The occurence of Polyteny in the dividing cells of three species of *Crotalaria* L. from two locations in Nigeria

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Abstract

The occurrence of polytene chromosomes in mitotic and meiotic tissues of three *Crotalaria* species is reported. A comparison of the frequency of occurrence of cells with these polytene chromosomes in the species of *Crotalaria* studied and their estimated index of polyteny are also presented. The origin of the polytene chromosomes and their probable functions are discussed.

Introduction

Chromosome polyteny, a situation where multistranded chromosomes result from failure of chromatids to separate, also described as polysomaty, has been reported in a number of organisms. They have been reported in salivary gland nuclei malphighian tubules, fat bodies, ovarian nurse cells and gut epithelia (Laird, 1980; Richards, 1980). Polytene chromosomes have been observed in maturing eggs of amphibians and beetles (Pai, 1976). They have been observed in nurse cells in *Drosophila* (Painter and Raindorp, 1939), in the intestinal cells of the larval mosquito (Berger, 1938), in dividing cells of Locusts (Croft and Jones, 1986), in *Chironomus tentans* (Egyhazi *et. al,* 1986), and *Sciara coprophila* (Eastman, 1980). They have also been reported in the apices of *Spinacia* (Berger, 1941), and tapetal cells of *Spinacia* (Witkuks, 1949). Huskins (1948) and Huskins and Steintz (1948) studied the problem of polytene chromosomes in *Rhoeo discolour* and attempted to deduce a relationship between polyteny and differentiation.

In all cases there is evidence that chromosomes undergo great multiplication or multiple replication without the individual strands separating. There is a good correlation between nuclear size, the amount of DNA, the degree of polyteny and the overall cell size. The cells with polytene chromosomes sometimes reach up to 10 times the size of normal cells.

In iliae epithelia of mosquito larvae there is evidence of reduction division within the polysomatic cells resulting in the imaginal epithelial cels, which are smaller than the cells from which they arose (Grell, 1946).

In all the reports, the function and destiny of these polysomatic cells have been suggested. They are barely known or studied in plants and until now its occurence in *Crotalaria* has never been reported. This report is aimed at providing first information about the occurrence of polyteny in the plant *Crotalaria* and creating the basis for further investigation into its potential in *Crotalaria* genetics.

Materials and Methods

Young flower buds of *C. retusa, C goreensis* and *C. cylindrocarpa* were collected from the wild from Ife and Port- Harcourt, in Nigeria, fixed directly in acetic acid-ethanol (1:3 v/v) fixative and stored in the refridgerator. Mature seeds of the three species were 'sown' in petridishes after soaking in concentrated sulphuric acid (H_2SO_4) for twenty minutes and rinsing in tap water. This acid treatment broke the dormancy of the seeds which germinated within 36 hours thereafter. Root tips were harvested between 9.00 a.m. and 10.00 a.m. on the fifth day and fixed directly in acetic acid-ethanol (1:3 v/v) fixative. The root tips were stored in small vials in a refridgerator until examined.

Anthers from the young flower buds were squashed on clean slides and stained with FLP orcein (Olorode, 1974). Clean cover slips were placed over them and the slides were allowed to stay for 24 hours before examination. The root tips were similarly squashed after hydrolysing in 18% HCl for ten minutes.

The number of cells with polytene chromosomes was noted and was also estimated as a percentage of the total number of dividing cells observed. The diameters of the cells were measured in microns at a magnification of X

1000 using a calibrated occular graticule. The index of polyteny was estimated as a ratio of size of cells with polytene chromosome to size of the regular cells with polytene chromosome to size of the regular cells around them. Diameter of regular cells and of the polysomatic cells were measured using the occular graticule and then reduced to ratios.

Results

The cells with polytene chromosomes were observed scattered among dividing cells and were found to be numerous in squashes that showed a high frequency of metaphase cells. Table 1 shows the frequency of occurrence of cells with polytene chromosome in the three *Crotalaria* species from the two locations.

Polyteny occurred regularly in the *Crotalaria* species studied and comparably in the two locations. All the cells contained a single or at most two granular chromatin material(s) each. Plates 1:(a), (b) & (c) show cells with polytene chromosomes from the different species. Plate 1a & b also show the size of one polysomatic cell relative to the surrounding cells.

Table 2 shows the mean diameter of the cells with polytene chromosomes and their estimated index of polyteny. *C. retusa* generally showed larger cell size and more numerous cells with polytene chromosomes than *C. goreensis* and *C. cylindrocarpa*. These polysomatic cells were smaller in *C. goreensis* which also recorded the fewest number (Table 1).

The relative degree of polyteny within each species has been estimated (Table 2).

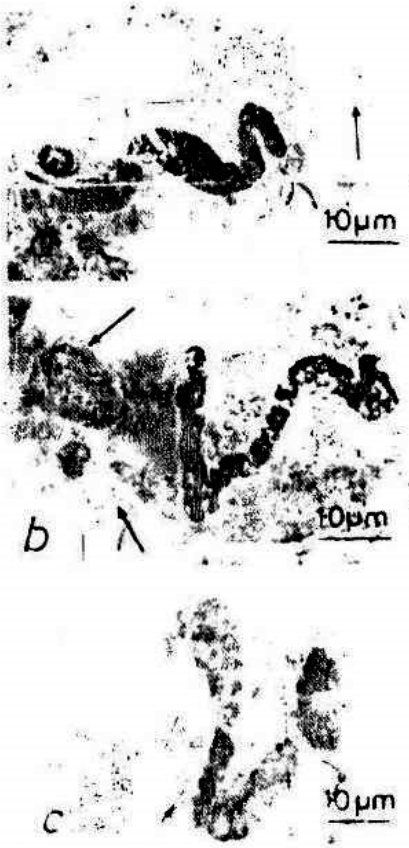

Plate 1: (a) A cell of *C. goreensis* showing polytene chromosome. (Elements coalesced into one).
(b) A cell of *C. retusa* showing polytene chromosome.
(c) Cell of *C. cylindrocarpa*. (Elements coalesced into two units). Arrows indicate the regular tissue cells).

Table 1: Frequency of cells with polytene chromosomes in anthers and root tips of three species of *Crotalaria* from two loctions. Percentage estimate in parenthesis.

Species	Location I (Ife)				Location 2 (Port Harcourt)			
	Anthers (meiotic)		Root tips (mitotic)		Anthers (meiotic)		Root tips (mitotic)	
	Total No. of cells	No. of cells with polytene chromo-somes	Total No. of cells	No. of cells with polytene chromo-somes	Total No. of cells	No. of cells with polytene chromo-somes	Total No. of cells	No. of cells with polytene chromo-somes
C. retusa	288	21 (7.29)	324	25 (7.87)	268	18 (6.71)	246	16 (6.50)
C. goreensis	272	19 (6.99)	291	18 (6.18)	280	16 (5.71)	262	15 (5.73)
C. cylindrocarpa	302	23 (7.78)	257	16 (6.23)	284	17 (5.99)	280	17 (6.07)

Table 2: Mean diameter of the cells with polytene chromosomes and their estimated index of polyteny in the three species of *Crotalaria* from two locations.

Species	Location 1 (Ife) Mean diameter of cells (μm) Anthers	Root tips	Level of Polyteny	Location (Port Harcourt) Mean diameter of cells (μm) Anthers	Root tips	Level of Polyteny
C. retusa.	160 ± 9.1	173.6 ± 9.1	20	151.4 ± 7.6	158.3 ± 67	18
C. goreensis	124.7 ± 7.0	128.8 ± 7.0	15	119.8 + 6.9	121.6 + 5.5	13
C. cylindrocarpa	131.4 ± 7.1	132.3 ± 6.1	16	128.7 + 6.8	125.4 + 81	15

Discussion

The general occurrence of polyteny in meristematic tissues and actively dividing cells is a significant phenomenon. It has been suggested that polyteny serves to multiply active genes which are involved in the formation of ribosome during cell division (Painter and Reindorp, 1939; Swanson, 1968). It has also been suggested that cells that have secretory functions such as salivary gland cells and intestinal cells of the larval mosquito and cells that maintain high metabolic levels such as egg cells and pollen mother cells in plants may have chromosomes of a polytene nature. The probable use of such multiplicity of chromatids (genes) in these tissues is to provide or supply abundant ribosome particles that are required by the dividing cells. Huskins (1947) suggested that genes in carrying out their active functions, accumulate chromatin as a by-product, and that such accumulation is manifested in the reproduction of the chromatin strands, increase in chromosome size and cell size. This will imply that the longer the cells remain active in the polytene state without chromatids separating, the greater will be the degree of polyteny. On the basis of this it will appear that the cell with polytene chromosomes are cells of actively dividing tissue in *Crotalaria* whose chromatid separation processes are delayed relative to the other cells.

Berger (1941) on the other hand suggested that the function of polyteny is to produce profusely numerous cells from a single cell, to meet the requirement in the tissue development during the metamorphosis of the Culex mosquito. This suggestion he buttressed with evidence of reduction division within such tissues. This suggestion does not seem to apply to the situation in *Crotalaria* since there was no evidence of reduction division within the tissue examined in this study.

The observation that the cells showing polyteny usually contained single or at most two giant chromatin materials can be explained using two other observations. One observation is that cells with polytene chromosomes were more frequent in squashes with numerous metaphase cells, while the second observation is that there is a tendency of the *Crotalaria* chromosomes to clump together without any noticeable adverse effect on the process of division and subsequent products. The nature of this clumping has not been analysed but it is believed that if this is sufficiently severe and if it involves the centromere, it might lead to coalescence of the entire genome resulting in a single chromatin 'block' per cell. It is also believed that further multiplication of the chromosome strands will proceed normally, with little or no interference. If cell division is suppressed by this kind of coalescence and if, as assumed, multiplication of chromosome strands within the 'block' is not prevented, then polyteny will result.

The results obtained in this study indicate that polyeny could be a fairly regular event in the growing or dividing tissues of the three species of *Crotalaria* although no specific function can be assigned to the phenomenon at this point.

Consequently further work has to be done to elucidate the probable function of these cells in the tissues of these *Crotalaria* species where they occur.

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