Chromosome Morphology in the Pasture Legume *Lotononis bainesii* Baker and the Related Species *Lotononis angolensis* Welwitsch ex Baker¹

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Lotononis bainesii Baker and *L. angolensis* Welwitsch ex Baker are self-pollinated, herbaceous perennial legumes native to southern Africa (Cameron 1985). Because these species are grazing tolerant and have high nutritional quality and palatability, they are under study for summer pasture improvement (Cameron 1985, Smith 1974). *Lotononis bainesii* has $2n=36$ chromosomes; *L. angolensis* has $2n=18$ chromosomes (Byth 1964, Pritchard and Gould 1964, Smith 1974). Byth (1964) reported that chromosomes of *L. bainesii* consistently formed 18 bivalents during metaphase I of meiosis. He speculated that *L. angolensis* might be a diploid and *L. bainesii* a tetraploid with a common basic chromosome number of nine. The present study was conducted to provide additional karyological information on the gross chromosome morphology and karyotypes of *L. bainesii* and *L. angolensis*.

Materials and methods

Seeds of *L. bainesii* cv. Miles (obtained from the Genetic Resource Center, CSIRO, Div. Trop. Crops and Pastures, Australia) and *L. angolensis* (A. Smith, Dept. of Agriculture and Water Supply, Natal, South Africa) were inoculated with "Lotononis Special 1" rhizobial inoculant (The Nitragin Company, Milwaukee, WI, USA) and sown in clay pots containing two parts of sand to one part shredded sphagnum moss and one part horticultural vermiculite. Plants were housed in a greenhouse with a seasonal temperature range of 10 to 35°C and natural daylength.

Conventional karyotypes

Actively growing root tips were collected between 0800 h and 1000 h and pretreated for 2 to 3 h with mono-bromonaphthalene, colchicine, paradichlorobenzene, or water. Several pretreating agents were used to find an optimum treatment for chromosome contraction and clarification of constriction regions (Sharma and Sharma 1980). Root tips were then fixed in 45% acetic acid for 2 h, hydrolysed in 1N HCl at 60°C for 10 to 15 min, and squashed in a drop of 45% acetic acid. Slides were frozen using dry ice and cover glasses were removed. Slides were rinsed in distilled water and stained with 1% propionic carmine, aceto-carmine, basic fuchsin, or (Merritt and Burns 1974) a 4% aqueous solution of Giemsa stain in a phosphate buffer at pH 7. After staining, slides were dipped once in distilled water, air dried, then dipped in xylene and mounted in permount. Well-squashed metaphase cells were photographed using an Olympus BH-2 microscope with high contrast Kodak technical pan film 2415 at

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100 ASA and printed at 2000 $\times$ magnification.

To form the karyotype, chromosome images from a single, well-spread cell were cut from the enlarged photomicrograph, matched in pairs according to their morphology, grouped according to centromere location, and assigned a number in order of decreasing length. Each chromosome was measured with a divider on photographic enlargements and translated to microns on a similarly enlarged micrometer scale. Measurements were made from 5 to 10 uniformly treated complements. The relative length (RL) of each chromosome was calculated as: $RL = (\text{chromosome length} / \text{total chromosome length}) \times 100$ (Krikorian *et al.* 1983). The arm ratio (r), expressed as the length of the longer arm (l) relative to the shorter one ($r = l/s$), was employed as an index to the centromere position (Levan *et al.* 1965). Total form length (TF), expressed as a percentage, was calculated using the formula: $TF\% = (\text{total length of all short arms} / \text{total length of chromosome complement}) \times 100$. Perfect symmetry of karyotypes is obtained when TF% is 50.

C-banded karyotypes

Traditional chromosome identification criteria, dependent on morphological features such as relative length, arm ratio, and primary and secondary constrictions (Noda and Kasha 1978, Bostock and Sumner 1980), were of limited use in characterizing the small chromosomes of the *Lotononis* species. Consequently, a C-banding technique was used to differentially stain the chromosomes. Initially, C-banding was attempted using the technique described by Bennett *et al.* (1977). This technique, developed for cereal chromosomes, was not successful for *Lotononis* and modifications were therefore made as follows. Root tips were pretreated with a saturated solution of para-dichlorobenzene for 24 h at 4°C, fixed in 45% acetic acid, and refrigerated overnight for not longer than 20 h. Roots were hydrolysed in 1N HCl for 50 to 60 min at room temperature and then transferred to 45% acetic acid. Denaturation and re-naturation procedures were adapted from the *Vicia faba* protocol of Hizume *et al.* (1980): preparations were denatured in 5% BaOH for 4 min at 60°C and renatured in 2XSSC solution for 1 h at 60°C. Remaining steps in the C-banding procedure were unchanged from Bennett *et al.* (1977).

Cells with well-spread metaphase or prophase chromosomes were photographed at 2000 $\times$ magnification. Chromosome images were cut from the prints, matched according to similarities in banding pattern, length, and centromere position, and used to form the banded karyotype. Observed bands were drawn on ideograms of the karyotypes of *L. bainesii* and *L. angolensis* in which arm ratios and relative sizes of the chromosomes were represented to scale.

Observations

Conventional karyotypes

Root tip cells of *L. bainesii* had 36 chromosomes (Fig. 1A) in eighteen pairs (Fig. 2A); *L. angolensis* had 18 chromosomes (Fig. 1B) in nine pairs (Fig. 2). There were two pairs of chromosomes with satellites in *L. bainesii* and a single pair with satellites in *L. angolensis*. Total form lengths of the two species were almost identical: TF% of *Lotononis bainesii* was 39.85% and TF% of *L. angolensis* was 39.38%.

Chromosomes were best differentiated when pretreated with a freshly prepared saturated solution of paradichlorobenzene in water. Staining techniques using 1% propionic carmine, aceto-carmine, or basic fuchsin were suitable for counting chromosomes under low magnification (100 $\times$) but only the Giemsa stain was suitable for photographing chromosomes under oil immersion. Differential Giemsa staining of some chromosomes aided in distinguishing certain homologs, particularly pairs numbered 2, 7, 10, 11 and 16 of *L. bainesii* and pairs 2

and 4 of *L. angolensis*.

On the basis of arm ratio, centromere position, and in some cases differential staining, metaphase chromosomes of *L. bainesii* were separable into two groups (Fig. 2A). Centromeres of chromosome pairs numbered 1 to 11 (Group 1) are median (Table 1); arm ratios of this group varied between 0.82 and 1.27. The longest Group 1 chromosome was 3.36 and the shortest 2.27 μm in length. Centromeres of chromosome pairs numbered 12 to 18 (Group

Fig. 1. Somatic metaphase chromosomes of *Lotononis bainesii* (left). Somatic metaphase chromosomes of *Lotononis angolensis* (right). $\times 2000$

Fig. 2. A, photo-ideogram of *Lotononis bainesii*. B, photo-ideogram of *Lotononis angolensis*. A and B: homologous pairs arranged according to centromere position and decreasing size. $\times 2000$

2) are submedian; arm ratios ranged from 0.9 to 1.27. The longest Group 2 chromosome was 3.54 and the shortest 2.72 μm in length. Mean length of metaphase *L. bainesii* chromosomes was 2.78 μm and mean satellite length was 0.45 μm .

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Fig. 3. A, somatic prophase cell of *Lotononis bainesii*: thirty-six chromosomes identified by C-bands. B, somatic prophase cell of *Lotononis angolensis*: eighteen chromosomes identified by C-bands. $\times 2000$

Fig. 4. A, karyotype of *Lotononis bainesii*. B, karyotype of *Lotononis angolensis*. A and B: chromosomes arranged according to C-bands into homologous pairs. $\times 2000$

Metaphase chromosomes of *L. angolensis* were also separable into two groups (Fig. 2B), using criteria as above. Centromeres of chromosome pairs numbered 1 to 7 are median (Table 2); arm ratio of these chromosomes ranged from 1.19 to 1.50. The longest chromosome was 7.72 and the shortest 6.36 μm . In the second group, chromosome pairs numbered 8 and 9 (submedian), the arm ratio varied from 1.99 to 2.36. Mean length of metaphase *L. angolensis* chromosomes was 6.96 μm and mean satellite length was 1.36 μm .

Table 1. Somatic metaphase chromosomes of *Lotononis bainesii*: size and centromere position

Chromosome pair	Total length (μm)	Relative length ^a	Long arm (μm)	Short arm (μm)	Relative index ^b	Position of centromere
Group 1						
1	3.36	6.64	2.00	1.36	1.47	m ^c
2	3.00	5.92	1.77	1.28	1.34	m
3	2.90	5.74	1.54	1.36	1.13	m
4	2.81	5.56	1.27	1.21	1.21	m
5	2.81	5.56	1.54	1.27	1.21	m
6	2.72	5.38	1.63	1.09	1.49	m
7	2.63	5.20	1.63	1.00	1.63	m
8	2.45	4.84	1.27	1.18	1.07	m
9	2.27	4.48	1.27	1.00	1.27	m
10	2.27	4.48	1.18	1.09	1.08	m
11	2.18	4.30	1.36	0.82	1.05	m
Group 2						
12	3.54	7.00	2.27	1.27	1.78	sm ^d
13	3.54	7.00	2.27	1.27	1.78	sm
14	3.09	6.10	2.18	0.90	2.42	sm
15	2.81	5.56	1.80	1.01	1.78	sm
16	2.72	5.38	1.72	1.00	1.72	sm
17	2.72	5.38	1.72	1.00	1.72	sm
18	2.72	5.38	1.72	1.00	1.72	sm

^a Percentage of the length of the total haploid genome.

^b Ratio of the long arm to the short arm.

^c m=median.

^d sm=submedian.

Table 2. Somatic metaphase chromosomes of *Lotononis angolensis*: size and centromere position

Chromosome pair	Total length (μm)	Relative length ^a	Long arm (μm)	Short arm (μm)	Relative index ^b	Position of centromere
Group 1						
1	7.72	12.31	4.54	3.18	1.42	m ^c
2	7.50	11.96	4.09	3.41	1.19	m
3	7.27	11.59	4.09	3.16	1.28	m
4	6.81	10.86	4.09	2.72	1.50	m
5	6.81	10.86	4.09	2.72	1.50	m
6	6.36	10.14	3.63	2.73	1.32	m
7	6.36	10.14	3.63	2.73	1.32	m
Group 2						
8	8.40	13.40	5.90	2.50	2.36	sm ^d
9	5.45	8.69	3.63	1.82	1.99	sm

^a Percentage of the length of the total haploid genome.

^b Ratio of the long arm to the short arm.

^c m=median.

^d sm=submedian.

C-banded karyotypes

Slides prepared according to the unmodified C-banding technique of Bennett *et al.* (1977) showed banding only in the longer chromosomes in a few cells. Generally, bands were poorly

Table 3. Relative length, arm ratio, and observed bands of C-banded prophase chromosomes of *Lotononis bainesii*

Chromosome pair	Relative length ^a	Arm ratio ^b	Observed bands
			Long arm/Short arm/Satellite ^c
Group 1			
1	7.75	1.42	1 small centromeric/1 terminal, 1 centromeric
2	6.72	1.15	1 dark centromeric/1 dark centromeric
3	6.61	1.63	1 dark centromeric/1 dark centromeric
4	6.15	1.25	1 centromeric/1 centromeric
5	5.24	1.47	1 large dark centromeric/1 dark terminal, 1 large centromeric
6	5.24	1.55	None/1 terminal, 2 interstitial
7	5.01	1.20	1 dark centromeric/1 dark centromeric
8	5.01	1.44	1 centromeric/1 faint terminal, 1 centromeric
9	3.87	1.05	None/1 interstitial
10	1.93	1.12	None/none
Group 2			
11	6.83	2.53	None/2 faint interstitial/2 small terminal
12	6.38	2.53	2 small proximal/1 interstitial/1 in nucleolar organising region
13	6.04	1.90	1 dark centromeric/1 dark centromeric
14	5.81	2.18	1 centromeric/1 centromeric
15	5.47	1.82	1 centromeric, 1 small proximal/1 centromeric
16	5.47	1.76	1 small centromeric/1 small centromeric
17	5.38	2.57	1 centromeric, 1 very small proximal/1 centromeric
18	3.64	2.20	1 dark centromeric/1 small centromeric, 1 dark near secondary constriction

^a Percentage of the length of the total haploid genome.

^b Ratio of long arm to the short arm.

^c Only chromosome pairs numbered 11 and 12 had satellites.

Table 4. Relative length, arm ratio and observed bands of C-banded prophase chromosomes of *Lotononis angolensis*

Chromosome pair	Relative length ^a	Arm ratio ^b	Observed bands
			Long arm/Short arm/Satellite ^c
Group 1			
1	14.45	1.64	None/1 large distal
2	11.71	1.14	None/1 large centromeric/none
3	11.32	1.23	None/1 terminal block
4	10.93	1.70	None/entire
5	10.54	1.45	1 dark centromeric/1 dark centromeric
6	10.15	1.60	1 large dark centromeric, 1 small proximal/ 1 dark centromeric
7	8.20	1.33	None/entire
Group 2			
8	11.71	2.75	1 large proximal/none
9	10.90	2.50	1 dark centromeric/1 dark centromeric

^a Percentage of the length of the total haploid genome.

^b Ratio of long arm to the short arm.

^c Only the chromosome pair numbered 2 had a satellite.

defined or absent on fully contracted chromosomes. Prophase chromosomes stained with the modified technique (Figs. 3A, B) were used to produce the banded karyotypes (Figs. 4A, B). Mean length of prophase chromosomes was $5.47\ \mu\text{m}$ in *L. bainesii* and $11.13\ \mu\text{m}$ in *L. angolensis*.

Figs. 5–6. 5, ideogram of the banded karyotype of *Lotononis bainesii* showing relative sizes and positions of C-bands. 6, ideogram of the banded karyotype of *Lotononis angolensis* showing relative sizes and positions of C-bands.

Bands differed in size, intensity, and distribution. Centromeric bands were generally darkly stained. In some chromosome arms, no bands were visible. With the aid of the banding pattern, it was possible to identify 18 chromosome pairs in the somatic prophase of *L. bainesii* (Figs. 4A, 5) and 9 pairs in *L. angolensis* (Figs. 4B, 6). Chromosomes of each species were again divided into two groups on the basis of arm ratio. In *L. bainesii*, chromosome pairs 1 to 11 (Group 1) were metacentric and pairs 12 to 18 were submetacentric (Table 3). Chromosome pairs 1 to 7 in *L. angolensis* were metacentric and pairs 8 and 9 were submetacentric (Table 4).

Discussion

The observed chromosome numbers of 36 for *L. bainesii* and 18 for *L. angolensis* confirm results reported by Byth (1964), Pritchard and Gould (1964), and Smith (1974). In the present study, extreme contraction of the metaphase chromosomes and lax configuration of late prophase chromosomes made it difficult to distinguish the centromere and chromosome arms, two of the main criteria used in the morphological identification of homologous pairs. In *L. bainesii*, chromosome pairs numbered 4, 5 and 9; 10, 12 and 13; 16, 17 and 18 were characterised by having similar lengths and, in some cases, the same arm ratio. In *L. angolensis*, chromosome pairs 4 and 5 and pairs 6 and 7 had similar lengths and arm ratios. Variation in chromosome morphology, including the presence or absence of satellites and some variation in staining properties, aided in the identification of metaphase chromosomes.

Both *Lotononis* species had a large proportion of chromosomes with median centromeres and TF% of the two species was strikingly similar. However, mean length of *L. angolensis* chromosomes was approximately twice the length of *L. bainesii* chromosomes at both prophase and metaphase. Chromosome size can vary with environmental and other influences (Swanson *et al.* 1981), even in the same tissue of a single root (Kimber *et al.* 1975). Differences in chromosome lengths can also result from differential reaction to pretreating agents and fixatives (Darlington and LaCour 1950). However, the relative difference in chromosome length between *Lotononis* species was consistent across techniques and chromosome stages.

Chromosome banding of *Lotononis* species appeared related to the amount of contraction of the chromosomes. Metaphase chromosomes were unsuitable for banding studies. Other workers similarly have obtained more distinct C-bands in prophase than in highly contracted metaphase chromosomes (Patterson and Petricianni 1973).

In *L. angolensis*, banding revealed heterochromatin arranged in large blocks. In chromosome pairs numbered 4 and 7, heterochromatin occupies an entire arm. In *L. bainesii*, bands of heterochromatin were more dispersed. Present evidence suggests that the diploid *L. angolensis* is not a progenitor of the related tetraploid *L. bainesii* or that a tremendous amount of repatterning of the chromosomes must have occurred in *L. bainesii*.

Summary

Lotononis bainesii Baker, a diploid, and *L. angolensis* Welwitsch ex Baker, a tetraploid, are self-pollinated, herbaceous perennial legumes native to southern Africa. Because these species are grazing tolerant and have high nutritional quality and palatability, they are under study for summer pasture improvement. Gross morphology of *L. bainesii* and *L. angolensis* chromosomes was examined using conventional and C-banding techniques. Ideograms are presented showing that the two species were distinctly different in chromosome morphology and C-banding patterns.

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