

## CYTOMETRIC DETERMINATION OF GENOME SIZE IN *RUSCUS* (*LILIACEAE*) FROM THE FLORA OF MADEIRA

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With 6 figures and 3 tables

**ABSTRACT.** The phytogeographical origin and relationship between the local and European or African flora are one of the biggest problems concerning the study of Macaronesian vegetation. Flow cytometry can play an important role as a quick source of information to address such problems. In Madeira two species of *Ruscus* occur, *R. streptophyllum* P.F.YEO and *R. hypophyllum* (L.) LOWE var. *lancifolius* LOWE. The *R. streptophyllum* is an endemic species of Madeira whereas *R. hypophyllum* var. *lancifolius* is common to Madeira and Southern of Europe. The flow cytometry was used to determine and compare the genome size of *R. streptophyllum* and *R. hypophyllum* in the local flora. The material was collected from three populations of *R. streptophyllum* and one population of *R. hypophyllum* var. *lancifolius*. The *R. streptophyllum* has 20,24 pg of DNA in the genome. The specific genome variation between populations of *R. streptophyllum* is less than 1%, which shows a great homogeneity in natural variations of this species. The *R. hypophyllum* var. *lancifolius* has 19,07 pg of DNA in the genome. The flow cytogram measurements indicate the presence of 6% more DNA in *R. streptophyllum* than in *R. hypophyllum*. The results demonstrate that genome size variations do exist in *Ruscus* and suggest the presence of two taxa and *R. streptophyllum* has a larger dimension of genome when compared with other European species. However, these results will require further examination.

KEY WORDS: *Liliaceae*; *Ruscus*; Madeira; Flow cytometry; Genome size.

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## INTRODUCTION

The genus *Ruscus* include two forms: one with unbranched stems (*Simplices* series) and other with branched stems (*Ramosae* series). The genus *Ruscus* has a very wide phytogeographical distribution in Europe, with several species growing from the Caucasus to Macaronesia. The taxa of *Simplices* series replace each other geographically, from east to west, in the following order: *R. colchicus* P.F.YEO, *R. hypoglossum* L., *R. hypophyllum* L. (LOWE) and *R. streptophyllum* P.F.YEO. However, *R. aculeatus* L. (*Ramosae* series) is the exception having a ubiquitous geographical distribution (YEO, 1968).

On the island of Madeira a wide diversity was noted in the field, in the genus *Ruscus* (VIEIRA, 1991). Two species (fig 1 & 2) have been described by several authors: *R. hypophyllum* (L.) LOWE var. *lancifolius* LOWE and *R. streptophyllum* P.F.YEO (LOWE, 1830; MENEZES, 1914; YEO, 1968). However, Yeo and more recent YEO, 1968; HANSEN & SUNDING, 1994; VICKERY, 1994 refer to *R. hypophyllum* var. *lancifolius* [= *R. hypophyllum* (L.) LOWE var. *lanceolatus* LOWE] as a synonym of *R. streptophyllum* (FIG. 1 & 2).

*R. streptophyllum* a rhizomatous perennial plant, with simple unbranched stems (fig. 1 & 3 a). The rhizomes are knotty, with short internodes, leaves reduced, forming sheathing rhizome-scales, cauline scales, inflorescence-bracts and flower-bracts. Stems are simple terminated by leaf-like cladodes. The opposite cladodes are coriaceous, with a regular system of nervures. Inflorescence adaxial, with two or three unisexual flowers, normally located near the middle surface of the cladode and facing down. A deflected stele, separated from the basis of cladodes, serves the inflorescence. Flowers evolve by bract, with an oblong-lanceolate shape, 4-11 x 1,5-2,5 mm. The fruit is a scarlet berry, subglobose, with endospermous seeds. *R. streptophyllum* is widely distributed and grows in the more dry places of *Clethro-Laurion* phytoassociation.

*R. hypophyllum* var. *lancifolius* differs from *R. streptophyllum* in that the cladodes are usually alternate and the inflorescence can be adaxial or abaxial (fig. 2 & 3 b). Flowers evolve by a lanceolate bract (3-5 x 10 mm). *R. hypophyllum* LOWE has a more restricted distribution, with references to Ribeiro Frio and Porto Moniz.

Despite such diversity both forms are considered as one species by Vickery (1994). In the present work we intend to evaluate this diversity by flow cytometry technique. This method was used to compare these two morphological forms found on Madeira. Flow cytometry can give a supplementary information to resolve this problem, because has been successfully applied to evaluate plant diversity (GREILHUBER & EBERT, 1994; MARIE & BROWN, 1993; RAYBURN & ANGER, 1990; MOWFORT, & GRIME, 1989).

## METHODS

*R. streptophyllum* was collected from the populations, in Ribeiro Frio, Chão da Ribeira,

Vale da Ribeira da Janela, and *R. hypophyllum* var. *lancifolius* from the native plant collection at S. Vicente. The cladodes for flow cytometric analysis was wrapped in humidifying paper, Whatman n°1, and conserved in hermetic bags, 12 x 8 cm, at +4 °C. To determine the genome size of *Ruscus* the samples were measured in the presence of standard tissues of *Pisum sativum* L., (*P. sativum* L. cultivar Express Long (2C = 8.37 pg, 40.5 % GC; MARIE & BROWN, 1993). The material was macerated, with a razor blade, in presence of 500 µl of buffer system MARIE (MARIE & BROWN, 1993). The samples were filtrated through a 40 µm filter in to an ependorff and 5 µl of RNase (Boehringer) and 25 µl of bromide etidium (Pharmacia) were added. The samples were keet in the dark for 20 minutes for the coloration of the material. After that the samples were transfered into the test-tube of Coulter Epics Elite Cytometer. The DNA measurements were carried out at 605 nm of emission spectra. The cytometric statistical data obtained are shown in tables and figures, where N is the number of events, M - mean, CV - sample full coefficient of variation, and SD standard deviation. The amount of DNA (2C) in the sample was determine by the correlation between the emission wavelengths of sample events and standard, with known genome size.

## RESULTS AND DISCUSSION

In the field, there is clear morphological variation within the genus *Ruscus*, similar to that described for the *R. strepophyllus* (fig. 1 & 3 a) and *R. hypophyllum* var. *lancifolius* (fig. 2 & 3 b).

**TABLE 1** - Geographical localization of *Ruscus* populations, collected for flow cytometric determination of genome size.

Sample	MADU N°	Location UTM	Origin	Status	Ecosystem
1	00110	CB 2323	R. Frio	wild	1
2	00111	CB 0133	Chão da Ribeira	wild	1
3	00113	CB 0830	S. Vicente	cultivated	-
4	00112	BB 9731	V.R. da Janela	wild	1

Note: The number 1 correspond the natural ecosystem, *Clethro-Laurion* phytoassociation, observed to sampled populations.

Samples collected from populations located in Ribeiro Frio, Chão da Ribeira, Vale da Ribeira da Janela and S. Vicente were used in the cytometric determination of the genome

size of these *Ruscus* forms. Table 1 shows the location of the *Ruscus* populations used for the cytometric determination of genome size. In the figures 4 & 5 the cytograms obtained for the *R. streptophyllus* and *R. hypophyllum* var. *lancifolius* are shown. The cytometrical parameters of the four *Ruscus* populations when compared with the standard plant are given in table 2. The nuclear DNA amounts comparison in the *Ruscus* samples (table 3) shows that the populations of *R. streptophyllus* from the R. Frio, Chão da Ribeira and V. da Ribeira da Janela are distinct from the sample collected at S. Vicente. The cellular genome of *R. streptophyllus* was 20,24 pg of DNA. The specific genome variation between its populations is less than 1%, which shows a great homogeneity within wild plant populations. The cytometric analysis also shows a great level of homogeneity within populations (personal unpublished data) in contrast to that the population recognised as *R. hypophyllum* var. *lancifolius* had a DNA amount of 19,07 pg in genome. The flow cytometric measurements indicate that *R. streptophyllus* has 6% more DNA than the *R. hypophyllum* var. *lancifolius*. Such difference in the genome size and cellular DNA amount is enough to sense the existence of distinct taxa (GREILHUBER & EBERT, 1994; BARANYI & GREILHUBER, 1995).

**TABLE 2** - Comparison of the cytometrical parameters of the *Ruscus* populations.

Sample	<i>Ruscus</i>				<i>Pisum sativum</i>		
	N	Mean	SD	CV	Mean	SD	CV
1	665,0	337,0	16,0	4,8	136,5	12,1	8,8
2	677,0	494,2	20,2	4,1	206,9	13,2	6,4
3	490,0	477,4	13,5	2,8	210,8	13,2	6,3
4	1096,0	366,2	14,1	3,9	148,0	10,8	7,3

Moreover, the simultaneous determination of the *Ruscus* genome size from Ch. da Ribeira, *R. streptophyllus*, and S. Vicente, *R. hypophyllum* var. *lancifolius* (fig. 6) permit us to detect the presence of two nuclear clusters, with distinct emission peaks in the area of yellow waves, and with a distinct correlation between the guanine and adenine nucleotids. Na occurrence that can be possible only with the presence of genetically distinct taxa in the sample (MARIE & BROWN, 1993; GREILHUBER & EBERT, 1994).

These results, when compared with the cytometric data for *Ruscus* populations from the Iberian Peninsula shows that *R. streptophyllus* also has a larger genome size than *R. aculeatus* and *R. hypophyllum* from the European Flora. Considering the genome size, it is clear that the *R. hypophyllum* var. *lancifolius* is closer with *R. hypophyllum* from the Iberian

Peninsula, than with the *R. streptophyllus*. These preliminary results suggest that the genome size variation within the *Ruscus* forms on Madeira are sufficient to class *R. streptophyllus* and *R. hypophyllum* as independent taxa. These conclusion agree with the results of compared cytometric analysis of Chão da Ribeira and S. Vicente *Ruscus* samples. However more detailed examinations will be needed and, in particular, from other sources of *R. hypophyllum* on Madeira.

**Table 3** - Genome size for different species of the genus *Ruscus* from the Island of Madeira and the European Continent

Sample	Specie	Origin	2C (pg)
1	<i>R. streptophyllus</i>	Ribeiro Frio	20,24
2	<i>R. streptophyllus</i>	Chão da Ribeira	20,30
3	<i>R. hypophyllum</i>	S. Vicente	19,07
4	<i>R. streptophyllus</i>	V. da R. da Janela	20,17
5	<i>R. hypophyllum</i> *	Coimbra	18,95
6	<i>R. hypophyllum</i> *	Montera del Torero	15,06
7	<i>R. aculeatus</i> *	Sierra Bermeja	19,10
8	<i>R. aculeatus</i> *	Montera del Torero	18,56

\* Unpublished data, MARINA HORJALES & M. DE LAS NIEVES REDONDO personal communication.



Fig. 1 - *Ruscus streptophyllus* P.F. YEO. At the Vale da Ribeira da Janela. Photograph of M.Â.A. PINHEIRO DE CARVALHO & I.C. VALE LUCAS.



Fig. 2 - *Ruscus hypophyllum* LOWE var *lancifolius* LOWE. At São Vicente Garden. Photograph of M.Â.A. PINHEIRO DE CARVALHO & I.C. VALE LUCAS.

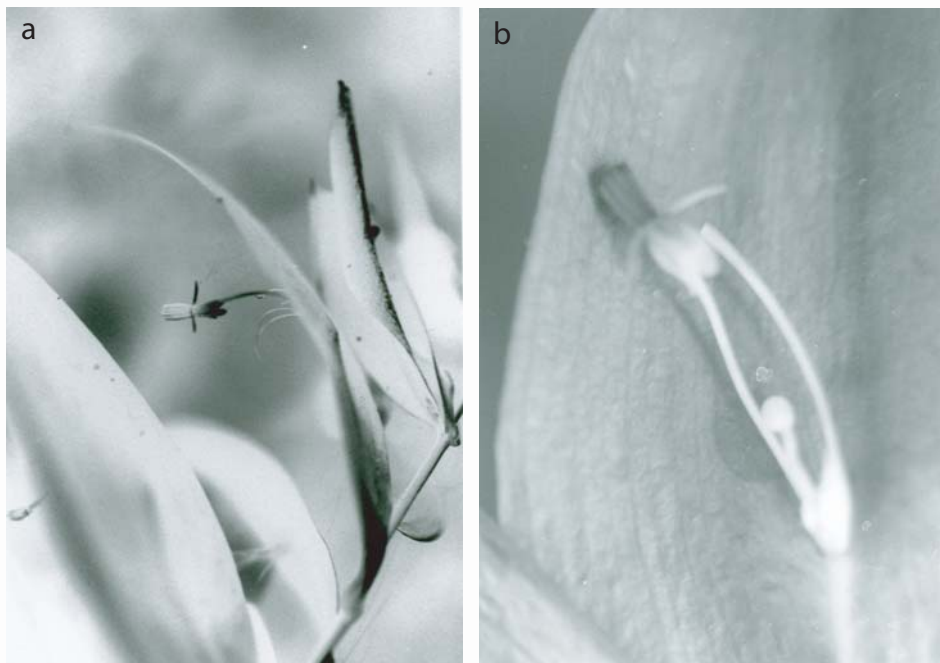


Fig. 3 - The inflorescence in *R. streptophyllum* P.F. YEO (a) and *Ruscus hypophyllum* LOWE var *lancifolius* LOWE (b), with the reduced bract-inflorescence. Photograph of M.Â.A. PINHEIRO DE CARVALHO & I.C. VALE LUCAS.

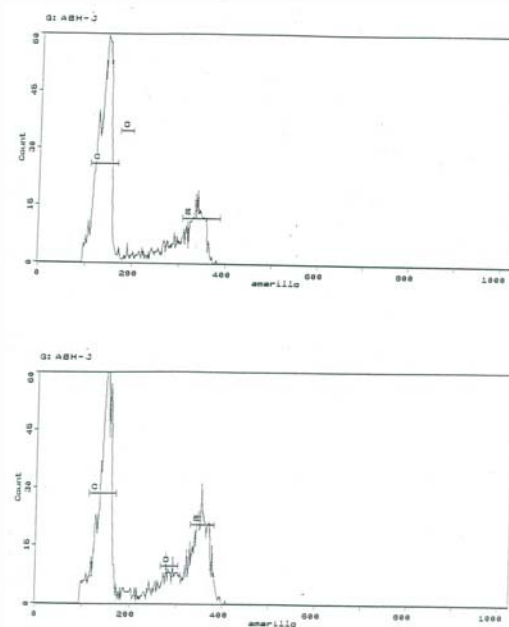


Fig. 4 - Flow cytometry histograms of DNA measurement in the Coulter Epics Elite Cytometer, wave length of emission 605 nm. Samples with *Ruscus streptophyllus* P.F. YEO from Ribeiro Frio (ruscus 1) and Chão da Ribeira da Janela (ruscus 2).

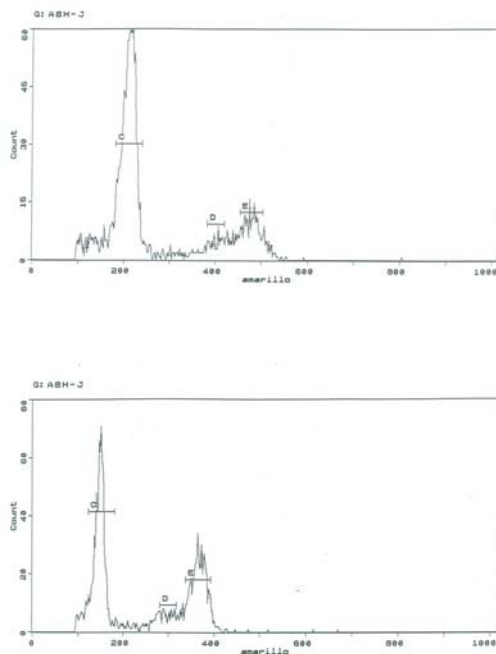


Fig. 5 - Flow cytometry histograms of DNA measurement in the Coulter Epics Elite Cytometer, wave length of emission 605 nm. Samples with *Ruscus streptophyllus* P. F. YEO from Vale da Ribeira da Janela (ruscus 4) and *Ruscus hypophyllum* LOWE var *lancifolius* LOWE from São Vicente (ruscus 3).

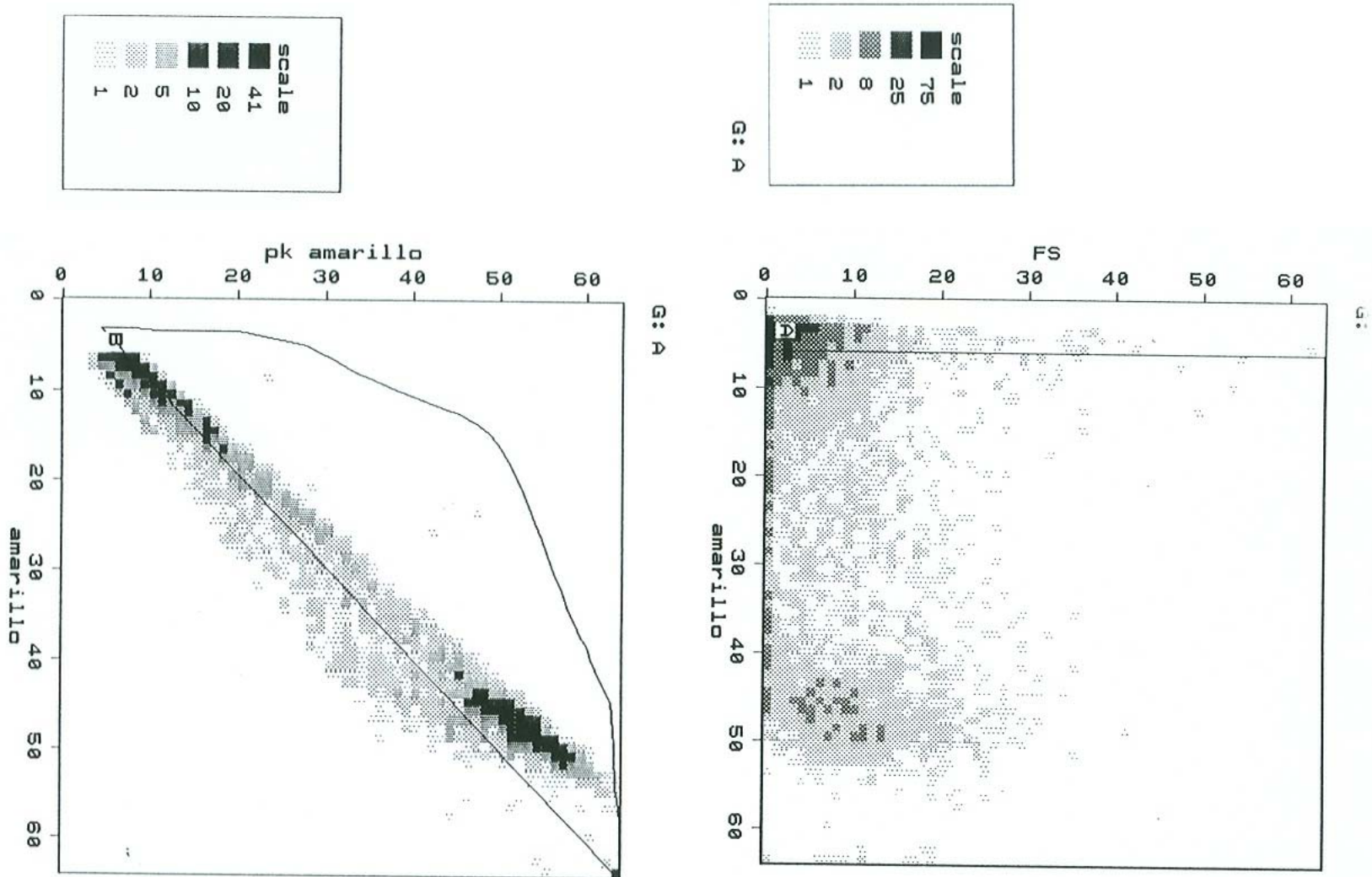


Fig. 6 - Flow cytometry histogram of DNA measurement in the Coulter Epics Elite Cytometer, wave length of emission 605 nm. Sample with *Ruscus hypophyllum* LOWE var *lancifolius* LOWE (a) and *Ruscus streptophyllum* P.F. YEO (b). Histogram shows the presence of two nuclear clusters in the sample.



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