Nuclear Genome Size Variations in *Citrus*

P. Ollitrault, D. Dambier
CIRAD-FLHOR, BP 5035
34032 Montpellier cedex 01
France

F. Luro
SRA San Giuliano
CIRAD-FLHOR/INRA
20230 San Nicolao
Corsica

C. Duperray
INSERM, U291
99, rue Puech Villa
34090 Montpellier
France

Fruits, vol. 49, n°5-6
p. 390-393 (English)
p. 475-476 (French)

Systematic analysis of genome sizes in Citrus *is important for programmes involving gene mapping and plant breeding via sexual crossing.*

introduction

Numerical taxonomic studies (Barrett and Rhodes, 1976) and molecular marker analyses (Green *et al.*, 1986; Ollitrault and Faure, 1992; Yamamoto *et al.*, 1993) have demonstrated that the wide variety of cultivated *Citrus* species can be clearly grouped around three basic taxa since *Citrus medica* (citrons), *Citrus reticulata* (mandarins) and *Citrus grandis* (pummelo) are generally considered to be the three ancestral species of currently cultivated *Citrus*. Despite the number of sympatric zones, this level of structuring suggests that many factors limit interspecific recombination. Some of these limitations are genomic, as shown by the structural heterozygosity (translocations, inversions, etc.) that has been noted in interspecific hybrids (Raghuvanshi, 1969; Gmitter *et al.*, 1992). In the present study, we looked for a quantitative component to account for the structural differentiation between nuclear genomes of various *Citrus* species.

material and methods

The relative nuclear genome sizes of four to five diploid cultivars of the eight main cultivated *Citrus* species were assessed (Table 1). For each cultivar, evaluations relative to the control triploid cultivar cv Tahiti lime were done in triplicate. Leaf samples from the test cultivar and control were minced finely in PBS buffer supplemented with dithiothreitol (1 mg/ml), Triton X100 (0.3%) and RNAase (10^{-3} U/ml). After filtration, 0.3 ml of the nuclear suspension was mixed with 0.3 ml extraction buffer supplemented with propidium iodide (200 mg/ml). Two thousand nuclei per sample were then analysed by Fascan flow cytometry connected to the Lysis 2 software programme. The size of the cv Tahiti lime nuclear genome was evaluated relative to chicken erythrocytes (2.33 pg/2C) so as to determine absolute sizes of genomes for each diploid cultivar considered. The data were analysed using hierarchical analysis of variance to determine possible size variations within each species and assess interspecific diversity.

results

The absolute size of the cv Tahiti lime nuclear genome was estimated at 1.17 pg/2C for 2N=3X=27 chromosomes. The coefficients of variation (c.v.) concerning the G0-G1 peaks for the different diploid samples was around 2.5-3% for most of the cultivars (Fig. 1). However the c.v. of peaks were systematically higher for some varieties of sweet orange sweet (*C. sinensis*), lime (*C. aurantifolia*) and mandarin, which resulted in higher variance for the genome size estimates. In six cases, there were significant variations in genome sizes (not higher than 3%) between cultivars of the same species (Table 1).

There was also very marked interspecific variability, i.e. reaching 10% between mandarin and citron (Figs. 1 and 2), the smallest and largest *Citrus* genomes respectively. The other species were divided between two other intermediate-sized groups. One included sweet orange and sour orange (*C. aurantium*) and the other lemon (*C. lemon*), lime, pummelo and grapefruit (*C. paradisi*). This division into four separate groups, revealed by the

Table 1
Estimated relative and absolute sizes of nuclear genomes in *Citrus* cultivars (/cv Tahiti lime).

Mean values for each species are presented in bold.
H.G. = homogeneous groups determined by the Newman-Keuls test.
F and p = statistics associated with the Newman-Keuls test.

Cultivars	Relative size	Absolute size	H.G.	Intra specific variability
***C. aurantium* (Sour orange)**	**0.643**	**0.752**		
Bigaradier Goutou	0.642	0.751	a	F = 2.39
Bigaradier Granito	0.644	0.753	a	p = 0.14
Bigaradier Maroc	0.641	0.756	a	N.S.
Bouquetier Nice	0.646	0.756	a	
***C. medica* (Citron)**	**0.696**	**0.814**		
Corse	0.696	0.815	a	F = 10.71
Digité	0.697	0.816	a	p < 0.01
Etrog	0.702	0.821	a	**
Poncire commun	0.690	0.807	b	
***C. limon* (Lemon)**	**0.665**	**0.778**		
Lisbonne	0.672	0.786	a	F = 11.93
Sweet	0.665	0.778	b	p < 0.01
Eureka	0.664	0.777	b	**
Meyer	0.660	0.772	b	
***C. aurantifolia* (Lime)**	**0.659**	**0.771**		
Brazil Sweet	0.646	0.756	a	F = 9.08
Kirk	0.666	0.779	b	p < 0.01
Mexican	0.666	0.779	b	**
Rangpur	0.660	0.772	b	
***C. sinensis* (Sweet Orange)**	**0.647**	**0.757**		
Parson Brown	0.646	0.756	a	F = 0.02
Shamouti	0.646	0.756	a	p = 0.99
Washington Navel	0.647	0.757	a	N.S.
Valencia late	0.647	0.757	a	
***C. paradisi* (Grapefruit)**	**0.666**	**0.779**		
Star Ruby	0.660	0.772	a	F = 7.36
Cecily	0.665	0.778	b	p = 0.01
Marsh	0.669	0.783	b	*
Thomson	0.670	0.784	b	
***C. grandis* (Pummelo)**	**0.669**	**0.783**		
Kao Pane	0.656	0.768	a	F = 25.01
Pink	0.666	0.779	b	p < 0.01
Seedless	0.673	0.787	bc	**
Inde	0.673	0.787	bc	
Sunshine	0.679	0.795	c	
***C. reticulata* (Mandarin)**	**0.630**	**0.737**		
Satsuma Wase	0.623	0.729	a	F = 9.14
Willow-leaf	0.624	0.730	a	p < 0.01
Cleopatra	0.627	0.733	a	**
Ponkan	0.636	0.745	b	

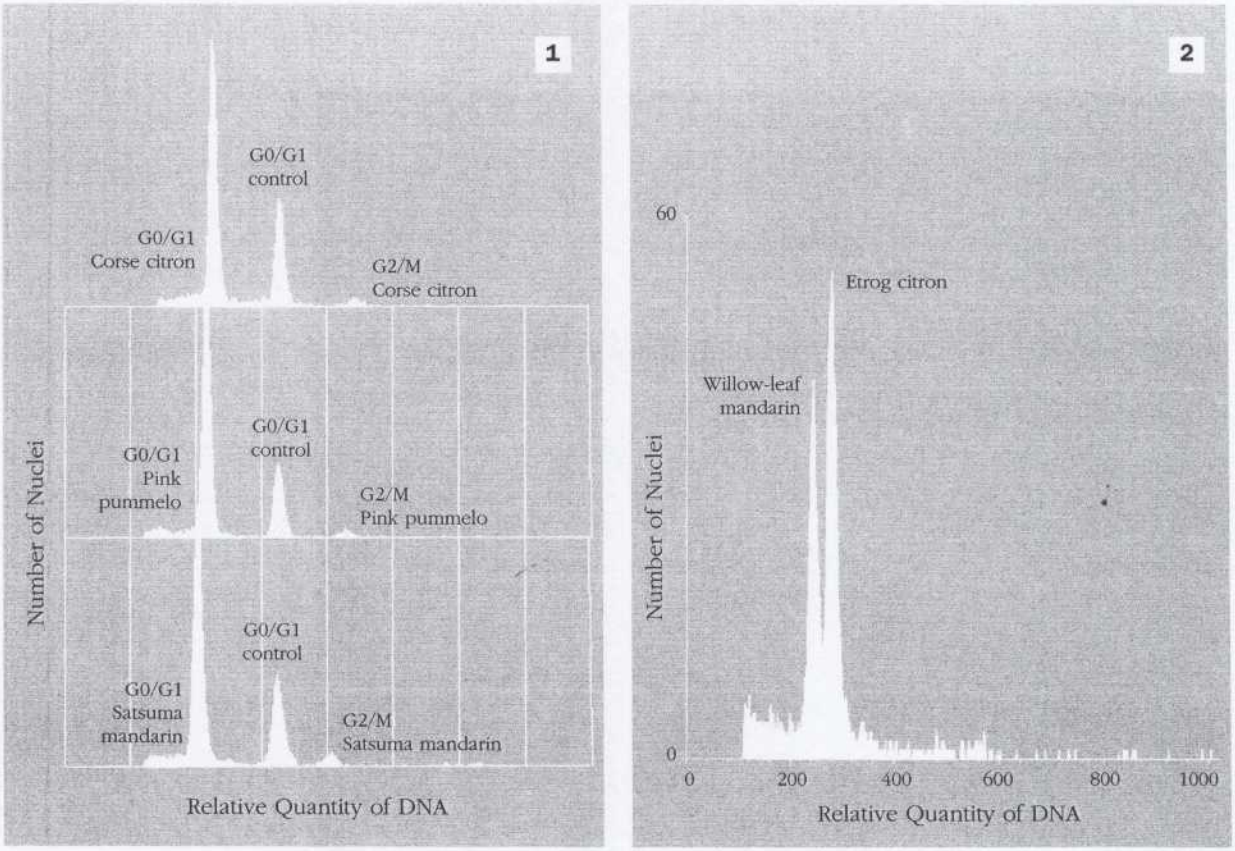

Figure 1
Estimated relative sizes of nuclear genomes in three diploid cultivars relative to cv Tahiti lime.

Figure 2
Genome size differentiation in cv willow-leaf mandarin and cv Etrog citron.

Newman-Keuls interspecific test results, was confirmed by the quadrimodal distributions of single observations (Fig. 3).

discussion

This is the first systematic genome-size study that has been carried out in *Citrus*. In *C. sinensis*, ARUMUGANATHAN and EARLE (1991) estimated a genome size of 0.76 pg/2C in comparision to chicken erythrocytes, but there are no other previous results for the other species covered here. Diploid cultivars seemed to have relatively small genomes (0.73-0.82 pg/2C). ARUMUGANATHAN and EARLE (1991) obtained similar results for many other fruit trees, e.g. apricot (0.61 pg/2C), peach (0.54 pg/2C), mango (0.91 pg/2C) and pear (1.03 pg/2C).

In *Citrus*, significant intraspecific diversity was noted, in species that have evolved solely by mutations (*C. lemon* and *C. paradisi*) and those that have diversified by sexual crosses and mutations (*C. grandis, C. aurantifolia, C. medica* and *C. reticulata*). Note that the pomelo variety Star Ruby, with a smaller nuclear genome than the other varieties of this species, was developed in a gamma irradiation programme (HENSZ, 1960).

Interspecific diversity was relatively high and the genome size variations noted are in line with phylogenic hypotheses. The extreme values obtained for *C. medica* and *C. reticulata* confirm their ancestral roles in cultivated *Citrus*; the estimated genome sizes for secondary species is in agreement with their assumed hybrid origins (OLLITRAULT and FAURE, 1992). The quantitative differentiation in the three basic taxa is evidence of advanced evolution towards real speciation. It undoubtedly helped maintain high linkage disequilibrium in *Citrus* and could explain some non-Mendelian segregations that have been observed in the progeny of interspecific hybrids (OLLITRAULT and FAURE, 1992). This differentiation and its impact should be taken into account in gene mapping studies and plant breeding programmes involving sexual crossing.

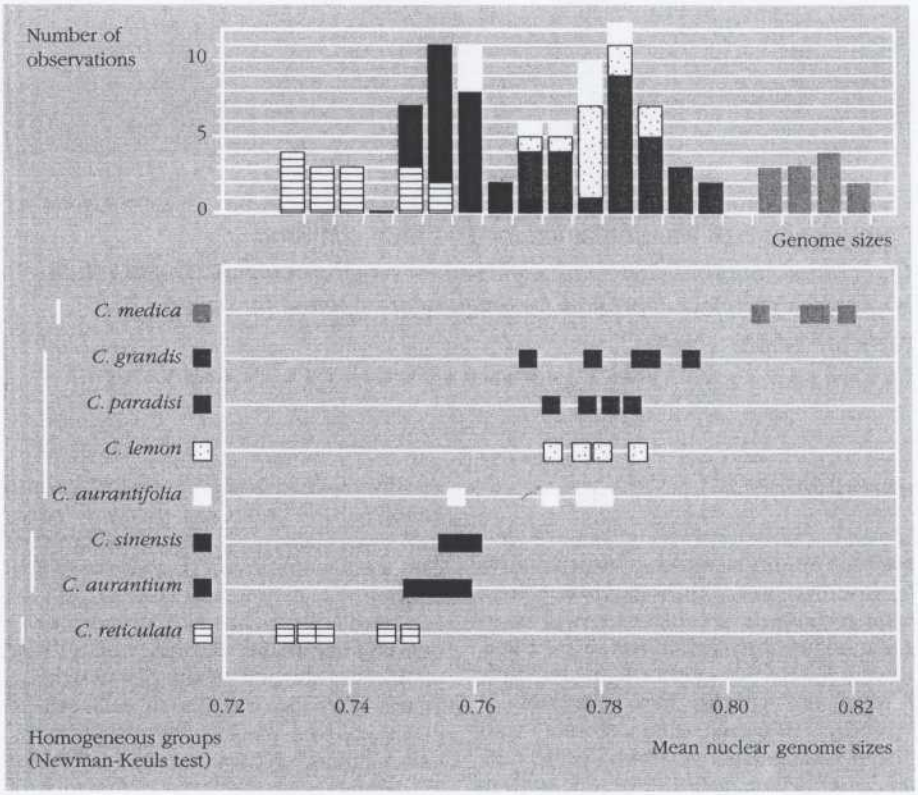

Figure 3
Variations in the size of nuclear genomes in the Citrus *genus (pg/2C).*

references

ARUMUGANATHAN K., EARLE E.D., 1991.
Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. 9 (3): 208-218.

BARRET H.C., RHODES A.M., 1976.
A numerical taxonomic study of affinity relationships in cultivated *Citrus* and its close relatives. Syst. Bot. 1: 105-136.

GREEN R.M., VARDI A., GALUN E., 1986.
The plastone of *Citrus*. Physical map, variation among *Citrus* cultivars and species, and comparaison with related genera. Theor. Appl. Genet. 72: 761-769.

GMITTER F.G., DENG X.X., HEARN C.J., 1992.
Cytogenetic mecanism underlying reduced fertility and seedlessness in *Citrus*. *In*: Proc. of VII Int. *Citrus* Cong., Int. Soc. of Citriculture. p. 113-116.

HENSZ R.A., 1960.
Effect of X-ray and thermal neutrons on citrus propagating material. J. Rio Grande Val. Hort. Soc. 14: 21-25.

OLLITRAULT P., FAURE X., 1992.
Système de reproduction et organisation de la diversité génétique dans le genre *Citrus*. Proceedings of the international conference "Complexe d'espèce, flux de gènes et ressources génétiques", Paris, France, 8-10 January 1992. Paris, France, Bureau des Ressources Génétiques ed., p. 133-151.

RAGHUVANSHI S.S., 1969.
Cytological evidence bearing on evolution in *Citrus*. *In*: Proc. 1st Citrus Symp., Chapman ed., Univ. of California, Riverside 1: 207-214.

YAMAMOTO M., KOBAYASHI S., NAKAMURA Y., YAMADA Y., 1993.
Phylogenic relationships of citrus revealed by RFLP analysis of mitochondrial and chloroplast DNA. Japan. J. Breed., 43: 355-365.