CHROMOSOME ANALYSIS IN THE KRUGER NATIONAL PARK WITH SPECIAL REFERENCE TO THE CHROMOSOMES OF THE GIRAFFE (GIRAFFA CAMELOPARDALIS GIRAFFA (BODDAERT)

by

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INTRODUCTION:

This paper represents the technique used and the chromosome analysis in a male and a female giraffe in the Kruger National Park. The taxonomic status of these animals is cited by Meester and Davis (1964) as Giraffa camelopardalis giraffa (Boddaert) and they are distributed, at present, in the Eastern Transvaal, adjoining parts of Southern Rhodesia and Mocambique, and have also been introduced into the Hluhluwe Game Reserve, Natal.

IMMOBILIZATION OF ANIMALS:

The animals were immobilized with the aid of a cross-bow and projectile syringe [Van Niekerk and Pienaar (1962)], using the drug combination M99, Hyoscine and Acetylpromazine. This technique is ideally suited to the collection of blood and marrow in big and potentially dangerous animals, as they are easily handled in safety while drugged, and can be released within two minutes after the collection has been completed by injecting an antagonist, Nalorphine Hydrobromide, intravenously. (See Fig. 1.)

METHODS:

A. PERIPHERAL BLOOD CULTURES:

A young male giraffe was darted by Dr. U. de V. Pienaar and chromosome preparations were obtained by the peripheral blood culture technique. The technique used was a modification of the technique described by Moorhead et al. (1960).

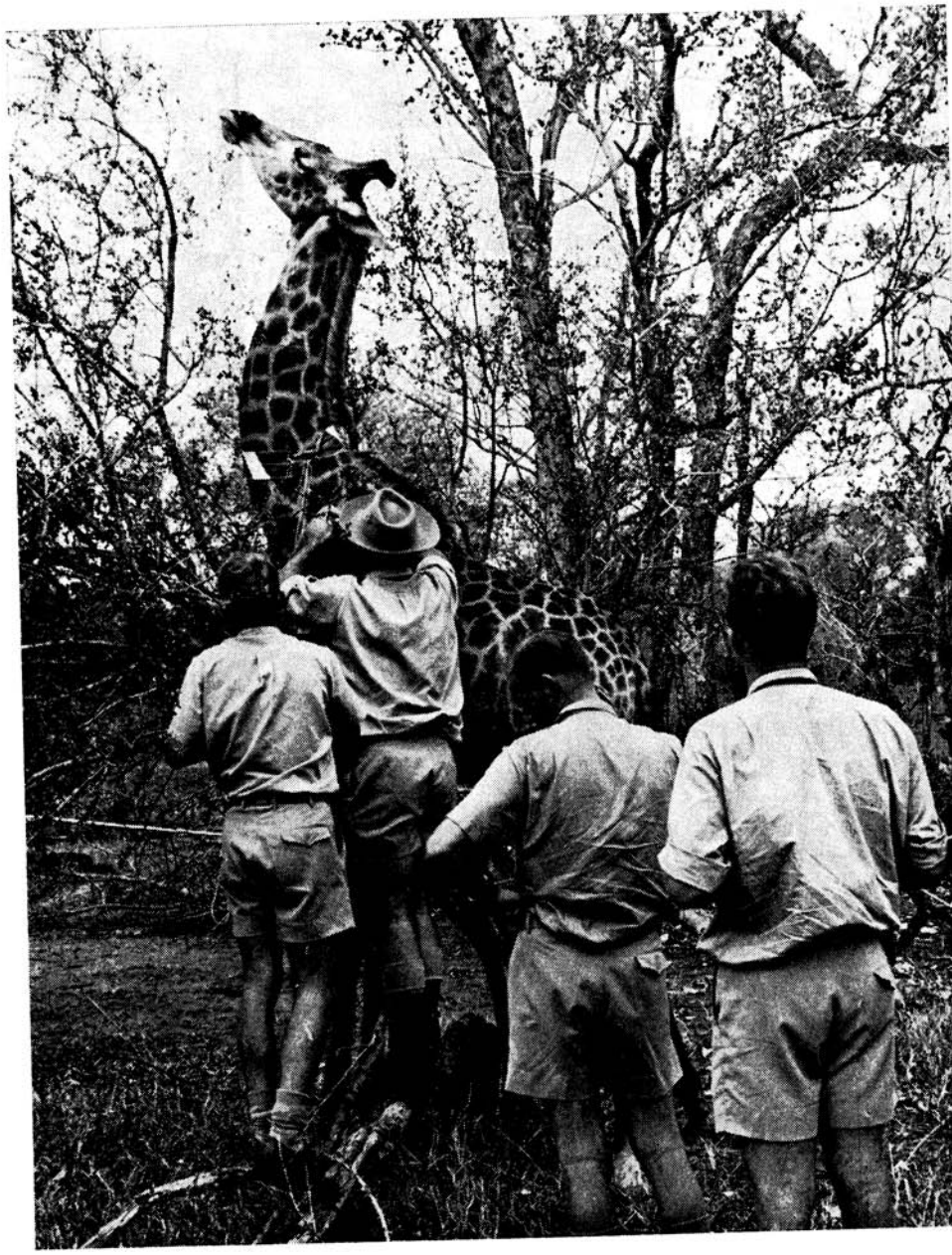

FIG. 1.—Giraffe which has been darted. Note how easily the animal can be handled under the influence of the drugs.

After thorough cleansing of the skin, 10 ml. of blood was drawn from an ear vein into a syringe which had been moistened with heparin. After withdrawing the needle from the vein it was corked with a sterile cork and the syringe inverted several times to ensure thorough mixing of the blood and heparin.

The syringe was then allowed to stand, protected from the light, with the needle uppermost so that the blood setiled out into a red cell layer and an upper leucocyte-rich plasma layer. After $1\frac{1}{2}$ hours the cork was removed from the needle and the upper half of the leucocyte-rich plasma layer was injected upwards into an inverted rubber-topped culture bottle containing 9 ml. of culture medium 199 and 0.2 ml. phytohaemagglutinin.

The leucocyte suspension was incubated at 37° C for 3 days, at Pretoriuskop and then transported to Skukuza for further preparation. This had to be done as no continuous electricity supply was available at Skukuza for the incubation. The final 3 hours that the cells spent in the culture bottle were in the presence of added colchicine at a final concentration of 10^{-6}M.

After 3 houers exposure to colchicine the cells were spun down in a test tube and most of the supernatant fluid was aspirated. The button of cells was then suspended in the remaining supernatant fluid after which distilled water was added slowly until three times the volume of cell suspension was added. This was allowed to stand for 7 minutes and then the hypotonic cell suspension was centrifuged, after which the supernatant fluid was removed.

Five ml. of acetic-alcohol (1 part glacial acetic acid to three parts methanol) was then carefully pipetted into the tube being careful not to disturb the button of cells. After at least 30 minutes the button of cells was broken up and the suspension centrifuged. All the acetic-alcohol was drawn off and fresh acetic-alcohol added. The suspension was once again centrifuged and about 2-3 ml. of acetic-alcohol was added into which the cellular button was broken and a fine suspension formed by repeated pipetting.

Three to 5 drops of the suspension were dropped onto a clean slide, and the fixative ignited by bringing the edge of the slide into contact with a flame. After drying, the slides were stained with Leishman's blood stain and rinsed with distilled water after 10 minutes.

B. SHORT TERM MARROW TECHNIQUE:

A young female giraffe was darted by one of us (N.F.) and marrow was removed for preparation. The technique for obtaining chromosome preparations was modified from the short term technique described by Kiossoglou et al. (1964).

As a certain amount of difficulty had been experienced with other species in that rapid clotting of marrow occurred, it was decided to use a technique worked out for the impala. About five minutes before marrow aspiration was performed, the giraffe was given 125,000 i.u. of heparin

FIG. 2.

Metaphase plate and karyotype prepared from this metaphase. Female Giraffe.

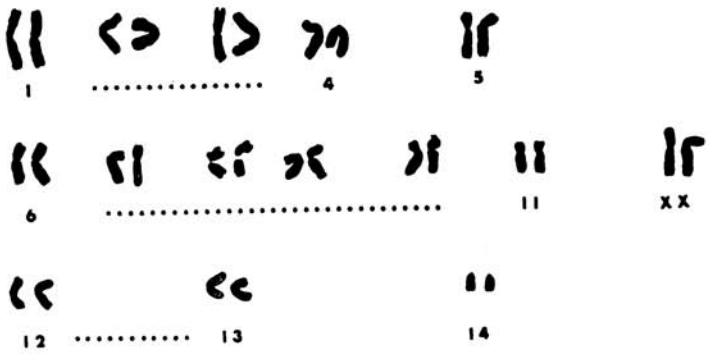

FIG. 3.

Metaphase plate and karyotype prepared from this metaphase. Male Giraffe.

intravenously. No difficulty occurred in penetrating the marrow cavity of the sternum and about 1 ml. of marrow was drawn into a syringe containing 1 ml. of a solution of 9 ml. saline and 1 ml. of 10% potassium oxalate (Onderstepoort personal communication).

The cell suspension was then carefully injected into a saline-oxalate mixture containing a final concentration of colchicine 10^{-6}M. The suspension was carefully protected from the light and the cells left in the colchicine mixture for 1 hour, after which preparation of the suspension proceeded as with the material obtained from peripheral blood culture. One minor difference being that 0.44% sodium citrate was used in the preparation rather than distilled water which was used to render the cells from the peripheral culture hypotonic.

RESULTS:

The following are the numbers of chromosomes counted per cell in the two animals:—

	NUMBER OF CHROMOSOMES PER CELL							NUMBER OF CELLS COUNTED
	<28	28	29	30	31	32	32>	
Male	—	1	1	18	—	—	—	20
Female	—	—	1	9	—	—	1	11

There is a modal number of 30 chromosomes per cell in both animals.

KARYOTYPES:

Three metaphase plates from the male and two metaphases from the female were fully analysed and karyotypes prepared. Figure 2 shows a metaphase plate plus karyotype from a female and figure 3 shows a metaphase plate and its karyotype from a male cell.

Each cell has 28 paired autosomes, these being mainly metacentric and submetacentric with one pair of acrocentric chromosomes which are small. It is possible to broadly divide the autosomes into five main groups but it is less easy to distinguish individual pairs in most groups.

The male has in addition to 28 autosomes, a large submetacentric X chromosome and a small metacentric Y chromosome. This Y chromosome is of interest in that it is metacentric and not acrocentric as is often found in other mammals.

The female has a pair of X chromosomes which rather resemble the single X found in the male and which also have to be distinguished from

chromosomes in the 1-4 group and chromosome No. 5.

DISCUSSION:

The technique of peripheral blood culture, if successful, produces a fine selection of countable metaphase plates, but under field conditions it suffers from a number of disadvantages—

(a) Until there is a continuous electricity supply at Skukuza, the distance between the incubator and the laboratory is a disadvantage.

(b) There is a strict necessity to work under sterile conditions when performing peripheral blood culture. This is not always an easy rule to adhere to in the bush under field conditions.

(c) Peripheral culture necessitates a three day wait before dividing cells are found in healthy animals. Thus any changes of technique after failure of a culture means a further wait of three days to see if the new technique is successful.

All the above disadvantages have to be carefully balanced against the many well-spread metaphase plates produced when a peripheral blood culture works well.

The short marrow technique, whilst not usually producing as many mitotic cells as the longer peripheral culture technique, does possess several distinct advantages.

(a) A strict sterile technique is not necessary unless the cell suspension is kept for longer than 6 hours.

(b) Preparations can be inspected for metaphase plates within 3 hours of sternal puncture and, if necessary, the technique can be repeated the same day in the event of a failure.

(c) No incubation is necessary. The cell suspension is kept at room temperature.

(d) No culture medium, phytohaemagglutinin or serum is necessary.

(e) The technique is a simple one, and does not appear to produce any discomfort to the tranquilized animal.

It would thus appear that the short term marrow technique possesses a number of advantages over the technically more difficult peripheral culture method as a source of material for chromosome analysis in the Kruger National Park.

SUMMARY:

The difficulties associated with, and the relative advantages of the technique of peripheral blood culture and the short term marrow technique are discussed. The chromosome number and karyotypes of a male and female giraffe are described.

ACKNOWLEDGEMENTS

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