined here for the first time. Our results for the remaining taxa (Table 1) can be compared with the literature data, which correspond to different samples to those analysed here. Cautious interpretation regarding taxon identity is also in order owing to the lack of voucher specimens and unknown source of voucher identification in the contributions by other authors.

Our EB-flow cytometric estimates are about 12 % lower than the PI measurements given by Belletti *et al*. (1998; 1C-values given in parentheses) in *C. chacoense* and *C. annuum* (both 3·83 pg), *C. frutescens* (3·97 pg), *C. chinense* (4·02 pg), *C. baccatum* var. *baccatum* (4·22 pg) and var. *pendulum* (4·20 pg), although the differences are as small as 3 and 8 % in *C. eximium* (4·35 pg) and *C. pubescens* (4·86 pg), respectively. This difference could be explained in part by methodological variables, such as the different dye and standards (*P. sativum* and *C. annuum*) used by Belletti *et al*. (1998). On the other hand, early Feulgen densitometric measurements in *Capsicum* by Bennett and Smith (1976; 1C DNA amounts given in parentheses) are about 30 to 40 % higher than those presented here for the following species: *C. annuum* (4·70 pg), *C. frutescens* (6·00 pg), *C. chinense* (5·90 pg), *C. baccatum* (5·65 pg), *C. eximium* (6·15 pg) and *C. pubescens* (6·45 pg). Using Feulgen densitometry, Mukherjee and Sharma (1990) also found a 50 % higher mean 1C-value (5·07 pg) than that observed here for *C. annuum*. In these cases, the source of such striking discrepancies cannot be explained. In addition, Bennett and Smith (1976) reported larger genomes in cultivated forms compared with wild ones of *C. annuum* (1·35-fold) and *C. baccatum* (1·31-fold), a fact that does not agree with our observations for the latter species (1·01-fold) where both types of samples were analysed.

Particularly in *C. annuum*, the most widely examined species in the genus, the low 1C-value (2·76 pg) obtained by Galbraith *et al*. (1983), compared with the present value of 3·38 pg, could be attributed to their use of MI, which probably produces biased DNA content estimates due to its GC-binding specificity (Schweizer, 1976). Arumuganathan and Earle (1991) found a mean 1C-value for this species of

TABLE 1. Capsicum *taxa studied and their chromosome number, voucher number, provenance, status, nuclear DNA 1C value (mean and % coefficient of variation) in pg and % of* Hordeum vulgare*, measured using ethidium bromide (EB) flow cytometry (for some Feulgen measurements see footnotes), haploid karyotype length (HKL) and heterochromatin (Hc) as a percentage of HKL*

		Ethidium bromide-flow cytometry								
Taxon, cytotype, and voucher number*	Provenance and status†	Number of seedling pairs tested (Ns)	Total number of runs (Nr)‡	1C DNA in: pg	% of standard	CV%	Genome size (Mbp)	Scheffé group§	HKL (μm)	Hc
C. chacoense Hunz. (2*n* = 24)										
Cytotype 1										
EAM 104	Argentina, Prov. Córdoba, Dept. Calamuchita, Falda del Sauce (w)	3	9	3·34	66·1	1·2	3273	a		
EAM 195	Argentina, Prov. Córdoba, Dept. Calamuchita, Falda de Los Reartes (w)	3	9	3·33	65·8	1·0	3263	a		
EAM 207	Argentina, Prov. Córdoba, Dept. Río Segundo, Pilar (w)	3	9	3·36	66·3	0·7	3293	a		
All		9	27	3·34	66·1	1·0	3273	a	65·0[1]	2·9[1]
Cytotype 2										
LMB, LG 525	Argentina, Prov. Córdoba, Dept. Sobremonte, San Francisco del Chañar (w)	2	6	3·36	66·4	0·9	3293	a	71·3[1]	2·4[1]
Cytotype?										
EAM, RN 213	Argentina, Prov. Salta, Dept. La Viña, Osma (w)	2	6	3·35	66·2	1·2	3283	a		
All samples		13	39	3·35	66·1	1·0	3283	a		
C. parvifolium Sendtn. (2*n* = 24)										
ATH 25233	Brazil, Est. Bahia, Munic. Senhor do Bomfin and Itiuba; leg. VC (w)	5	15	5·77	113·9	0·8	5655	e	82·4[1]	5·3[1]
C. frutescens L. (2*n* = 24)										
EAM 200	Brazil, Est. Rio de Janeiro, Rio de Janeiro, bought at market place; leg. ATH (c)	3	9	3·40	67·2	1·1	3332	a	66·6[2]	5·6[2]
C. chinense Jacq. (2*n* = 24)										
Cytotype 1										
EAM 199	Brazil, Est. Rio de Janeiro, Rio de Janeiro, bought at market place; leg. ATH (c)	3	9	3·43	67·8	0·9	3361	a	61·3[2]	3·9[2]
Cytotype 2										
EAM 201	Brazil, Est. Amazonas, Manaus, bought at market place; leg. ATH (c)	3	9	3·41	67·4	0·9	3342	a	61·4[2]	5·5[2]
All samples		6	18	3·42	67·6	0·9	3352	a		
C. annuum L. (2*n* = 24) var. *annuum*										
Cytotype 1										
EAM 193	Argentina, Prov. Córdoba, Dept. Capital, Córdoba; leg. RJ (c)	3	9	3·45	68·1	1·1	3381	a		
EAM 203	Austria, Vienna, bought at market place (c)	3	9	3·36	66·5	1·0	3293	a		
All		6	18	3·41	67·3	1·0	3342	a	68·5[2]	1·8[2]
Cytotype 2										
EAM 204¶	Argentina, Prov. Jujuy, Dept. Capital, Jujuy, bought at market place in Salta; leg. GB (c)	3	9	3·32	65·7	1·2	3254	a	70·4[2]	2·9[2]
All samples		9	27	3·38	66·7	1·9	3312	a		
C. baccatum L. (2*n* = 24) var. *baccatum*										
EAM, RN 210	Argentina, Prov. Salta, Dept. La Viña, Osma (w)	3	9	3·71	73·3	1·2	3636	b	?	?
var. *pendulum* (Willd.) Eshbaugh										
Cytotype 1										
EAM 192	Argentina, Prov. Córdoba, Dept. Capital, Córdoba (c)	3	9	3·68	72·7	1·4	3606	b		

3·28 pg, which is comparable with that obtained here by applying PI, a fluorochrome without base preference (Dolezel, 1991). Furthermore, Arumuganathan and Earle (1991) and Mukherjee and Sharma (1990), respectively, observed a 1·34-fold (seven cultivars) and a 1·25-fold (23 cultivars) intraspecific variation in genome size. These

TABLE 1 *Continued*

Taxon, cytotype, and voucher number*	Provenance and status†	Ethidium bromide-flow cytometry: Number of seedling pairs tested (Ns)	Total number of runs (Nr)‡	1C DNA in: pg	% of standard	CV%	Genome size (Mbp)	Scheffé group§	HKL (μm)	Hc
EAM 209	Bolivia, Dept. Cochabamba, Cochabamba; CFP 259 (c)	3	9	3·74	73·9	1·0	3665	b		
All		6	18	3·71	73·3	1·2	3636	b	73·5[2]	7·3[2]
Cytotype 2										
EAM 205	Bolivia, Dept. Cochabamba, Cochabamba, bought at market place in Salta; leg. GB (c)	3	9	3·67	72·5	1·1	3597	b		
EAM 206	Argentina, Prov. Jujuy, Dept. Capital, Jujuy, bought at market place in Salta; leg. GB (c)	3	9	3·68	72·7	1·3	3606	b		
All		6	18	3·68	72·6	1·2	3606	b	74·3[2,a]	7·6[2,a]
var. *umbilicatum* (Vellozo) Hunz. et Barboza										
EAM 197	Argentina, Prov. Córdoba, Dept. Capital, Córdoba; leg. RM (c)	3	9	3·76	74·3	0·6	3685	b	74·3[3,b]	9·1[3,b]
All samples		18	54	3·71	73·2	1·4	3636	b		
C. eximium Hunz. (2*n* = 24)										
EAM, RN 216	Bolivia, bought at market place in Salta (w)	2	6	4·06	80·2	0·9	3979	c	?	?
C. pubescens R. et P. (2*n* = 24)										
GB 79**	Argentina, Prov. Salta, Dept. Rosario de Lerma, Rosario de Lerma (c)	3	9	4·45	87·9	1·1	4361	d		
EAM 198	Bolivia, Dept. Santa Cruz, Santa Cruz, bought at market place in Salta; leg. GB (c)	2	6	4·44	87·7	0·4	4351	d		
EAM 202	Peru, Región Central, Chanchamayo; leg. GV (c)	3	9	4·47	88·3	1·1	4381	d		
EAM 208	Bolivia, Dept. Cochabamba, Cochabamba; leg. AMP (c)	3	9	4·50	88·9	2·0	4410	d		
All samples		11	33	4·47	88·2	1·4	4381	d	80·5[2]	19·0[2]
C. campylopodium Sendtn. (2*n* = 26)										
Cytotype 1										
ATH 25116	Brazil, Est. Rio de Janeiro, Munic. Petropolis, prox. Belvedere; leg. VC (w)	1	3	5·74	113·3	0·9	5625	e	85·4[1]	27·5[1]
Cytotype 2										
ATH 25130	Brazil, Est. Minas Gerais, Munic. Caratinga, Mata de Jaó (w)	3	9	4·53	89·5	1·3	4439	d	79·1[1]	23·2[1]

1C DNA amounts were calculated from the internal standards, *Hordeum vulgare* (1C = 5·063 pg) for ethidium bromide measurements and *Allium cepa* (1C = 16·75 pg) for Feulgen measurements, respectively. Genome size was calculated from the equivalence 1 pg = 980 Mbp.

x, mean (average ratio); CV%, coefficient of variation as a percentage of mean, with reference to the total number of runs for EB data and to the number of nuclei of the test sample (Nn; with ten nuclei measured per plant) for Feulgen data.

Arrangements of samples in cytotypes after Moscone *et al.* (1995, 1996).

* Abbreviations of collectors' names: GB, G. Barboza; LMB, L. M. Bernardello; VC, V. Casali; LG, L. Galetto; ATH, A. T. Hunziker; RJ, R. Juliani; EAM, E. A. Moscone; RM, R. Münch; RN, R. Neumann; AMP, A. M. Planchuelo; GV, G. Vilcapoma; CFP, registration no. of Centro Fitotécnico Pairumani (Cochabamba).

† *w*, Natural habitat/wild origin; *c*, cultivar.

‡ Three runs per seedling pair.

§ $P > 0{\cdot}05$

¶ 1C DNA content by Feulgen densitometry: $x = 3{\cdot}16$ pg, 18·85 % of standard (CV = 7·53 %), Ns = 12, Nn = 120.

** 1C DNA content by Feulgen densitometry: $x = 4{\cdot}56$ pg, 27·20 % of standard (CV = 5·04 %), Ns = 6, Nn = 60.

[1] Moscone (unpubl. res.); [2] Moscone *et al.* (1996); [3] Moscone (1999); [a, b] Average values that include data of additional samples not considered in the present study, i.e. *C. baccatum* var. *pendulum* ATH 25382 and *C. baccatum* var. *umbilicatum* CORD 75.

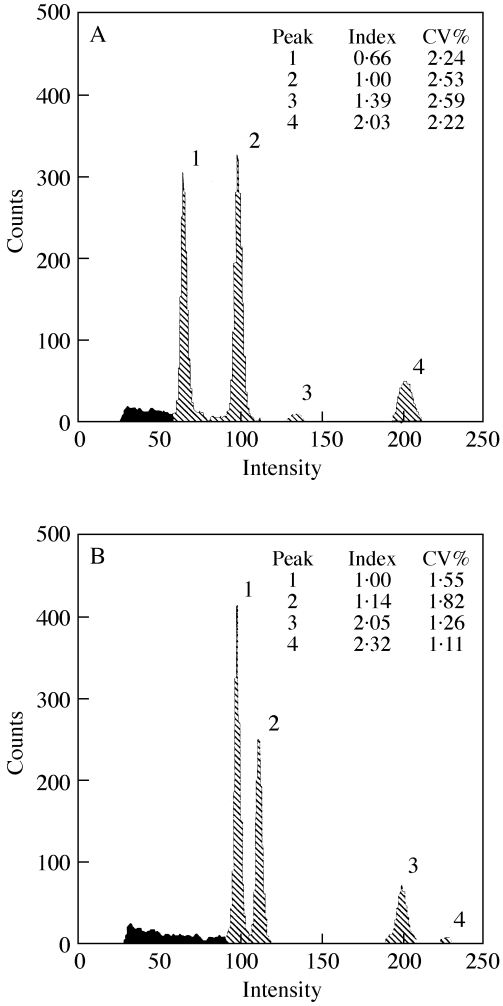

FIG. 1. Ethidium bromide flow histograms of two *Capsicum* taxa and *H. vulgare* 'Ditta' (in A, peaks 2 and 4; in B, peaks 1 and 3) as standard, showing genome size variation among the *Capsicum* accessions. A, *C. chacoense* LMB, LG 525 (peaks 1 and 3). B, *C. parvifolium* ATH 25233 (peaks 2 and 4). Vertical and horizontal axes represent the number of nuclei counted and the intensity of the fluorescence channel, respectively. Peaks 1and 2 correspond to G1, and peaks 3 and 4 to G2 nuclei. Index, Ratio mode *Capsicum* sample/mode *H. vulgare* G1 and G2 peaks; CV%, coefficient of variation of G1 and G2 peaks as a percentage.

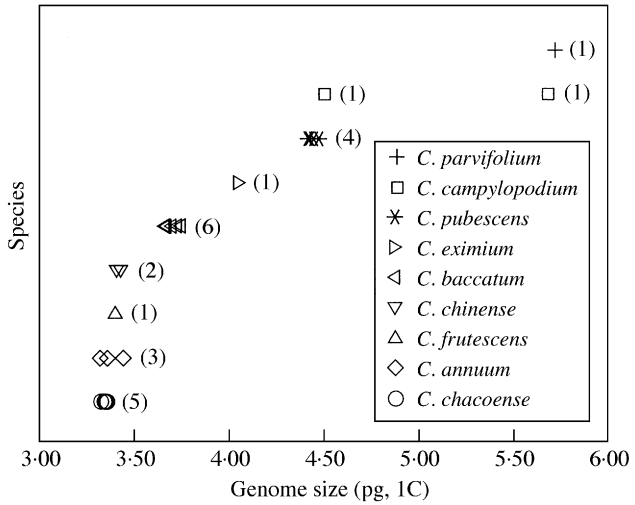

FIG. 2. Distribution of DNA 1C-values of 25 samples and nine species of *Capsicum* measured by ethidium bromide flow cytometry. The number of samples for each species is given in parentheses.

results contrast with the present findings of just 1·04-fold variation between the extreme 1C-values of our *C. annuum* samples, which was non-significant after one-way ANOVA.

Intraspecific variation in nuclear DNA content

The generally high constancy of nuclear DNA amount observed between samples of a given plant species without chromosome variation (cf. Greilhuber, 1998; Bennett *et al*., 2000*c*) is confirmed by the present results and those of Belletti *et al*. (1998), which demonstrate a non-significant variation of DNA amount below the species level in *Capsicum*, with few taxonomically interesting exceptions. In this sense, although both cytotypes of *C. campylopodium* are indistinguishable by exomorphological features (A. T. Hunziker, pers. comm.), they differ not only in DNA content, but also in chromosome length, heterochromatin amount and karyotype asymmetry (Table 1; E. A. Moscone, 1989, unpubl. res.). Similarly, Belletti *et al*. (1998) report a highly significant difference in genome size between *C. baccatum* var. *praetermissum* (Heiser et Smith) Hunz. (cited as *C. praetermissum*) and two other varieties of this species, *C. baccatum* var. *baccatum* and var. *pendulum* (both cited as subspecies). In the first variety, basic karyotype information including heterochromatin quantity is required to appraise its taxonomic rank.

Systematics and karyotype evolution

Together with chromosome data obtained using Giemsa C-, fluorochrome- and $AgNO_3$-banding techniques (Moscone *et al*., 1993*a*, *b*, 1995, 1996; Moscone, 1999), nuclear DNA content measurements in *Capsicum* demonstrate differences in genome size between taxa and contribute to their taxonomic grouping. In general, the karyological information supports previous conclusions on systematic affinities and phylogenetic relationships in *Capsicum* based on other methodological approaches, including chloroplast and nuclear non-coding DNA sequence studies (Hunziker, 1950; McLeod *et al*., 1983; Pickersgill, 1991; Walsh and Hoot, 2001).

Capsicum chacoense, *C. frutescens*, *C. chinense* and *C. annuum* var. *annuum* are all white-flowered taxa that are closely related according to their similar DNA amount, which is comparatively low (Table 1, Scheffé group a). In addition, they all display little heterochromatin (Table 1) of the GC-rich type, simple heterochromatic banding patterns (heterochromatin group I; Moscone *et al*., 1993*a*, *b*), and one or two AgNORs per basic chromosome set (Moscone *et al*., 1995: AgNOR groups A–B). The latter three species are included in the *C. annuum* complex, where poorly developed crossing barriers and links at morphological and isozymic levels suggest that they could be conspecific, particularly *C. frutescens* and *C. annuum* (cf. Pickersgill,

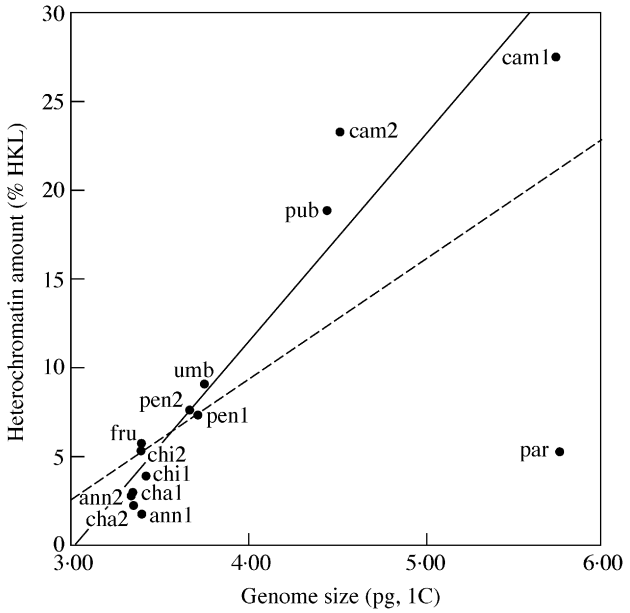

FIG. 3. Relationship between genome size (1C-value in pg) and heterochromatin amount [expressed as a percentage of the haploid karyotype length (HKL)] in *Capsicum*. Solid line (r = 0·878, P = 0·0001) and dashed line (r = 0·728, P = 0·003) represent the correlation without and with the *C. parvifolium* data, respectively. cha, *C. chacoense*; par, *C. parvifolium*; fru, *C. frutescens*; chi, *C. chinense*; ann, *C. annuum* var. *annuum*; pen, *C. baccatum* var. *pendulum*; umb, *C. baccatum* var. *umbilicatum*; pub, *C. pubescens*; cam, *C. campylopodium*. Cytotypes are indicated as 1 or 2 after the abbreviated species name.

1991; Hunziker, 2001). Although crosses between the *C. annuum* complex and *C. chacoense* give fertile hybrids, the latter species is distinguished by its woody habit, isozyme profile and DNA sequence changes (McLeod *et al*., 1983; Pickersgill, 1991; Walsh and Hoot, 2001).

On the other hand, *C. baccatum*, another white-flowered species but one that has yellowish-green spots in the throat of its corolla, shows an increased DNA content (Scheffé group b). The available chromosome banding data in *C. baccatum* var. *pendulum* and var. *umbilicatum* indicate more GC-enriched heterochromatin and a higher degree of complexity in the heterochromatic banding pattern (still placed in heterochromatin group I; Moscone *et al*., 1993*a*, *b*; Moscone, 1999) in relation to the species previously mentioned. Furthermore, it has three to four AgNORs in the basic complement (Moscone *et al*., 1995; Moscone, 1999: AgNOR group C). Therefore, the present results support the separate placement of *C. baccatum* within the white-flowered group, although its inclusion in a subgroup together with *C. chacoense* has been proposed on the basis of weak molecular evidence (cf. McLeod *et al*., 1983; Walsh and Hoot, 2001).

Capsicum eximium, whose purple or white flowers have yellowish-brown spots in the inner throat, is intermediate between *C. baccatum* and *C. pubescens* in DNA content (Scheffé group c). Although a lack of information about heterochromatin amount and number of active NORs in *C. eximium* prevents further karyological comparisons, the genome size supports its placement close to *C. pubescens* within the purple-flowered group, as was suggested by isozyme and crossing studies (McLeod *et al*., 1983; Pickersgill, 1991). On the other hand, DNA sequencing data indicate an unclear relationship between the latter two species (Walsh and Hoot, 2001).

Capsicum pubescens and *C. campylopodium* cytotype 2, grouped together by their comparatively large genome size (Scheffé group d), also have a large amount of heterochromatin of GC- and AT-rich types and complex heterochromatic banding patterns (heterochromatin group II; Moscone *et al*., 1993*a*, *b*). Nevertheless, the former species has purple flowers with a white inner throat, $2n$ = 24, mainly GC-enriched satellite DNA and two AgNORs in the basic complement (Moscone *et al*., 1995: AgNOR group D), whereas the latter species is white-flowered with yellow spots on the corolla throat, has $2n$ = 26, mainly AT-rich heterochromatin and only one AgNOR per genome (Moscone *et al*., 1995: AgNOR group E). Their links remain uncertain owing to the lack of isozyme data and crossing experiments, not only in the second species but also in the other members of *Capsicum* with x = 13, whose taxonomic status should be revised. For example, *C. rhomboideum* (Dunal) Kuntze (as *C. ciliatum*) with $2n$ = 26 is far apart from the *Capsicum* species with $2n$ = 24 according to DNA sequence analysis (Walsh and Hoot, 2001).

Finally, *C. parvifolium*, a tree with flower colour polymorphism (white and purple, both with yellowish-green spots in the throat), and the herbaceous *C. campylopodium* cytotype 1 are similar in that they have the largest genomes (Scheffé group e) and also one AgNOR in the haploid complement (Moscone *et al*., 1995: the former species in AgNOR group A). However, both species have different chromosome numbers, and different quantities (heterochromatin group I and II, respectively, Moscone *et al*., 1993*a*) and types (GC-rich in *C. parvifolium*; Moscone *et al*., 1993*b*) of heterochromatin. Thus, there are some karyological similarities, although the relationship seems to be distant, with isozyme, DNA sequence and crossing relationships not yet known.

The present results lend further support to our working hypothesis on chromosome evolution in *Capsicum* (cf. Moscone *et al*., 1993*a*, *b*, 1995, fig. 14), which become robust after DNA amount information. In this sense, the gradual increase in DNA content observed in different species or groups, always related to the increase in heterochromatin amount except in *C. parvifolium*, is considered as an evolutionary advancement. Thus, the woody and perennial *C. chacoense* exhibits all the karyological features regarded as plesiomorphic in the genus, such as small genome size, x = 12, two AgNORs in the basic complement, little GC-enriched heterochromatin and a simple banding pattern in a symmetrical karyotype. On the other hand, the herbaceous and probably annual *C. campylopodium* represents the most apomorphic condition by displaying the largest genomes and the derived x = 13, only one AgNOR per basic chromosome set, the highest heterochromatin amount mainly of AT-rich type and complex banding patterns in asymmetrical karyotypes. In the genus, the basic position of *C. chacoense* and the advanced status of the species with x = 13, particularly

C. campylopodium, have been discussed extensively by Moscone *et al*. (1993*a*).

Of the 92 genera included in Solanaceae (Hunziker, 2001), only 12 have been measured for nuclear DNA content (cf. Bennett *et al*., 2000*a*; Stiefkens and Bernardello, 2000), with 1C-values of the species with diploid complements ranging from 0·6 pg (588 Mbp) in *Solanum chacoense* Bitter and *S. tripartitum* Dunal, both $2n = 24$ (Bennett *et al*., 2000*a*) to 24·8 pg (24 304 Mbp) in *Cyphomandra hartwegii* (Miers) Walp. var. *ramosa*, with $2n = 24$ (Pringle and Murray, 1991). The species of *Capsicum*, together with the majority of diploid *Nicotiana* species, display the second largest genomes in the family after members of *Cyphomandra*. Among diploid species, the interspecific genome size variation found here in *Capsicum* is similar to that recorded in *Petunia* (1·67-fold; White and Rees, 1985), although more extreme values have been reported between $2n = 24$ species of *Solanum* (5·17-fold; Bennett *et al*., 2000*a*), *Nicotiana* (4·13-fold; Narayan, 1987) and *Cyphomandra* (3·67-fold; Pringle and Murray, 1991). In addition, there is apparently a discontinuous distribution in DNA content within *Capsicum*, as described in *Cyphomandra* (Pringle and Murray, 1991) and *Nicotiana* (Narayan, 1987). Presently, the scarce information on DNA amount in the family as a whole prevents its use in systematic considerations on the intergeneric relationships of *Capsicum*, which is placed in subtribe Capsicinae Yamazaki (tribe Solaneae) according to the system proposed by Hunziker (2001). DNA content data on other members of this subtribe, such as *Athenaea* Sendtn., *Aureliana* Sendtn. and *Vassobia* Rusby, are therefore needed.

Nuclear DNA content and heterochromatin

The present results suggest that in *Capsicum* heterochromatin has been gained by addition rather than by euchromatin transformation, as was proposed in other plant groups (cf. Greilhuber, 1995). It has been demonstrated that DNA content in the angiosperms varies as a consequence of the change and proportion of repeated DNA sequences in the nuclear genome, particularly tandem repeats or satellite DNAs that make up heterochromatic C-bands on the chromosomes (cf. Flavell, 1986; Bennett and Leitch, 1995; Greilhuber, 1995). In contrast to *Cyphomandra* (Pringle and Murray, 1993) and *Nicotiana* (Narayan, 1987), genome size and heterochromatin amount are strongly correlated in *Capsicum*, although the range of variation is eight-fold broader in the latter parameter. On the other hand, *C. parvifolium*, with the largest genome, has scarce heterochromatin. Therefore, increases not only in highly repetitive sequences of defined heterochromatic bands, but in cases also in dispersed DNA repeats, i.e. euchromatin increase, can be supposed for *Capsicum*.

As in several members of *Nicotiana* (Narayan, 1987), in the core $2n = 24$ *Capsicum* species whose karyotypes are almost invariably composed of 11 metacentric plus one subtelocentric chromosome pairs of decreasing size, changes in DNA content and heterochromatin are obviously not associated with gross changes in chromosome morphology or karyotype reorganization. Thus, during the evolution of this group, DNA additions should have occurred equally over both chromosome arms with retention of karyotype symmetry, as was proposed in *Cyphomandra* (Pringle and Murray, 1993), although in *Capsicum* large heterochromatic bands are often distributed on just one arm throughout the complement. The lack of subtelocentric chromosomes in the karyotype of *C. parvifolium*, the species with the largest genome, could be explained by transformation of the original subtelocentric present in other $2n = 24$ species into a metacentric by additional DNA spread on the short and long arm as well. The genus *Capsicum* is, to a large extent, an example of 'karyotypic orthoselection' in plants, which preserves highly similar karyotypes throughout a higher taxon despite changes in genome size (Brandham and Doherty, 1998).

On the other hand, the increased genome size of *C. campylopodium* is related to an increase in heterochromatin amount and karyotype repatterning, both typical features of the marginal $2n = 26$ *Capsicum* taxa analysed to date, which are thought to have arisen recently by centric fission and be subject to an active process of speciation (Moscone *et al*., 1993*a*). Although the presence of two intraspecific cytotypes in *C. campylopodium* was not accompanied by exomorphological differentiation, this phenomenon could indicate a starting point of diverging evolutive paths: on the one hand, cytotype 1, where additional DNA is related to large heterochromatic bands distributed over one chromosome arm throughout a comparatively more asymmetrical complement and, on the other hand, cytotype 2, where large heterochromatic blocks are generally located on both arms giving rise to a less asymmetrical karyotype (Moscone *et al*., 1993*a*, 1995; E. A. Moscone, unpubl. res.).

ACKNOWLEDGEMENTS

We thank Renate Obermayer for technical assistance, and several botanists who have provided material. E.A.M. wishes to acknowledge fellowship support from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina, for study leave at the Institute of Botany, University of Vienna, Austria. This work was supported by the Austrian Fonds zur Förderung der wissenschaftlichen Forschung, grant no. P12674-GEN to J. Greilhuber, and by Secretaría de Ciencia y Tecnología, Universidad Nacional de Córdoba, Argentina, grant no. 194/00 to E.A.M.

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