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THE RIBOSOMAL RNA GENE NUMBER AND THE LENGTH OF THE NUCLEOLAR SECONDARY CONSTRICTIONS IN *BELLEVALIA ROMANA* AND *B. DUBIA* (LILIACEAE): A POSSIBLE CORRELATION

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INTRODUCTIONS

RITOSSA and SPIEGELMAN (1965) in *Drosophila melanogaster*, WALLACE and BIRNSTIEL (1966) in *Xenopus laevis* and PHILLIPS *et al.* (1971) in *Zea mays* demonstrated that the genes coding for ribosomal RNA are located in chromosomal regions which encompass the nucleolar organizer and that a strict correlation exists between nucleolar organizer number and rDNA amount. In various species the location of the rRNA genes has been made possible by cytological hybridization of 18S and 28S rRNA to metaphase chromosomes and in most cases the autoradiographic silver grains have been observed in correspondance of the nucleolar secondary constrictions. For example, there is an obvious location of rRNA genes in the NORs in the human chromosome complement (HENDERSON *et al.* 1972), in other primates (HENDERSON *et al.* 1974*a*, 1974*b*), in the Indian muntjac (PARDUE and HSU 1975) and also in plants such as *Vicia faba* (SCHEURMANN and KNALMANN 1975). On the other hand some authors also found cytological evidence of rDNA cistrons in different regions of the chromosome complement (PARDUE *et al.* 1970; AVANZI *et al.* 1972; KANO *et al.* 1976).

In some instances, a variation in length of NOR associated secondary

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Abbreviations used: NOR = Nucleolar organizer region. MAK = Methylated albumin Kieselguhr. SDS = Sodium dodecyl sulphate. SSC = 0.15 M NaCl + 0,015 Na citrate.

constrictions has also been related to variation in rDNA cistron number. MILLER and BROWN (1969), working on *Bufo marinus*, found in individuals with a long nucleolar secondary constriction a significantly higher percentage of ribosomal DNA. Furthermore these authors, studying mutans of the Mexican axolotl (*Ambystoma mexicanum*) carrying homozygous partial NOR deletion, found only 55% of the rDNA present in wild type. In *Mus musculus* it has been found that the chromosomes 12, 16 and 18 carry rDNA cistrons; however, cytological hybridization show significantly more silver grains on chromosome 12, having a long and prominent secondary constriction, than on chromosomes 16 and 18 (ELSEVIER and RUDDLE 1975).

The aim of this paper is to correlate the length of the nucleolar secondary constrictions with the ribosomal gene number as calculated by rRNA/DNA filter hybridization in *Bellevalia romana* and *B. dubia* (Liliaceae).

MATERIALS AND METHODS

Plant material.

Bellevalia romana and *B. dubia* plants were collected in the same locality near Crotone (Calabria, Italy).

Cytological technique.

Karyological analyses were carried out on meristematic cells of actively growing root-tips. After a pretreatement with 0.3% colchicine for four hours the roots were fixed in 3 : 1 absolute ethanol-acetic acid and Feulgen stained.

Preparation and fractionation of labeled rRNAs.

Growin roots of a bulb of *Bellevalia romana* or *Allium cepa* was immersed in 20 ml of distilled water containing 2 mCi of uridine 5-H^3 (New England Nuclear, 28 Ci/mmol). The solutions were shaken for 72 hours with a magnetic stirrer and then « chased » for 6 hours with unlabeled uridine (4 mg in 25 ml of distilled water). RNAs were extracted from roots according to the procedure described by RITOSSA and SPIEGELMAN (1965). 18S and 25S ribosomal RNA were separated from other nucleic acids by MAK column chromatography (MANDELL and HERSCHY 1960). The specific activities of *Bellevalia romana* and *Allium cepa* rRNA's were 63,000 and 35,000 c.p.m./μg respectively.

DNA extraction.

Nuclear preparations from leaves of four individuals for the two species of *Bellevalia* examined were made according to STERN (1971). After nuclear disruption by SDS 2% and two steps of deproteinization with 24 : 1 chloroform-isoamyl alcohol, the DNA was purified by Cs_2SO_4 density gradient in the

Spinco 50 fixed-angle rotor at 22° for 48 hours at 44,000 r.p.m. after the procedure described by FLAMM *et al.* (1969). The O.D. 260/280 and 260/230 ratios of the purified DNAs were 1.82 and 2.2 respectively.

Hybridization of rRNA to DNA.

Hybridization experiments were performed according to the procedure of GILLESPIE and SPIEGELMAN (1965). 50 μg of denaturated DNA were passed through nitrocellulose filters (Schleicher and Schuell, BA 85, 27 mm). The hybridization of the rRNA to DNA was carried out by immersing the DNA-loaded filters in 3 ml of a 2 X SSC solution containing 5 μg of rRNA-H^3. The incubation was for 12 hours at 60°C. After RNAase (Wortington) digestion, the washed and dried filters were counted in a Mark I (Nuclear Chicago) scintillation counter. For further details of the method see MAGGINI (1975).

Measurement of amount of DNA/nucleus.

Feulgen stained telophase nuclei in root meristems were measured in a Barr and Stroud integrating microdensitometer at 546 mμ. The amounts of DNA/2C nucleus of the two *Bellevalia* species were measured with respect to *Allium cepa* telophase nuclei, assuming that the latter species contains 33.5×10^{-12} g/telophase nucleus (VAN'T HOF 1965). For both *Bellevalia* species and for *Allium cepa* was made the mean of the determinations of 50 telophase nuclei.

RESULTS AND DISCUSSION

Bellevalia romana has a diploid chromosome number $2n=8$, with four short nucleolar secondary constrictions on type A and C chromosomes (Fig. 1a); *B. dubia*, with similar chromosome complement ($2n=8$), has six secondary constrictions clearly longer than those of *B. romana* and carried by three pairs of homologous chromosomes of type A, C and D (Fig. 1b). *B. dubia* has also different biotypes variable for the heteromorphism of the nucleolar regions (MAGGINI 1972), but for the present work we used four individuals possessing the karyotype shown in Fig. 1b.

The difficulties of evaluating the length of nucleolar secondary constrictions on colchicine arrested metaphases are well-known. Variability in NOR carrying secondary constrictions length is observed in different metaphases of the same root-tip, depending on the different times of arrest of the cells. However we observed numerous metaphase plates both with and also without colchicine pretreatment and by these observations and by previous works on the same species (D'AMATO 1947; GARBARI 1968; MAGGINI 1972) there is sufficient evidence of a significant difference in the length of the nucleolar secondary constrictions between *Bellevalia romana* and *B. dubia*.

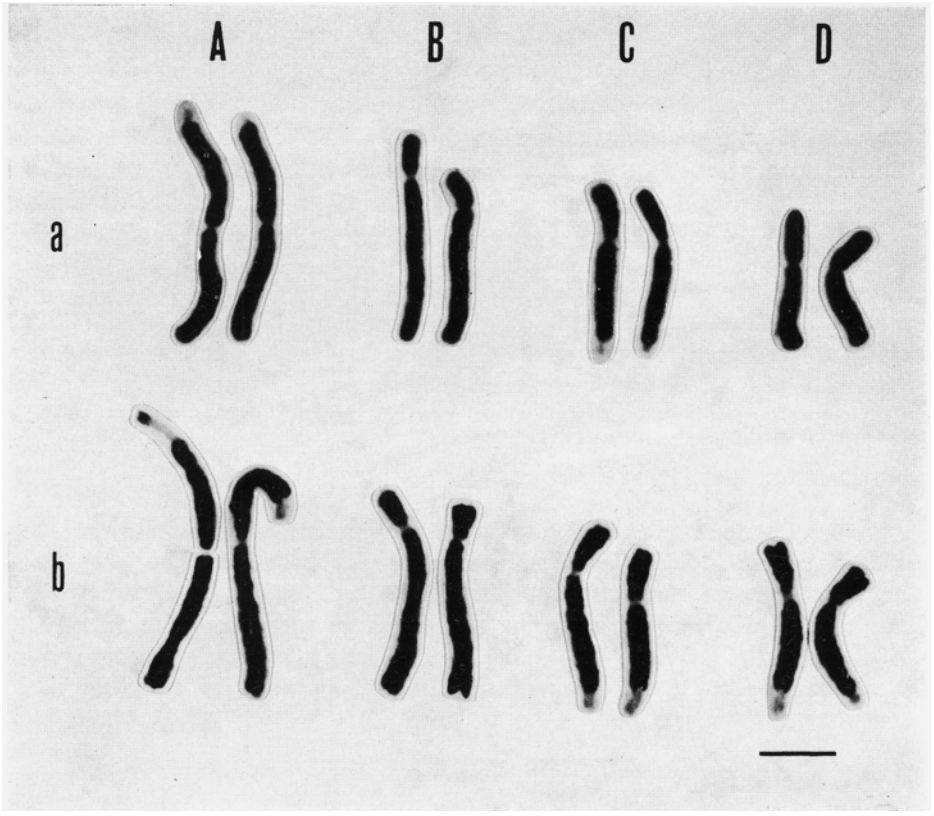

Fig. 1. — Chromosome complement of *Bellevelia romana*. (a) showing four nucleolar secondary constrictions and of *Bellevalia dubia*. (b) with six nucleolar secondary constrictions. Bar represents 5 μm.

The secondary constrictions observed in the two species appear to be really nucleolar since a correspondence exists between their number and the maximum number of nucleoli in interphase nuclei (MAGGINI 1972). This is in accord with studies by many authors in various species (HEITZ 1931; MCCLINTOCK 1934). Furthermore the study of DOERSCHUG (1976) on maize strains carrying from one to seven additional nucleolar organizer fragments obtained by a reciprocal translocation between chromosome 6 and a B chromosome, provides evidence that rDNA genes are located within the nucleolar organizing body. Unfortunately it is impossible to exclude in *Bellevalia romana* and in *B. dubia* the presence of rDNA cistrons in regions of the chromosome complement outside the NORs as demonstrated by cytological hybridization in *Rhynchosciara hollaenderi* (PARDUE

et al. 1970), in *Phaseolus coccineus* (AVANZI *et al.* 1972) and in the rat (KANO 1976).

In order to quantify, in terms of number of genes, the percentages of rRNA/DNA hybridizations, we have measured the nuclear DNA content of the two species by comparative Feulgen microdensitometry. *B. romana* and *B. dubia* have a similar amount of DNA per 2C nucleus (24 X 10^{-12} g). In Table I are reported the saturation values of the hybridization experiments performed with *B. romana* rRNA-H^3. *B. romana* shows 0.044% as DNA hybridized and *B. dubia* reaches the value of 0.135%. Hybridization experiments were also performed with *Allium cepa* rRNA-H^3. The only slightly lower values observed in these experiments are in agreement with the high homologies of the ribosomal RNA observed in species of the Liliaceae family (MAGGINI 1975). Assuming 2 X 10^6 daltons as molecular weight of 18S + 25S rRNA, the percentages of hybridization observed lead to an approximate estimate of 3,200 genes for the ribosomal RNA in *B. romana* and of 9,800 genes in *B. dubia* (Table I).

TABLE I

rDNA gene number and amount of DNA per 2C nucleus.

	B. romana	*B. dubia*
Chromosome number	8	8
NORs number	4	6
% rDNA expt. 1[a]	0.044	0.135
s	± 0.002	± 0.003
% rDNA expt. 2[b]	0.040	0.129
s	± 0.003	% 0.004
pg DNA/2C nucleus	24	24
rDNA genes number	3,200	9,800

a) experiments performed with rRNA-H^3 of *B. romana.*
b) experiments performed with rRNA-H^3 of *A. cepa.*

If a simple correspondence between the NORs number and the amount of ribosomal DNA exists, we would expect in *B. dubia* 4,800 genes, while in fact we find almost twice as many genes, i.e. three times the number of the *B. romana* genes. We conclude by our cytological and molecular hybridization results that a positive correlation exists between the length of the nucleolar secondary constrictions and the number of the ribosomal RNA genes in the *Bellevalia* genus.

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SUMMARY

Karyological analyses and rRNA/DNA hybridization experiments were carried out in *Bellevalia romana* and *B. dubia* (Liliaceae). *B. romana* ($2n=8$) has four short NORs and 3,200 genes for ribosomal RNA; *B. dubia* ($2n=8$) has six NORs clearly longer than those of *B. romana* and 9,800 rRNA genes. The gene numbers are calculated on the basis that both species possess 24×10^{-12} g/2C nucleus. A correlation between cytological and molecular hybridization results is made.