Delpinoa, n.s., **23-24:** 129-136. 1981-1982

Quinacrine fluorescence analysis of the chromosomes of *Macrozamia Miq.* (Cycadales, Zamiaceae)

ALDO MORETTI

Istituto di Botanica dell'Università di Napoli,
Via Foria, 223 - 80139 Napoli, Italy.

Riassunto.

Mediante colorazione con chinacrina, un fluorocromo che evidenzia le regioni eterocromatiche, sono stati esaminati i cromosomi di *Bowenia* Hook., *Lepidozamia* Regel e *Macrozamia* Miq., tre generi australiani di Cycadales.

I cromosomi di tutte le specie di *Macrozamia* hanno mostrato fluorescenza intensa a livello dell'eterocromatina centromerica; fluorescenza ridotta è stata a volte osservata a livello dell'eterocromatina dei segmenti distali dei cromosomi di *M. diplomera* e *M. secunda. M. communis* e *M. pauli-guilielmi* ssp. *pauli-guilielmi* si distinguono dalle altre specie per la mancanza di fluorescenza intensa sulla coppia di cromosomi telocentrici. I cariotipi delle specie di *Macrozamia* sono risultati coincidenti nel numero (2n=18) e nella morfologia (8M, 8S, 2T).

Bowenia e *Lepidozamia* non hanno mostrato alcun tipo di fluorescenza, risultando pertanto cariologicamente distinti da *Macrozamia.*

I differenti risultati ottenuti per i tre generi e tra le specie di *Macrozamia* sono stati discussi per le loro implicazioni tassonomiche.

INTRODUCTION.

Cycads are a primitive group of Gymnosperms at present confined to the tropical and subtropical regions of both hemispheres. The ten genera extant today are subdivided into the families of the Boweniaceae, Cycadaceae, Stangeriaceae and Za-

Key words: *Cycadales, Zamiaceae, Macrozamia;* Quinacrine fluorescence; Cytotaxonomy.

miaceae (JOHNSON, 1959; STEVENSON, 1981). On account of their very ancient origin, dating back to the Paleozoic, the cycad genera appear from their morphology and distribution to be relic forms with many lost relatives (JOHNSON, 1959).

The karyotypes of the genera confirm the primitiveness of the group. Although within each genus the species karyotypes are very similar, with *Zamia* the only exception (NORSTOG, 1980; MORETTI & SABATO, 1983), and although many genera have the same chromosome number, the karyotype patterns differ clearly among the genera (MARCHANT, 1968). Therefore, it is very difficult to obtain support for the explanation of the taxonomic and phylogenetic relationships among the cycad genera from the comparative analysis of the karyotypes. In this regard, the study of the chromosome heterochromatic regions can be very useful. Such a study has been done in cycads only on one Asian species of *Cycas* (TANAKA & HIZUME, 1980) and on two African species of *Encephalartos* (MOGFORD, 1979). The great quantity of heterochromatin found in these species suggests the extension of the investigation to other cycad genera.

With this aim, several species belonging to the Australian genera *Bowenia, Lepidozamia* and *Macrozamia* were investigated in the present work. The quinacrine fluorescence technique was used to study the occurrence of heterochromatin.

MATERIALS AND METHODS.

Cycad species examined in this investigation are listed in Table 1.

For the cytological preparations actively growing root-tips were kept for 20 hours in 10 ppm solution of IPC (isopropyl-N-phenyl carbamate, SIGMA Chem. Co.) at 15°C to shorten the chromosomes which in cycads are very long. The IPC solution was prepared as reported by NORSTOG (1980). After pretreatment in IPC, root-tips were fixed for 2 hours in 1/3 acetic acid/ethanol and were then washed with distilled water.

All preparations were stained with quinacrine dihydrochloride (BDH) as suggested by VOSA (1970). The terminal meriste-

Genus	Species
Bowenia Hook. f.	*B. serrulata* (W. Bull) Chamberlain
	B. spectabilis Hook. f.
Lepidozamia Regel	*L. hopei* Regel
	L. peroffskyana Regel
Macrozamia Miq.	*M. communis* L. Johnson
	M. diplomera (F. Muell.) L. Johnson
	M. heteromera C. Moore
	M. miquelii (F. Muell.) A. DC.
	M. moorei F. Muell.
	M. pauli-guilielmi ssp. *pauli-guilielmi* W. Hill & F. Muell.
	M. riedlei (Fisch. ex Gaudich.) C. A. Gardn.
	M. secunda C. Moore
	M. spiralis (Salisb.) Miq.

TABLE 1 — Cycad species examined. All the plants are in cultivation in a greenhouse at the Botanical Garden of Naples (Italy).

matic parts (2 mm) of the root-tips were kept for a few minutes in 45% acetic acid and squashed in this solution until a fine suspension of cells was obtained. A glycerin-albumen coated cover-slip was pressed on the suspension. The cover-slips were separated by inverting the slides in a ridged dish of absolute ethanol and the preparations were stained for 5 minutes at room temperature in 0.5% quinacrine in absolute ethanol. After rinsing briefly in absolute ethanol and air drying, the preparations were mounted in distilled water and the cover-slips were ringed with a rubber solution to prevent dehydration.

The observations were made with a Leitz Dialux microscope using a mercury vapour lamp as a source of ultraviolet light, with a BP 436/7 exciter filter and a LP 490 barrier filter. The micrographs were taken on Ilford HP5 film.

RESULTS AND DISCUSSION.

Among the three genera of cycads examined only *Macrozamia* displayed quinacrine fluorescent bands. The chromosomes of all the species of *Macrozamia* showed enhanced quinacrine fluorescent bands at the centromeric regions (an exemplifying karyotype is shown in Fig. 1). *M. communis* and *M. pauli-guilielmi* ssp. *pauli-guilielmi* distinguish themselves from the other species in that their pair of telocentric chromosomes appeared quite devoid of fluorescent bands (Fig. 2). In some chromosomes of *M. diplomera* and *M. secunda,* moreover, reduced quinacrine fluorescence in the form of dark bands at the distal regions was sometimes seen (Fig. 3).

It has been demonstrated several times (WEISBLUM, 1973; WEISBLUM & DE HASETH, 1972; VOSA, 1975), that the quinacrine fluorescence of heterochromatic regions is enhanced by the presence of repetitive sequences of adenine-thymidine nucleotides and is reduced by guanine-cytidine nucleotides. On this basis it can be argued that most of the heterochromatin revealed by quinacrine in the *Macrozamia* species is composed of adenine-thymidine nucleotides.

The karyotypes of all the *Macrozamia* species are similar in chromosome number ($2n=18$) and morphology, and the chromosome complements consist of four pairs of metacentrics, four pairs of submetacentrics and one pair of telocentrics; it cannot be exclude however that one or two pairs of submetacentric chromosomes are formed by acrocentric chromosomes. These complements are in agreement with those reported by MARCHANT (1968) for *M. communis, M. heteromera, M. miquelii, M. pauli-guilielmi* and *M. secunda;* this author also reported the putative presence of some acrocentric chromosomes (see the figures of his paper). In the same paper, MARCHANT reports on the occurrence of terminal heterochromatic knobs on some chromosomes in all karyotypes examined by Feulgen staining after a cold treatment and/or a treatment in alpha-bromonaphthalene. Considering the similar location, the terminal heterochromatic knobs of MARCHANT might correspond to the dark distal regions of reduced fluorescence observed in the present work (Fig. 3).

Fig. 1 - The quinacrine stained chromosomes of *M. secunda* showing enhanced fluorescence at the centromeric regions. (x 1100).

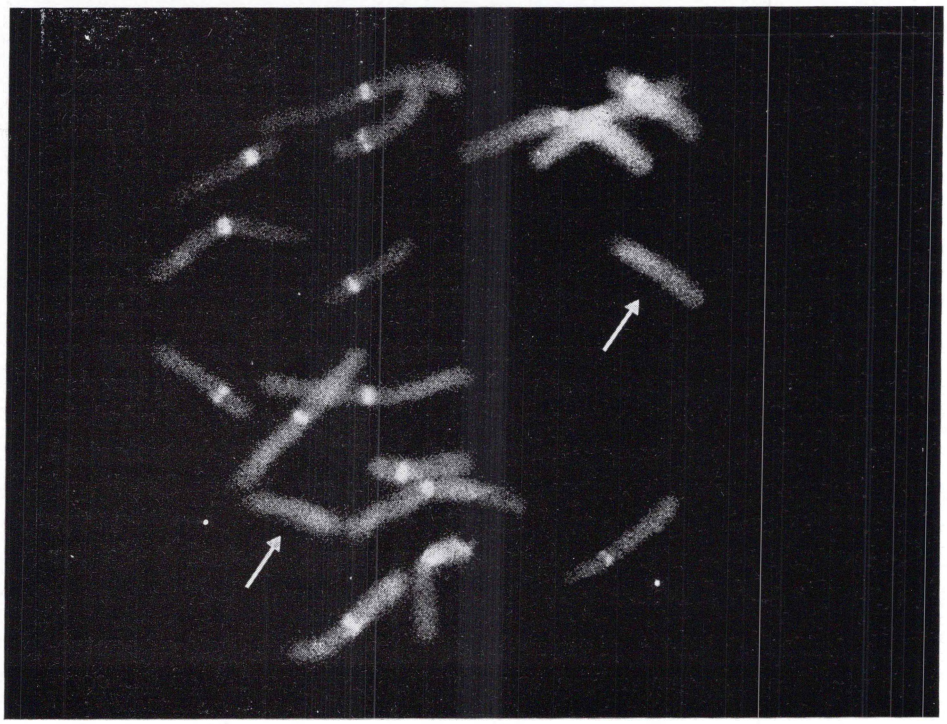

Fig. 2 - The quinacrine stained chromosomes of *M. communis* showing enhanced fluorescence at the centromeric regions except that on the pair of telocentrics (arrowed). (x 1200).

Fig. 3 - The quinacrine stained chromosomes of *M. secunda.* Besides enhanced fluorescence at centromeric regions, note on the distal regions of some chromosomes the dark bands (arrowed) of reduced fluorescence. (x 1300).

Although *Bowenia* and *Lepidozamia* species did not show any quinacrine fluorescence, the different responses to quinacrine displayed by the three genera examined appear to be of taxonomic interest. *Lepidozamia* (Zamiaceae), in the past included into the *Macrozamia* genus (Zamiaceae), is today considered an independent genus (JOHNSON, 1959); *Bowenia,* once considered a representative of the Zamiaceae, has been recently included in the new family Boweniaceae (STEVENSON, 1981). The karyological differences resulting between *Macrozamia* on the one hand and *Bowenia* and *Lepidozamia* on the other should confirm the systematic separations proposed on a morphological basis for *Bowenia* and *Lepidozamia.*

A further result of taxonomic interest is the lack of centromeric fluorescence on the telocentric chromosomes of *M. com-*

munis and *M. pauli-guilielmi* ssp. *pauli-guilielmi* (Fig. 2). In this case, the karyological data do not corroborate the systematic position of the two species; in fact they are included separately into the two sections of *Macrozamia; M. communis* into the section *Macrozamia* and *M. Pauli-guilielmi* into the section *Parazamia* (JOHNSON, 1959).

Acknowledgment — I'm very grateful to Dr C. G. Vosa of the Botany School of Oxford (England) for his helpful advise and criticism during the course of this work which was done in part in his laboratory.

SUMMARY

The chromosomes of several species of the Australian cycads *Bowenia* Hook., *Lepidozamia* Regel and *Macrozamia* Miq. have been examined by fluorochrome quinacrine in order to detect the heterochromatic regions.

The chromosomes of all the examined species of *Macrozamia* showed enhanced fluorescence at level of the centromeric heterochromatin; reduced fluorescence has been seen at level of the heterochromatin of the distal segments on some chromosomes of *M. diplomera* and *M. secunda.* The pair of telocentric chromosomes of *M. communis* and *M. pauli-guilielmi* ssp. *pauli-guilielmi* did not show any fluorescence type. The karyotypes of the *Macrozamia* species resulted similar in number (2n=18) and morphology (8M, 8S, 2T).

The chromosomes of *Bowenia* and *Lepidozamia* did not show any fluorescence type.

The different results obtained among the three genera and among the *Macrozamia* species have been discussed for their taxonomic implications.

REFERENCES

JOHNSON L. A. S., 1959. *The families of cycads and the Zamiaceae of Australia.* Proc. Linn. Soc. N. S. W., **84**: 64-117.

MARCHANT C. J., 1968. *Chromosome patterns and nuclear phenomena in the cycad families Stangeriaceae and Zamiaceae.* Chromosoma, **24**: 100-134.

MOGFORD D. J., 1979. *Heterochromatin in* Encephalartos. Cytologia, **44**: 951-954.

MORETTI A. & S. SABATO, 1983. *Karyotypes of* Zamia paucijuga *Wieland* (*Zamiaceae*). Pl. Syst. Evol., in press.

NORSTOG K., 1980. *Chromosomes number in* Zamia (*Cycadales*). Caryologia, **33**: 419-428.

STEVENSON D. W., 1981. *Observations on ptyxis, phenology, and trichomes in the Cycadales and their systematic implications.* Amer. J. Bot., **68** (**8**): 1104-1114.

TANAKA R. & M. HIZUME, 1980. *C banding treatment for the chromosomes of some gymnosperms.* Bot. Mag. Tokyo, **93**: 167-170.

VOSA C. G., 1970. *Heterochromatin recognition with fluorochromes.* Chromosoma, **30**: 366-372.

VOSA C. G., 1975. *The use of Giemsa and other staining techniques in karyotype analysis.* Curr. Adv. Pl. Sci., **6**: 495-510.

WEISBLUM B., 1973. *Fluorescent probes of chromosomal DNA structure: three classes of acridines.* Cold Spring Harb. Symp. quant. Biol., **38**: 441-449.

WEISBLUM B. & P. L. DE HASETH, 1972. *Quinacrine: a chromosome stain specific for deoxyadenylate-deoxythymidylate-rich regions of DNA.* Proc. nat. Acad. Sci. U.S.A., **69**: 629-632.