Cytological Studies in the Cycads: Sex Chromosomes in *Cycas*

BY

A. ABRAHAM AND P. M. MATHEW

(*Botany Department, Kerala University, Trivandrum, India*)

With one Plate and eight Figures in the Text

ABSTRACT

The chromosomes of *Cycas pectinata* are studied from root tip and pollen mitoses. The male and female plants showed the same number of chromosomes ($2n = 22$) with almost identical chromosome morphology. The only difference is that in the female plant two chromosomes of the somatic complement (pair III) bear satellites, while in the male the same pair is heteromorphic with only one of its members bearing a satellite. This becomes further clear when two types of haploid complements are noticed in pollen mitosis, one type possessing a satellited chromosome, and the other showing none bearing a satellite.

The pair of chromosomes which is heteromorphic in the male is assumed to be associated with sex determination in *Cycas*, the male being the heterogametic sex with XY type of sex chromosomes.

INTRODUCTION

SINCE the discovery of sex chromosomes in dioecious organisms, a number of plants have been cytologically investigated to determine the type of chromosome mechanism which may be responsible for the expression of morphological differences between the sexes. Different types of sex chromosome complexes have been recognized, including (1) female XX, male XY as in *Melandrium album* (Blackburn, 1923) and *Humulus lupulus* (Jacobson, 1957), (2) female XX, male XY_1Y_2 as in *Humulus japonicus* (Winge, 1923; Jacobson, 1957), *Rumex acetosa* (Kiharo and Ono, 1923 *a*, *b*) and *Rumex hastatulus* (Smith, 1955), (3) female XY, male XX as in *Fragaria elatior* (Kihara, 1926), and (4) female XX, male XO as in *Dioscorea sinuata* (Meurman, 1925).

In dioecious Gymnosperms very little has been done on the cytology of sex determination. This is probably due to the fact that in many of the dioecious Gymnosperms the male and female individuals are identical in their external vegetative features, and as they normally take many years to become reproductively mature, one has to wait for a long period to discern the sex of a particular plant. However, in *Ginkgo biloba* evidence has been furnished as to the presence of heteromorphic chromosomes associated with sex determination (Newcomer, 1954; Lee, 1954), and an XY type of sex mechanism has been assumed in this plant (Lee, 1954). The work reported here was undertaken to study the general morphology of the chromosomes in the male

[Annals of Botany, N.S. Vol. 26, No. 102, April, 1962.]

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

and female plants of *Cycas*, and to examine in particular whether the differences in the sexes of *Cycas* could be correlated with any corresponding chromosomal difference.

Material and Methods

Material for this study was obtained from male and female plants of *Cycas pectinata* growing in the Botanical garden of the University College. Somatic chromosome studies were made from both root-tip mitosis and pollen mitosis. In the initial stages of this investigation root tips were taken from seedlings of undetermined sex. Later the work was made easier by the availability of suckers from both male and female plants. The suckers were separated from the parent plants and transplanted in separate pots, where they yielded sufficient root-tip material after 4 months. For pollen-mitosis studies microsporophylls at the correct stage were collected from two male plants.

Both root tips and microsporophylls were fixed in Carnoy's fluid (absolute alcohol—glacial acetic acid—chloroform in the ratio 3:1:1). A trace of iron acetate was added to the fixative, and this was found to increase the staining capacity of the chromosomes. Root tips were treated in 8-oxyquinoline and simultaneously cooled at 0° C. for 3–4 hours prior to fixation. In the case of root tips, several fixation periods ranging from 2 days to 2 weeks have been tried, and a fixation time of about 1–2 weeks was found necessary to yield good results. Fixed materials kept under low temperature in a refrigerator for even longer periods produced better results. Acetocarmine squash technique was followed in making cytological preparations. No cooling or pretreatment with 8-oxyquinoline was done in the case of microsporophylls. The microsporangia were hydrolysed in normal HCl for 15–25 minutes just before smearing, and this was found useful in softening the thick walls of the microspores.

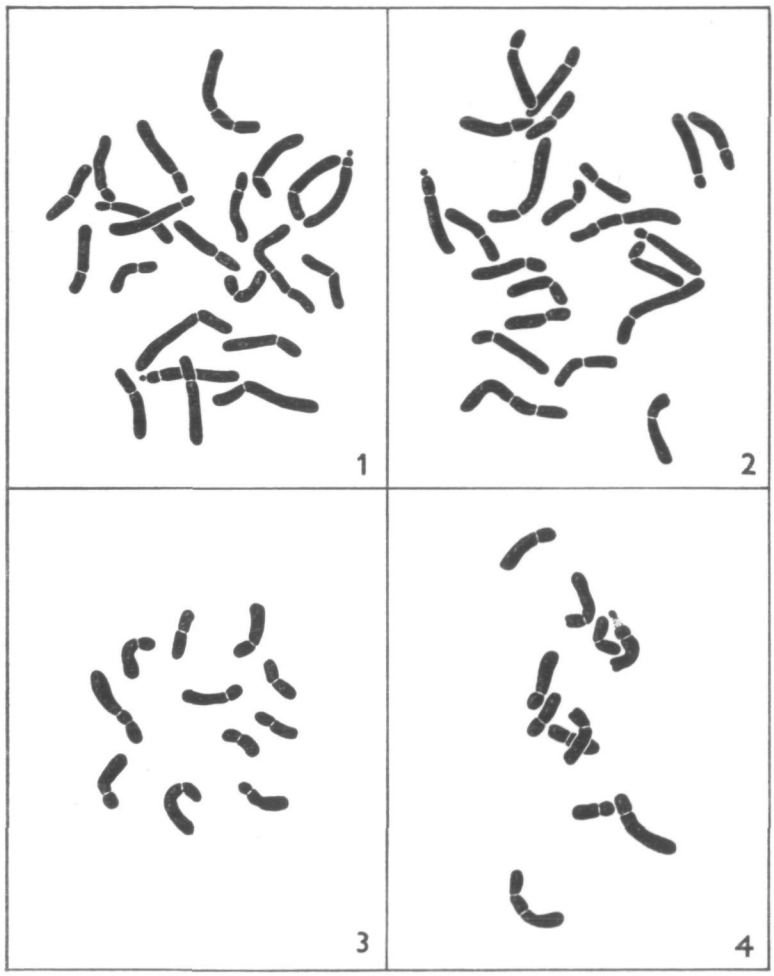
Results

Twenty-two chromosomes were counted in the root-tip cells of the female plants (Pl. I, Fig. 1). They were fairly large, ranging from 7·5–15 μ in length, and consisting of 2 pairs of long, 6 pairs of medium-sized, and 3 pairs of short chromosomes. One of the longest pairs showed two constrictions, one median and the other submedian. Except for one of the shortest pairs which had median constriction, all the others possessed either submedian or subterminal constrictions. Both the members of a medium-sized pair of chromosomes carried satellites at the end of the short arm (see chromosome at 6 o'clock and 2 o'clock in Pl. 1, Fig. 1 and Text-fig. 1).

The male plant also had 22 chromosomes in root-tip cells (Pl. 1, Fig. 2) showing similar morphological characters to those in the female, except in the case of the pair III (Text-fig. 6) where only one member is satellited, not both as in the female (see chromosome at 9 o'clock in Pl. 1, Fig. 2 and Text-fig. 2).

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

The haploid complements, as observed in several pollen grains during pollen mitosis, consisted of 11 chromosomes. They were a little more condensed



(TEXT-FIGS. 1–4. × 1,000. Explanatory diagrams of Figs. 1–4 in Plate I)

FIG. 1. *Cycas pectinata* female plant showing 22 chromosomes. Two chromosomes are satellited (chromosome at 6 o'clock and 2 o'clock).

FIG. 2. *C. pectinata* male plant showing 22 chromosomes. Only one chromosome bears satellite (chromosome at 9 o'clock).

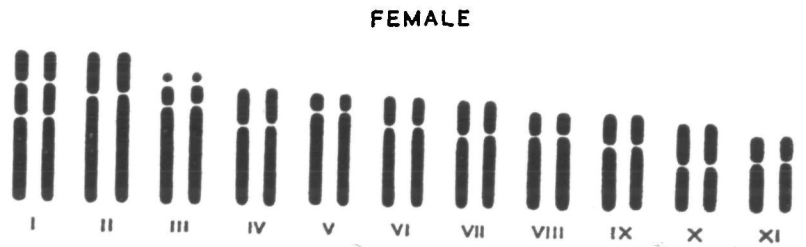
FIG. 3. Pollen mitosis in *C. pectinata*. In the haploid complement there is no satellite-bearing chromosome.

FIG. 4. Another pollen mitosis in *C. pectinata*. One chromosome is satellited.

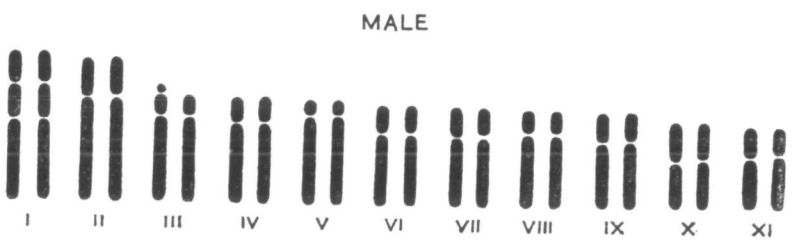
than those in the root-tip cells. In each haploid set examined there were 2 long, 6 medium-sized, and 3 short chromosomes. On close examination of several haploid complements it was seen that in about half of them there was no satellite-bearing chromosome (Pl. 1, Fig. 3 and Text-fig. 3), while in the

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

other half a medium-sized chromosome with submedian constriction possessed a satellite (Pl. 1, Fig. 4 and Text-fig. 4).



5



6

7 8

(Text-figs. 5–8. Diagrammatic representation of Figs. 1–4 in Plate 1 and Text-Figs. 1–4)

Fig. 5. Diploid complement of the female plant. Both the members in the pair III of chro mosomes are satellited.

Fig. 6. Diploid complement of the male plant. Pair III is heteromorphic, one of its members bein g satellited and the other non-satellited.

Fig. 7. A haploid set from pollen mitosis showing no satellite-bearing chromosome.

Fig. 8. Another haploid set from pollen mitosis. Chromosome III bears a satellite.

Discussion

Previous cytological data show that 22 is the commonly occurring somatic chromosome number in the genus *Cycas* (Darlington and Wylie, 1955) as reported in *C. circinalis*, *C. rumphii*, and *C. revoluta* (Sax and Beal, 1934).

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

However, Nakamura (1929) has reported $2n = 22$ and 24 for *C. revoluta*. The present finding of $2n = 22$ in *C. pectinata*, for which there is no previous cytological record, also agrees with those reported in the other three species of *Cycas*. Except for the one report of $2n = 24$ in *C. revoluta*, it may be considered that all the species of *Cycas* so far investigated are diploids on the basic number 11.

A karyotype analysis of the male and female individuals of *C. pectinata* reveals that recognizable chromosomal difference exists between the two sexes. The idiogram of the female individual shows that both the members of the pair III are satellited (Text-fig. 5), while that of the male individual clearly shows that the same pair is heteromorphic, with only one of its members bearing a satellite (Text-fig. 6). A more or less similar feature has been reported in *Ginkgo biloba* (Lee, 1954), where four satellited chromosomes (2 pairs) are present in the female, while in the male only three chromosomes show satellites. In the male the heteromorphic pair with one of its members lacking a satellite has been assumed to constitute the sex chromosomes, and based on this an XY type of sex determination is suggested in *Ginkgo biloba*, the female being homogametic.

Comparing the results obtained during pollen mitosis in several pollen grains it has been possible to recognize two types of haploid complements, one type possessing a satellited chromosome (chromosome III in Text-fig. 8), and the other consistently lacking the satellite in the corresponding chromosome (Text-fig. 7), and this appears to make it further clear that in the male of *Cycas pectinata* there is a heteromorphic pair of chromosomes.

In the absence of any other observable chromosomal difference between the two sexes, one is reasonably inclined to correlate the chromosomal difference discussed above with the dioecism existing in *Cycas*. The male plant, which possesses a heteromorphic pair of chromosomes, is considered to be heterogametic with XY type of sex chromosomes, Y being non-satellited, and the female is homogametic (XX).

Literature Cited

Blackburn, K. B., 1923: Sex Chromosomes in Plants. Nature, **112**, 687–8.

Darlington, C. D., and Wylie, A. P., 1955: Chromosome Atlas of Flowering Plants. George Allen and Unwin Ltd., London.

Jacobson, P., 1957: The Sex Chromosomes in *Humulus*. Hereditas, **43**, 357–70.

Kihara, H., 1926: Über die Chromosomenverhältnisse bei *Fragaria elatior*. Zeits. Ind. Abst. Vererb., **41**, 41–42.

—— and Ono, T., 1923*a*: Cytological Studies on *Rumex* L. I. Chromosomes of *Rumex acetosa* L. Bot. Mag., **37**, 84–90.

—— —— 1923*b*: Cytological Studies on *Rumex* L. II. On the Relation of Chromosome Number and Sexes in *Rumex acetosa* L. Bot. Mag., **37**, 147–9.

Lee, C. L., 1954: Sex Chromosomes in *Ginkgo biloba*. Amer. Jour. Bot., **41**, 545–9.

Meurman, O., 1925: The Chromosome Behaviour of some Dioecious Plants and their Relation with Special Reference to the Sex Chromosomes. Soc. Sci. Fenn. Comm. Bilo, **2**, 1–105.

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

*NAKAMURA, T., 1929: Mem. Coll. Sci. Kyoto, Ser. B, **4**, 353.
NEWCOMER, E. H., 1954: The Karyotype and Possible Sex Chromosomes of *Ginkgo biloba*. Amer. Jour. Bot., **41**, 542–5.
*SAX, K., and BEAL, J. M., 1934: J. Arnold Arbor., **15**, 225.
SMITH, B. W., 1955: Sex Chromosomes and Natural Polyploidy in Dioecious *Rumex*. Jour. Hered., **46**, 226–32.
WINGE, O., 1923: On Sex Chromosomes, Sex Determination and Preponderance of Female in Some Dioecious Plants. Compt. Rend. Trav. Lab. Carlsberg, **15**, 1–26.

* *Vide* Darlington, C. D., and Wylie, A. P., 1955.

EXPLANATION OF PLATE

(Figs. 1–4. All photographs at a magnification of ×1,000)

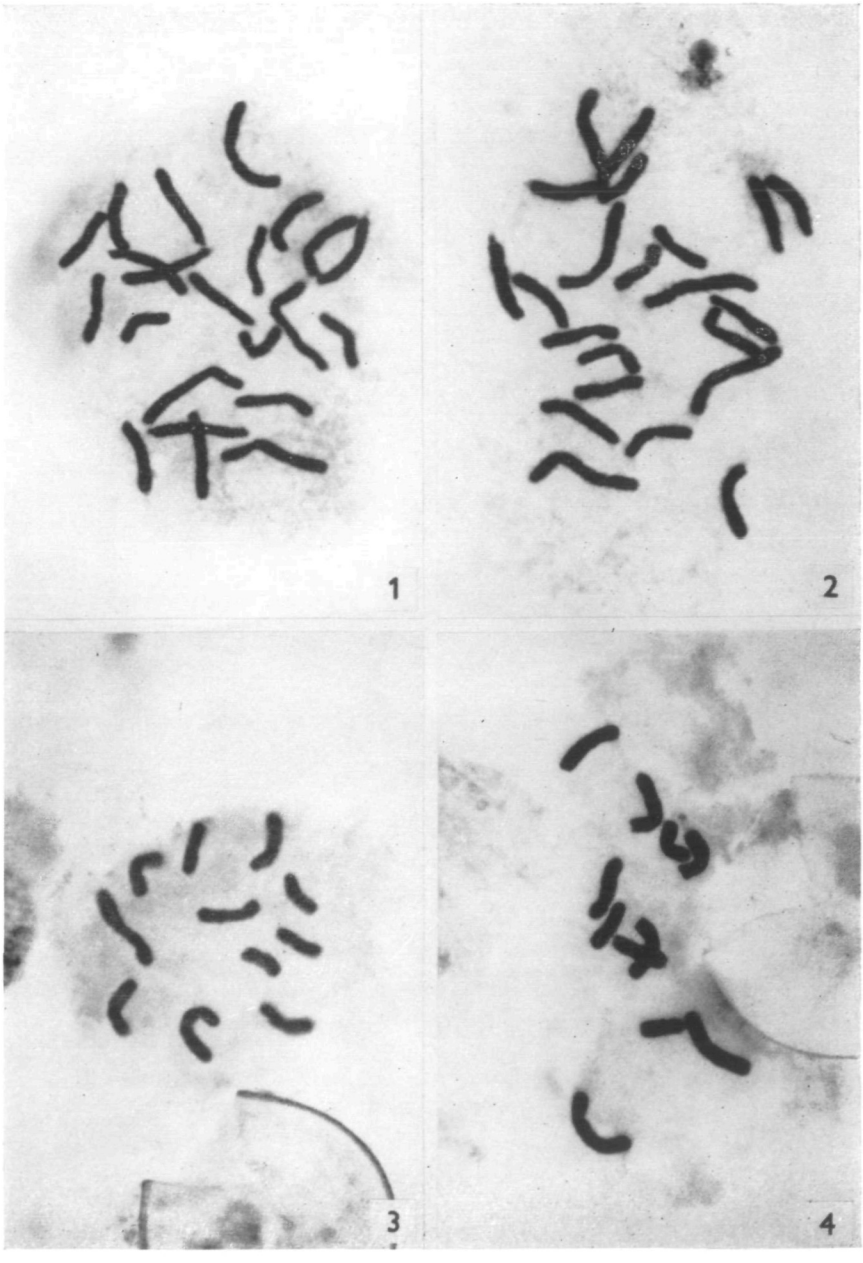
FIG. 1. Somatic mitosis in a root-tip cell of *Cycas pectinata* (female plant) showing 22 chromosomes. Two of the medium-sized chromosomes possess satellites.

FIG. 2. Root-tip mitosis in *C. pectinata* (male plant) showing 22 chromosomes. Note there is only one satellite-bearing chromosome.

FIG. 3. Pollen mitosis in *C. pectinata*. There are 11 chromosomes in the haploid complement, and none of them bears a satellite.

FIG. 4. Another haploid complement from pollen mitosis showing 11 chromosomes. One of the medium-sized chromosomes bears a satellite.

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011



A. ABRAHAM AND P. M. MATHEW

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011

Downloaded from aob.oxfordjournals.org by guest on January 26, 2011