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MORPHOLOGICAL AND KARYOTYPE STUDIES IN EIGHT SPECIES OF THE GENUS *CROTALARIA* LINN

by

K. E. AKPABIO
Department of Botany and Microbiology
University of Cross River State
Uyo, Nigeria

and

O. OLORODE
Department of Botany
Obafemi Awolowo University
Ile-Ife
Nigeria.

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ABSTRACT

Morphological analyses on vegetative characters of the eight species were made. Interspecific variation in vegetative and floral morphology is very significant and has a strong bearing on taxonomy. Karyotype analyses were also made using root tip mitoses in the eight species. Chromosome number of 2n = 16 was recorded for the eight species but **C. doniana and C. naragutensis** have accessory chromosomes in addition. The Karyotype is fairly symmetrical and would not be a strong taxonomic feature without morphological considerations. It has been concluded that speciation in the genus probably arose by structural changes of chromosomes and changes in individual genes or gene complexes within the ancestral population and that the distinctness of the individual species depends upon the absence of interspecific gene flow.

INTRODUCTION

Crotalaria L. is a large leguminous genus containing over 500 named species (Polhill, 1981). The genus is a member of the family Fabaceae and is found predominantly in the tropics and subtropics (Hutchinson, 1964), mostly in Africa, India and Madagascar.

Morphological studies have been carried out on a good number of its species (Polhill, 1968, 1981, Pilbeam and Bell, 1979). Cytological work has also been done on a number of species and chromosome numbers of $2n$ = 14, 16, 18, 28, 32 and 36 have been published for various species of the genus (Polhill and Raven, 1981; Gupta and Gupta 1978; Gill and Husaini, 1985). The occurrence of B–chromosome has been reported in a number of species (Gupta and Gupta, 1977).

In the present report the morphology and the karyology of *Crotalaria retusa, C. goreensis. C. cylindrocarpa, C. doniana C. calycina, C. naragutensis, C. comosa* and *C. spectabilis* have been studied. All the species listed, excepting *C. spectabilis*, are widely distributed in the southern part of Nigeria. *C. spectabilis* is a close relative of *C. retusa*, but it seems to be alien, used mainly as a cultivated ornamental species.

MATERIALS AND METHODS

The plant materials (eight *Crotalaria* species) were collected from wild populations in Southern Nigeria. The eight species are the dominant species in Nigeria south of the savanna. Matured seeds and voucher specimens as well as vegetative and floral data of each species were taken directly from the field. Seeds of all the species were planted out in garden soil in plastic buckets in the Screenhouse and in the field at the Botanical garden, University of Ife. Quantitative measurements of vegetative characters were taken. Table 1 shows the list of vegetative characters studied.

TABLE 1

List of characters investigated

1. Total plant height
2. Leaf or leaflet blade length
3. Leaf or leaflet blade breadth
4. Leaf petiole length
5. Inflorescence length
6. Flower length
7. Anther length
8. Pod length
9. Pod circumference
10. Number of seeds per pod
11. Seed length
12. Seed breadth
13. Flower colour analysis
14. Seed colour analysis.

Karyotype studies were made from temporary preparations of root tips from seeds. Seeds were germinated on filter paper in Petridishes after 10 min.. treatment with conc. H_2SO_4. The acid treatment enables the seeds to germinate within 48hrs of 'sowing'.

Root tips were harvested, prefixed in 0.002M aqueous solution of 8-hydroxyquinoline for 3 hrs, then transferred directly into acetic acid - ethanol (1:3v/v) fixative and stored in the refrigerator for at least 24 hrs before examination. The root tips were then squashed in FLP orcein stain and examined under microscope.

Photomicrographs of good metaphase preparations were taken for karyotype analysis.

RESULTS AND OBSERVATIONS

Table 2 and 3 show the quantitative data of the eight species. The field data from the wild compares closely with the data from the botanical garden. The variation between the two sets of data was not significant at 95% confidence level. All data were based on 25 plants of each species.

TABLE 2

Quantitative data (cm) on vegetative characters in the eight species of Crotalaria

Species	*Plant height*	*Leaf or leaflet blade length*	*Leaf or leaflet blade breath*	*Leaf petiole length*	*Inflorescence length*
Crotalaria retusa	80.75±0.59	10.25±0.26	4.27±0.21	–	24.11±6.09
C. calycina	220.75±41.06	15.37±0.58	3.74±0.21	–	23.47±1.85
C. spectabilis	116.75±5.37	9.98±0.351	4.59±0.15	–	44.06±2.75
C. naragutensis	114.13±3.84	9.80±0.38	6.37±0.15	4.02±0.08	19.38±4.01
C comosa	112.38±1.85	11.64±0.74	3.19±0.12	4.10±0.05	31.18±2.13
C. cylindrocarpa	100.50±3.19	9.41±0.10	4.29±0.18	5.98±0.06	11.14±1.49
C. goreensis	100.25±1.19	8.17±0.22	3.06±0.12	5.59±0.10	7.78±0.83
C. doniana	102.75±1.98	10.75±0.25	5.14±0.13	7.69±0.12	24.82±7.71

TABLE 3

Quantitative data on flowers, fruits and seeds of 8 species Crotalaria

Species	*Flower length (cm)*	*Anther length (mm)*	*Pod length (cm)*	*Pod circum-ference (cm)*	*No. of Seeds/pod*	*Seed length (mm)*	*Seed breadth (mm)*
Crotalaria retusa	3.04±0.05	3.07±0.08	3.63±0.09	3.17±0.07	17.50±2.43	4.14±0.16	3.19±0.11
C. calycina	3.45±0.05	8.04±0.05	5.08±0.11	4.72±0.07	77.22±1.48	6.00±0.08	4.45±0.05
C. spectabilis	2.86±0.04	3.30±0.06	6.04±0.13	5.02±0.12	21.75±2.60	4.85±0.05	3.74±0.06
C. naragutensis	2.06±0.09	3.05±0.05	4.31±0.10	2.26±0.05	31.94±3.85	3.69±0.21	2.46±0.05
C. comosa	3.04±0.06	5.95±0.05	6.37±0.07	4.50±0.08	85.61±9.33	2.12±0.08	1.59±0.08
C. cylindrocarpa	2.07±0.05	3.04±0.04	2.95±0.05	2.70±0.09	18.8330.86	3.87±0.07	2.76±0.05
C. goreensis	1.53±0.06	1.92±0.07	1.67±0.07	2.14±0.05	9.00±0.82	3.10±0.08	2.13±0.05
C. doniana	2.69±0.08	2.89±0.09	3.44±0.09	3.79±0.15	13.28±1.97	5.09±0.07	4.05±0.06

Figures 1 – 5 show some morphological characteristics of the reproductive branch. Flowers are typically papilionaceous and yellow with various designs on the petals. Three species, *C. retusa, C. calycina* and *C. spectabilis* are unifoliolate and sessile while the other five species have three leaflets at the end of petioles of varying lengths.

Crotalaria doniana

A tall woody shrub up to 120 cm in height. Used mainly as an ornamental with dense inflorescence bearing bright yellow flowers with purple markings across the middle of the standard petals (Figure 1).

C. retusa

A (small) woody herb with bright yellow flowers in terminal racemes. Widespread in Nigeria across vegetation zones and grows up to 80 cm in height with numerous lateral branches (Figure 2).

TABLE 4

Qualitative characteristics of flowers and seeds in the eight species of Crotalaria.

Species	Flower colour	Seed colour
C. retusa	Bright yellow with purple streaks on standard and wing petals.	Brown.
C. calycina	Golden yellow without any Streaks.	Greenish-black
C. spectabilis	Bleach yellow with purple streaks on Keel and wing petals.	Black
C. naragutensis	Yellow with purple streaks on Keel petal alone.	Grey
C. Comosa	Bleached yellow with purple streaks on all petals.	Greenish
C. goreensis	Yellow with purple to reddish blotch on tip/margin of standard and wing petals.	Red
C. doniana	Bright yellow with purple markings across the standard petal alone.	Yellowish-brown
C. cylindrocorpa	Bright yellow with reddish blush on standard and wing petals.	Yellow.

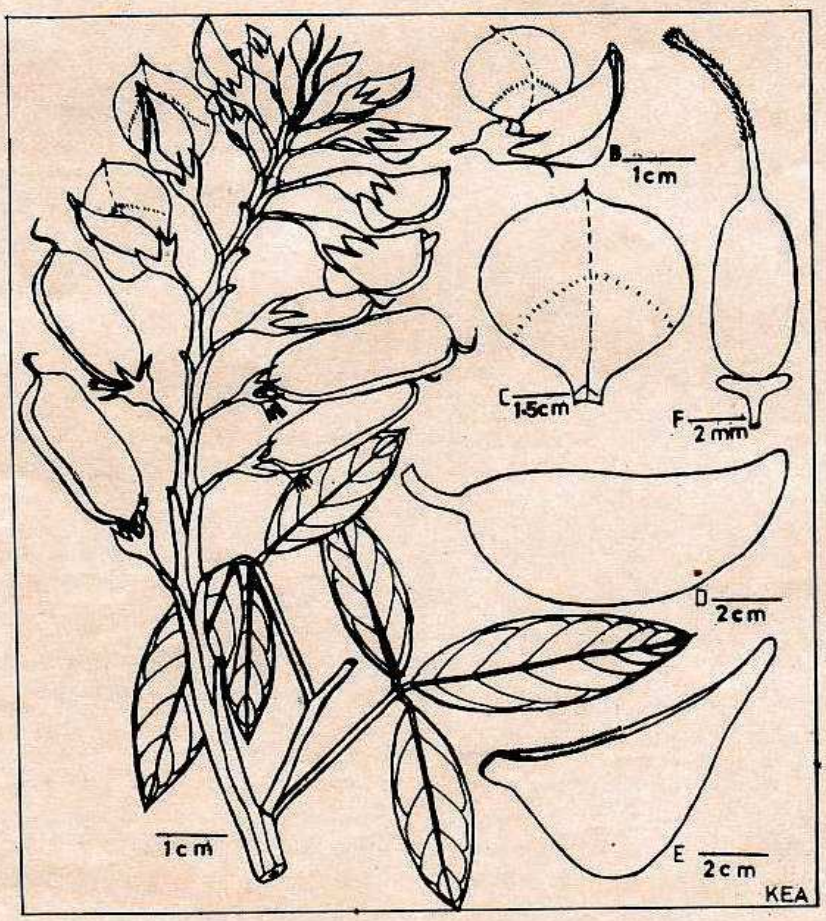

Figure 1: *Crotalaria doniana. A, Inflorescence; B, Flower; C. Standard petal with purple marks across the middle; D, Wing petal; E, Keel petal; F Pistil.*

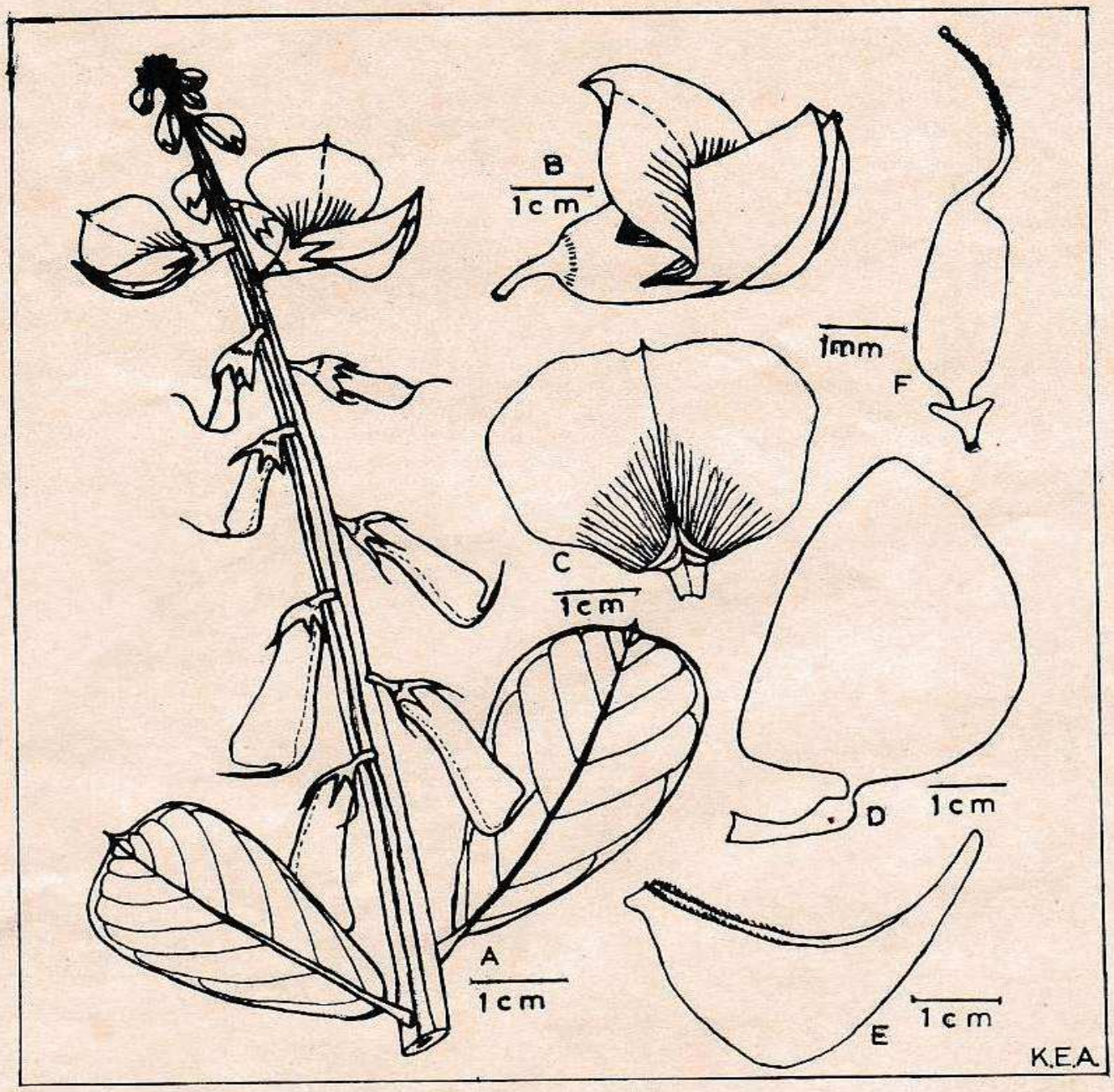

Figure 2: *Crotalaria retusa. A, Inflorescence; B, Flower; C, Standard petal with purple striation towards the base; D, Wing petal; E, Keel petal (untwisted beak); F, Pistil.*

TABLE 5

Classification of the genome of the eight species of Crotalaria according to chromosome size and centromeric position

Species	Number of chromosome (2n)*	Large			Medium			Small			
		Met.	Subm.	Acro.	Met.	Subm.	Acro.	Met.	Subm.	Acro.	B. chromosomes
C. retusa	16	1			3			4			
C. comosa	16	2		1			3	1		1	
C. doniana	16 + 2B	2	2		1	1		2			2*
C. goreensis	16	2	1		1	1	2	1			
C. cylindrocarpa	16	4			4						
C. calycina	16	1			2	2		3		1	
C. naragutensis	16 + 2B		1						2		2*
C. spectabilis	16	3			4			1			

Values in this table represent pairs of chromosomes.
**These values are absolute numbers not pairs.*
Met = *metacentric chromosomes*
Subm = *submetacentric chromosomes*
Acro. = *acrocentric chromosomes*

C. calycina

A woody hairy plant up to 200 cm in height. The inflorescences are very lax with about 6–8 bleached yellow flowers. The Keel petals are twisted and the fused sepals have thick white hairs (Figure 3).

C. spectabilis

Closely related to C. retusa but with stipules. Up to 110 cm in height; inflorescences with golden yellow flowers with purple veins on keel and wing petals.

C. naragutensis

A herbaceous woody plant up to 115 cm in height. Flowers typical with purple striations covering the entire keel petals (Figure 4).

C. comosa

A woody shrub up to 110 cm in height and bearing long slender leaflets. Profuse branches with terminal racemes. Flowers are bleached yellow with purple-brown striations on all the petals.

C. cylindrocarpa

A tall woody shrub up to 100 cm in height. Inflorescence lax with few flowers and cylindrical fruits (Figure 5).

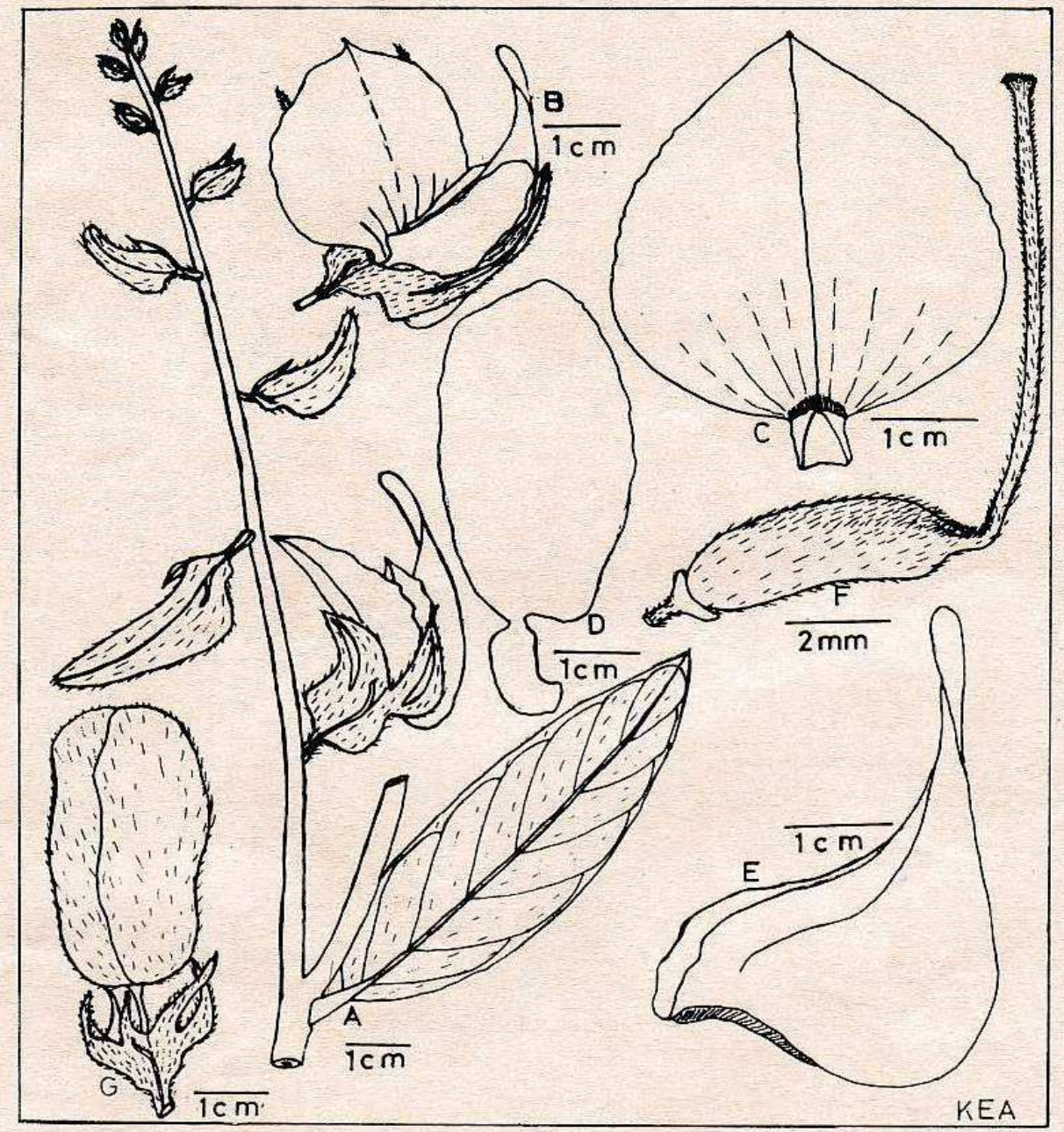

Figure 3: *Crotalaria calycina. A. Flowring shoot showing villous calyx and leaf; B, Flower; C, Standard petal inconspicously striate; D, Wing petal; E, Keel petal with twisted beak; F, Pistil with hairs; G, Fruit (pod) with hairs.*

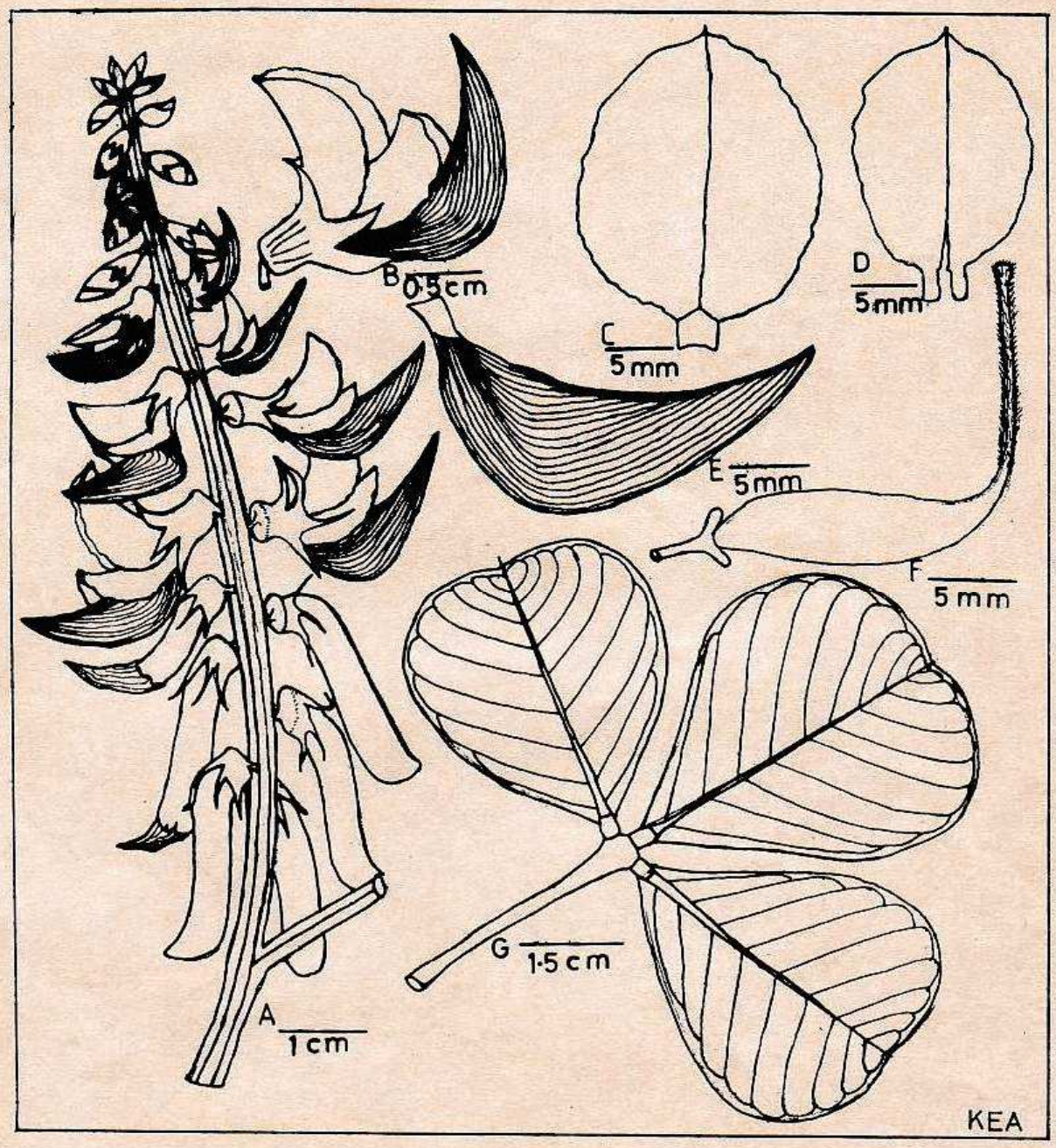

Figure 4: *Crotalaria naragutensis. A. Flowering shoot (inflorescence); B, Flower; C, Standard petal; D, Wing petals fused at the top, E, Keel showing purple striation; F, Pistil; G, Leaf.*

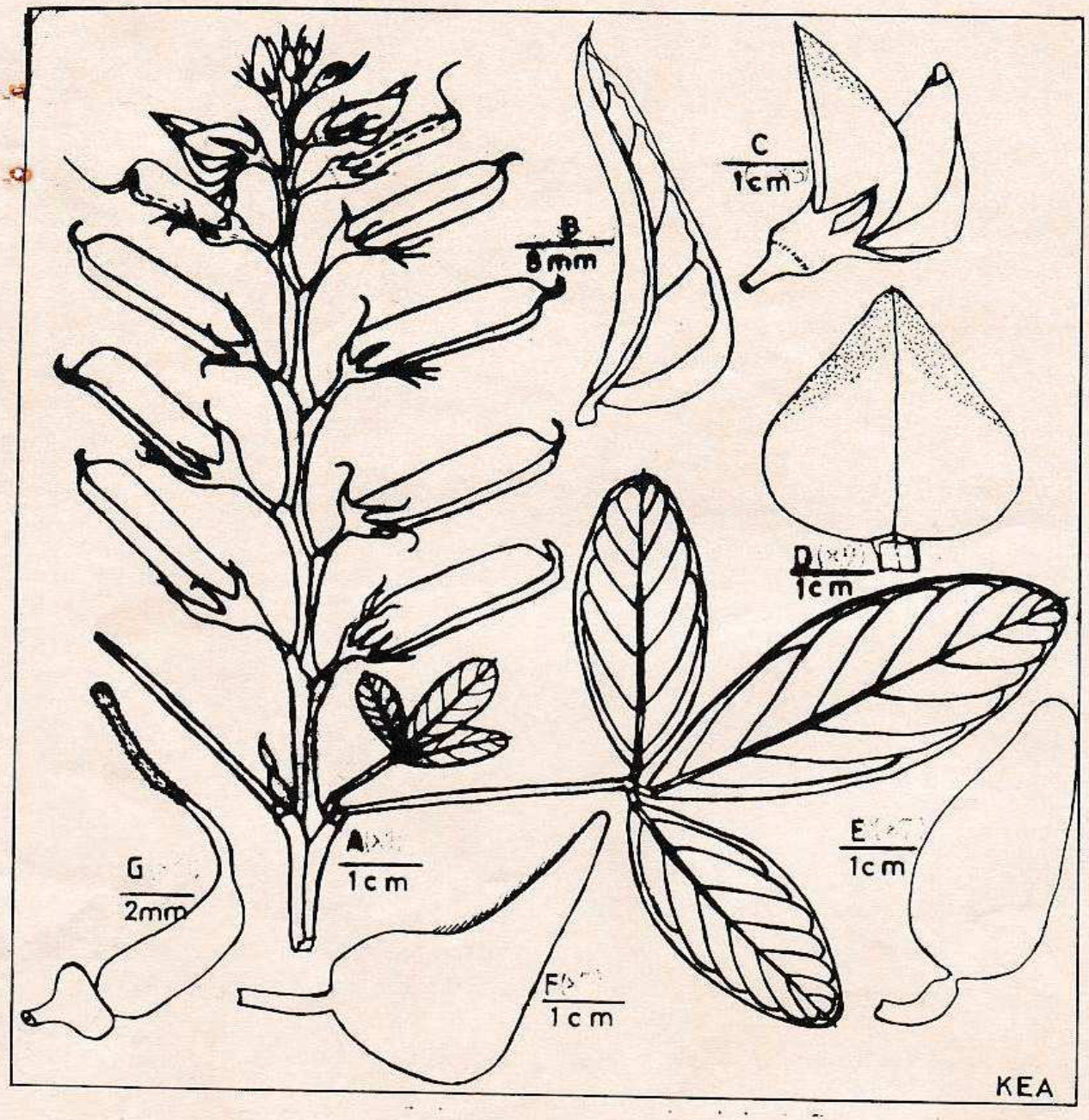

Figure 5: *Crotalaria cylindrocarpa. A, Flowering twig (inflorescene); B, Stipule showing shape and venation; C, Flower; D, Standard petal showing purple blotch; E, Wing petal; F, Keel petal; G. Pistil.*

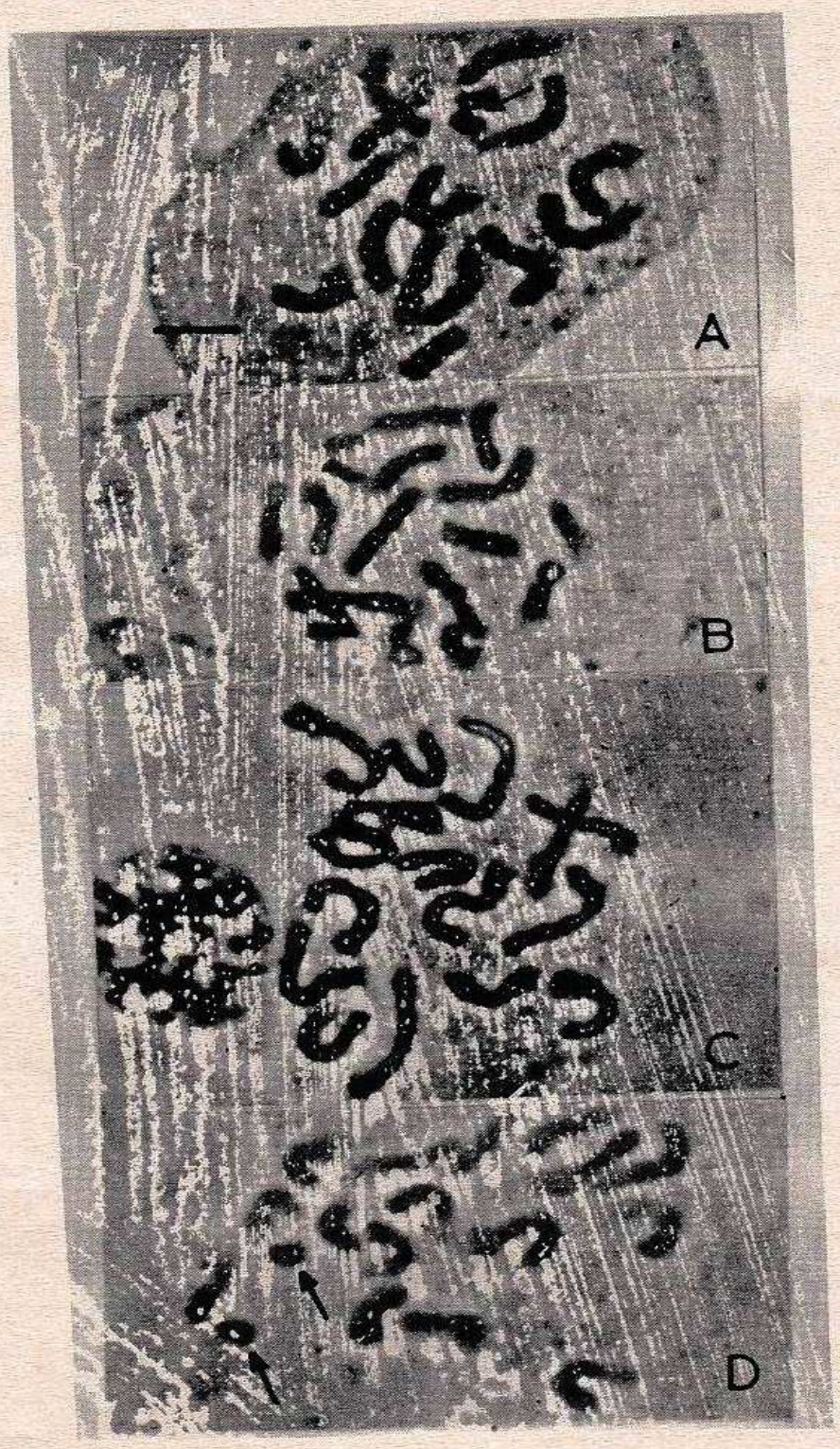

Figure 6: *Mitotic chromosomes of Crotalaria species. A = C. doniana (2n = 16 + 2B); B = C. cylindrocarpa, C = C. goreensis. D = C. naragutensis (2n = 16 + 2B); Arrows indicate B – chromosomes. The bar represents one micron (1 um).*

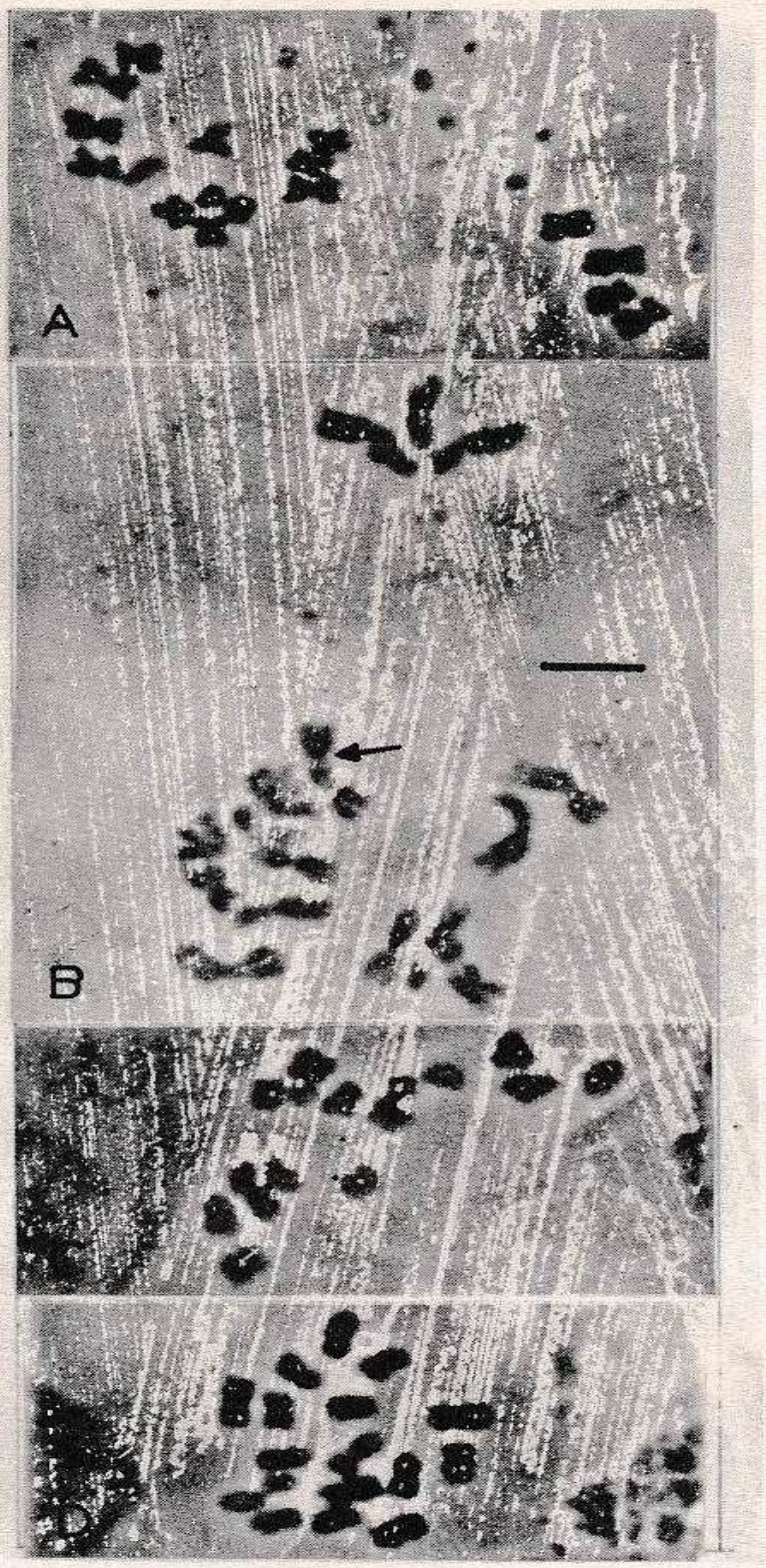

Figure 7: *Mitotic chromosomes of Crotalaria species. A = C. retusa; B = C. spectabilis; C = C. calycina; D = C. comosa. Arrow indicates secondary construction. The bar represents one micron (1 μm).*

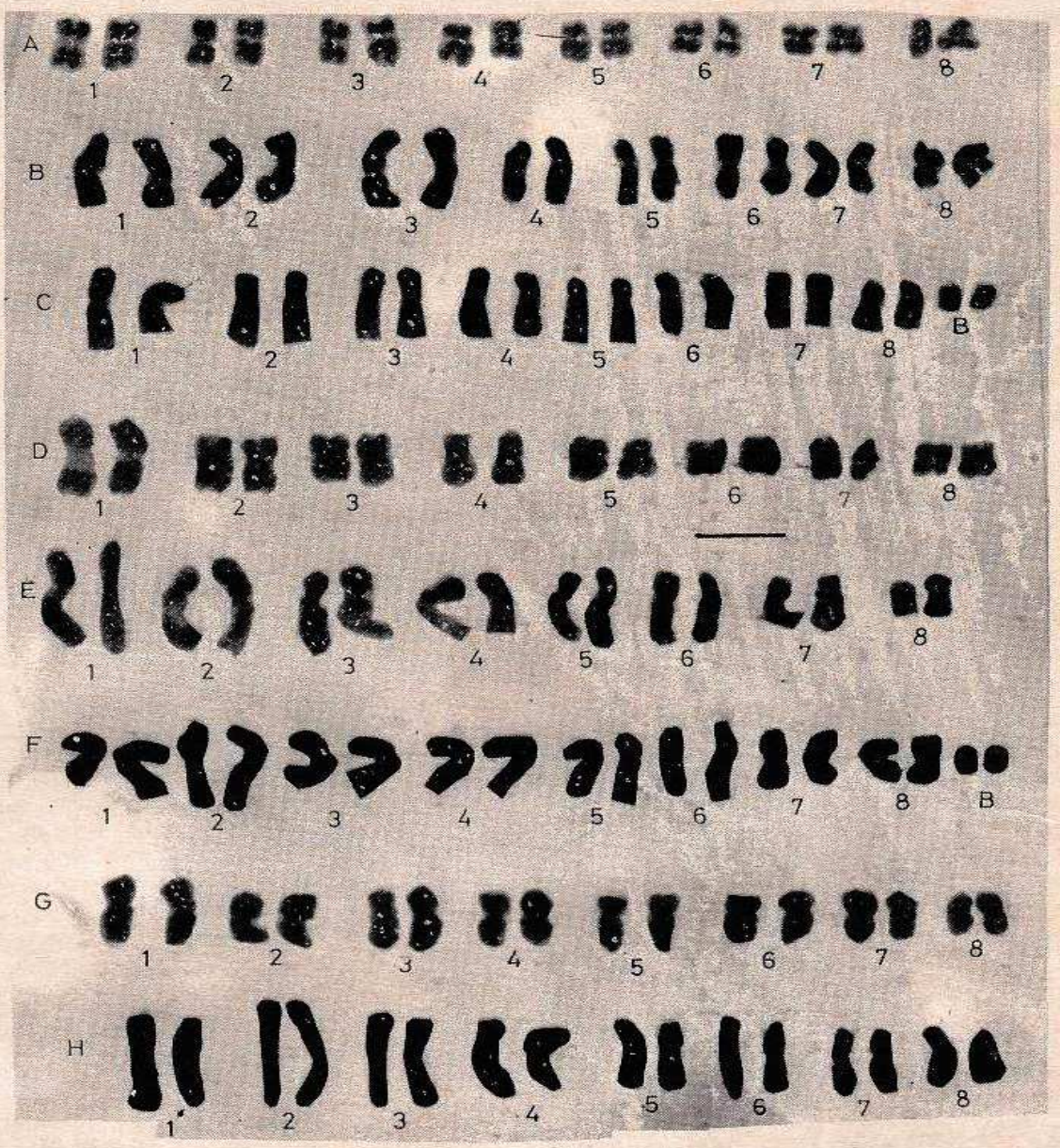

Figure 8: *Karyotypes of the eight species of Crotalaria.*

A = C. retusa
B = C. spectabilis
C = C. naragutensis
D = C. cylindrocarpa
E = C. goreensis
F = C. doniana
G = C. comosa
H = C. calycina

C. calycina

Letter B in Karyotype indicates the accessary chromosomes. The bar represents on micron (1 μm).

C. goreensis

A closely related species to *C. cylindricarpa* up to 100 cm in height. Has a dense habit with leafy stipules. Branches numerous with short dense terminal racemes. Flowers are similar to *C. cylindrocarpa,* yellow with marginal purple-reddish blotch on the standard petals.

Stipules

Stipules, present on *C. spectabilis, C. cylindrocarpa* and *C. goreensis,* are leafy and arise at the base of leaf petioles and branches.

Variation in quantitative data of inflorescences, flowers, fruits and seeds are presented in Tables 2 and 3. The markings/striations on the different petals and the seed colour variations are significant attributes of each species shown on Table 4. There was a very high positive correlation (0.81 at 95% confidence level) between inflorescence length and flower length. All the flowers possess dimorphic, monadelphous anthers, the larger anthers alternating with the smaller anthers. Variation in length of anthers is also presented in Table 3.

Interspecific variation in pod size was significant but intraspecific variation was insignificant thus the small standard deviation (Table 3). The largest pods were recorded in *C. comosa* and *C. calycina* while the smallest pods were recorded in *C.* goreensis.

The pod size and shape together with the seed size and colour form good diagnostic features for the recognition of the different species.

Karyotype Studies

The mitotic chromosomes of the eight species of *Crotalaria* are shown in Figures 6 and 7. The chromosome number of 2n = 16 was recorded in all except *C. doniana* and *C. naragutensis* where accessory chromosomes were also observed (Table 5, Figures 6A, 6D, and 8C and F).

The chromosome of each species can be classified as large, medium or small and these can be subgrouped as metacentric, submetacentric and acrocentric. Large chromosomes predominate in *C. spectabilis, C. doniana, C. goreensis* and C. cylindrocarpa while medium sized and small sized chromosomes predominate in the other four species. On a relative scale, chromosomes of *C. doniana, C. goreensis* and *C. calycina* are larger than the chromosomes of the other five species. The classification of the chromosome of different species is presented in Table 5 and the Karyotype is shown in Figure 8.

The nucleolar organiser region seems to be very short and very much terminal, such that it is difficult to detect. The satellite is also undetectable or hardly visible at metaphase, except *in C. spectabilis* where it appears to be located on one of the large submetacentric chromosomes (Figures 7B and 8B 3). Its location cannot be resolved in the other seven species.

One significant observation was the late condensation of some chromosome arms, a condition which resulted in the formation of heterochromatic regions. The phenomenon was observed in all the chromosomes of *C. doniana* except the accessory chromosomes, in some chromosomes, of *C. naragutensis, C. calycina* and *C. cylindrocarpa* (Figures 6A and D). It was not observed in *C retusa, C. goreensis* and *C. spectabilis.* The significance of these uncondensed regions is not yet understood.

Discussion

The individual species of *Crotalaria* are relatively uniform in gross morphology across various geographical regions. But marked variations in characters exist between the different species. The distinctness of the individual species is probably due to the general genetic stability and absence of interspecific gene flow. The genus is composed largely of mandatory selfers. All attempts to hybridise different species failed. Intraspecific hybridization however succeeded.

One of the major diagnostic generic characters of *Crotalaria is the inflated pod. This* character is constant and is not affected by environment nor the species. The variation in size and shape of pods is a very strong species characteristic such that no two species of the eight studied have pods that are similar in size and shape. This fact makes these traits very useful in species delimitation.

The eight species possess leaf forms ranging from simple leaves to compound leaves with three free leaflets (Polhill, 1971; Hutchinson and Dalziel, 1958). The number of leaflets was definite in all but *C. naragutensis.* This species possess a variety of leaf types (leaves with three, four, five leaflets and leaflets at various stages of dissection). There is no previous record of this kind of situation in *Crotalaria.*

The most significant floral observation was the variation in the pattern of streakings/striations on the various petals. As shown in Table 4 no two species presented the same pattern. Seed characteristics showed a continuum of variation. However the eight species can also be easily recognised on the basis of seed colour and size. The variation in colour of seeds is probably due to genetic factors concretely manifested in biochemical pathways which result in production and accumulation of different chemicals (pigments) in the seed coat. These pathways may be characterised by specific sequential reactions controlled by particular genes, the number of which vary with the number of reactions themselves, (Swanson, 1968).

Karyotype Studies

From the present study and those of earlier workers on other species (Gupta and Gupta, 1975; 1976; 1977; 1978) it appears that the genomes of *Crotalaria* species are fairly similar but differ in basic morphological and overall details. The chromosome number of 2n = 16 is recorded for all except two species, which in addition exhibit the presence of accessory chromosomes.

Though accessory chromosomes have been associated with the production of certain character conditions in other organisms (Jones 1975), there is no noticeable morphological or character condition that can be associated with the presence of accessory chromosome in the *Crotalaria* species.

An examination of the Karyotype of the eight species shows that Karyotypic asymmetry was of a low order within each species. The majority of chromosomes among the eight species are metacentric or submetacentric; very few are acrocentric and none are telocentric

The distribution of chromosome forms among the species indicates that there has been many structural changes within the genus. This speculation suggests that speciation in the genus *Crotalaria* arose by structural changes or rearrangements of chromosomes within the ancestral population coupled with changes in individual genes or gene complexes. This will be ascertained by interspecific hybridization and cytological analysis of the expected hybrids.

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