Karyotyping the Arabian Camel (*Camelus dromedarius*), Using C-Banding Technique

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The development of various staining techniques has been essential for the precise identification of chromosome morphology. Since the chromosomes of each species are unique, the C-banding staining has contributed immensely to the understanding of chromosome structure in eukaryotes. This technique was originally discovered by Pardue and Gall (1970) and was later modified by Arrighi and Hsu (1971). These bands are so termed because they involve the selective staining of constitutive heterochromatin in the region of the centromere. Hence, the bands are called C-bands in relation to the constitutive heterochromatin. It is now known that C-band chromatin contains a special type of DNA composed of very high repetitions of short sequences (Pardue and Galls 1970). Although constitutive heterochromatin is visible in untreated prophase chromosomes, it is better observed in condensed metaphase chromosomes by C-banding (Arrighi and Hsu 1970, Craig-Holmes and Shaw 1971, Sumner 1972).

The technique was used to study various mammalian families like the Muridae (Prescott *et al.* 1973, Bostock and Christie 1974, Schnedl and Czaker 1974, Dev *et al.* 1975), Bovidae (Evans *et al.* 1973, Bruere *et al.* 1974, Buckland and Evans 1978, Di Berardino and Iannuzzi 1981, Switonski 1987, Berland *et al.* 1988) and Cervidae (Jorge and Benirschke 1977, Van Tuinen *et al.* 1983, Goldoni *et al.* 1984, Mayr *et al.* 1987). This technique is yet to be used in the Camelidae apart from a single report by Bunch *et al.* (1985) describing G- and C- banding pattern homologies as well as chromosomes with NORs for Bactrian camels, llamas, and guanacos. Hence, in the present study the C-banding technique is used for the identification of *C. dromedarius* chromosomes *in vitro*.

Materials and methods

Venous blood was obtained from 10 male and 10 female camels from the King Saud University Animal Farm, under the sterile hood and using aseptic techniques, 10 ml of sterile complete growth medium (RPMI-1640) were put into a 25 cm² sterile culture flask, then 0.2 ml of sterile phytohemagglutinin (PHA) was added to the flask. An aliquot of 0.5 ml of blood was drawn from well suspended blood sample using a 1 ml disposable syringe fitted with 21G needle and added into the flask. The flask was inverted several times before incubating at 37°C in an incubator supplied with continuous flow of 5% CO₂ and 80% humidity. After 72 hr of incubation and 1 hr before harvesting the cell culture, 2 drops of colcemid (10 µg/ml) were added to the culture flask in order to synchronize the dividing cells at the metaphase stage. The cultured cells were then harvested by vigorous shaking the flask before transferring the cell suspension into centrifuge tube. The cell suspension was then centrifuged at 100 g for 10 min. The supernatant removed under vacuum and the pellet of cells were resuspended in 5 ml of 0.075 M KCl and incubated in a water bath at 37°C for 15 min as a hypotonic treatment. The cell suspension was then centrifuged and the cells were

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fixed in 3:1 absolute methanol-glacial acetic acid for a minimum of 30 min. The fixation procedure was repeated three times before spreading the cells on the slides.

The original method described by Arrighi and Hsu (1971) involves primary treatment with an alkali, sodium hydroxide (NaOH) to denature the chromosomal DNA and subsequent incubation in a salt solution. In modified method described by Sumner (1972), a milder alkali, barium hydroxide ($\text{Ba}(\text{OH})_2$), is used instead of NaOH. Both of these methods produce a similar characteristic pattern of C-bands. The later method may provide an advantage, because they facilitate better control of the denaturation of the chromosomes except for the centromere (constitutive heterochromatin) bands. The chromosomes preparations were obtained from peripheral blood cultures as described as above were allowed to age for 1–2 weeks before treating them with freshly prepared and filtered 2.5% barium hydroxide solution for 8 min at room temperature. After thorough rinsing in several changes of distilled water to remove as much salt deposits as possible, the slides were incubated for 2 hr at 60°C in

Fig. 1. C-banded karyotype of male *C. dromedarius*. $\times 5000$

Fig. 2. C-banded karyotype of female *C. dromedarius*. $\times 5000$

$2\times$ SSC (0.3 M sodium chloride containing 0.03 M tri-sodium citrate), rinsed briefly with distilled water and stained with 5% Giemsa stain for 5–15 min. Finally the slides were again rinsed briefly in distilled water, blotted, allowed to dry thoroughly, soaked in xylene and mounted in DPX. They were examined for metaphase stages under Nikon microscope with $25\times$ plan-chromatic objective and $10\times$ eyepieces. Photomicrographs of selective metaphase chromosomes were taken under bright field illumination, using $100\times$ oil immersion objective and $10\times$ eyepiece. The arm ratios were calculated according to the proposal of Levan *et al.* (1964) using an electronic caliper, Fowler Ultra-Cell II (Fowler Co.).

Results

The diploid chromosome number ($2n$) for the camel, *C. dromedarius* are 74 and the findings of C-band technique are present in Figs. 1 and 2. The chromosomes were divided according to the position of the centromere, into three types: Type 1 comprises acrocentric autosomes number 1–31, type 2 metacentric autosomes number 32, 33, 34 and the X and Y chromosomes

and type 3 represents one pair of submetacentric autosomes number 35.

All the acrocentric autosomes have a paracentric C-band which varies greatly in width. The amount of centromeric heterochromatin in bi-armed chromosomes was generally reduced relative to acrocentric autosomes. An interstitial C-band was not found in any of the chromosomes of the dromedary.

Table. 1. The relative mean lengths and arm ratio measurements in mm of the 74 mitotic chromosomes of Arabian camel *C. dromedarius*

Type of Chromosome	Total Length	Length of long arm (q)	Length of short arm (p)	Arm ratio q/p	Type of Centromere
1	13.79	11.54	2.25	5.13	A
2	12.21	10.52	1.69	6.22	A
3	11.78	10.03	1.75	5.73	A
4	10.87	9.09	1.78	5.11	A
5	10.19	8.54	1.65	5.18	A
6	9.49	7.83	1.66	4.72	A
7	8.90	7.54	1.36	5.54	A
8	7.88	6.64	1.24	5.35	A
9	7.67	6.55	1.12	5.85	A
10	7.35	6.09	1.26	4.83	A
11	7.20	5.66	1.54	3.67	A
12	6.96	5.88	1.08	5.44	A
13	6.38	5.14	1.24	4.14	A
14	6.02	4.76	1.26	3.77	A
15	5.11	4.10	1.01	4.10	A
16	4.35	3.38	0.97	3.48	A
17	4.14	3.35	0.79	4.24	A
18	3.96	3.18	0.78	4.10	A
19	3.77	3.04	0.73	4.16	A
20	3.57	2.79	0.78	3.57	A
21	3.45	2.78	0.67	4.14	A
22	3.40	2.69	0.71	3.78	A
23	3.37	2.68	0.69	3.88	A
24	3.27	2.51	0.76	3.30	A
25	3.10	2.41	0.69	3.49	A
26	3.00	2.31	0.69	3.34	A
27	2.90	2.34	0.56	4.17	A
28	2.88	2.29	0.59	3.88	A
29	2.82	2.26	0.56	4.03	A
30	2.69	2.07	0.62	3.33	A
31	2.53	1.97	0.56	3.51	A
32	7.38	4.66	2.72	1.71	M
33	6.27	3.39	2.88	1.17	M
34	6.19	3.73	2.46	1.51	M
35	5.04	3.64	1.40	2.6	SM
36	2.68	1.48	1.20	1.2	M
X	10.04	5.83	4.21	1.38	M
Y	4.69	2.74	1.95	1.4	M

Table 1 presents the mean length of long and short arm ratio measurements of the 74 chromosomes of the dromedary which has been constructed on the basis of the position of the centromere and are also shown in Fig. 3. The fundamental number (FN) of chromosomes was calculated according to the hypothesis of Matthey (1945) that counts acrocentric as one

and meta- and submetacentric chromosomes as two. Hence, the FN for the dromedary amounts to 86 and the fundamental number of autosomes (FNa) 82.

Discussion

The diploid number of *C. dromedarius* was found to be 74 chromosomes, which has been previously been reported for the guanaco, the Bactrian camel and the vicugna in addition to the dromedary (Benirschke 1955, Taylor *et al.* 1968, Koulischer *et al.* 1971, Bunch *et al.* 1985). However, Capanna and Civitelli (1965) have reported 72 for the vicugna and guanaco, but the vicugna was found to have $2n=74$ and more metacentric autosomes than the Bactrian camel (Koulischer *et al.* 1971). By means of the present technique it was possible to classify the chromosomes of the dromedary into 31 pairs of acrocentric autosomes, 4 pairs of metacentric autosomes, one pair of submetacentric autosomes and metacentric X and Y sex chromosomes. However, some authors have experienced some difficulties in classifying

Fig. 3. An idiogram of Arabian camel, *C. dromedarius* chromosomes construed on the basis of the position of centromeres. A, acrocentric, M, metacentric and SM, submetacentric.

chromosomes of the Camelidae including Taylor *et al.* (1968) who found difficulties in classifying Y and smaller chromosomes of the dromedary and Koulischer *et al.* (1971) who were unable to classify chromosomes of the Bactrian camel and vicugna due to indiscernible morphology.

Bunch *et al.* (1985) have described G- and C-banding pattern homologies and chromosomes with NORs for the Bactrian camel, the llama and the guanaco that have a $2n$ of 74 and a karyotype of 33 pairs of acrocentric autosomes, 3 pairs of submetacentric autosomes, a large submetacentric X and a very small acrocentric Y chromosomes. There were no differences in either of the G- and C-band patterns in these animals, but the Bactrian camel has 5 pairs of nucleolus organizer chromosomes and both guanaco and llama six. These authors concluded that such identical patterns of chromosomes linear differentiation of the Camelidae might suggest that their karyotype must have been stable for many thousands of years.

Based on the C-banding technique carried out in the present study, the C-banded chromosomes of the Arabian camel proved to be different from those of other Camelidae. These include 5 pairs of metacentric and submetacentric autosomes, 31 pairs of acrocentric autosomes, a medium metacentric X and a small metacentric Y chromosomes.

Summary

The chromosomes of the Arabian camel or the one humped camel, *Camelus dromedarius*, L. have been studied using cell cultures of peripheral blood lymphocytes. Using C-banding technique, the diploid chromosome number for the *C. dromedarius* was found to be 74, with a karyotype consisting of 62 acrocentric, 8 metacentric and 2 submetacentric autosomes. The X chromosome is large, metacentric and the Y chromosome is a small metacentric sex chromosome. The fundamental number has been calculated to be 86 chromosomes.

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