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Q-bands of some chromosomes of the White Rhinoceros (*Diceros simus*)

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The chromosome number of the White Rhinoceros (*Diceros simus*) was established to be 84. Thirteen pairs of autosomes and the X chromosome were identified by Q-bands.

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Five species of *Rhinoceros* (order Perissodactyla) are alive today (MORRIS 1965), but only three species have been karyotyped (Table 1). The karyotype of the White Rhinoceros, *Diceros simus*, was first studied by HEINICHEN (1967, 1971) who stated the chromosome number to be 2n=82. HSU and BENIRSCHKE (1973) reported this species to have the chromosome number of 2n=84, which is verified in the present paper.

The chromosome number 84 is the highest number in Perissodactyla. As the lowest chromosome number in Perissodactyla is 32, in the Mountain zebra, *Equus zebra* (BENIRSCHKE et al. 1964), evolution has resulted in a great variety of chromosome numbers in this order. To study this chromosome evolution in more detail the Q-band patterns of the White Rhinoceros chromosomes were analysed.

Materials and methods

Materials were obtained from the fetal membranes and placenta of a normal male foal of the White Rhinoceros. The foal was born in the Copenhagen Zoo after a gestation period of about 18 months. Material for tissue culture was taken from the chorioallantoic membrane and from the great vessels of the umbilical cord. The cells were cultured in Leighton tubes at 37°C added TCM 199 and fetal bovine serum, 30%. Colcemid solution, two drops 0.004% v/w, was added per 5 cc Medium 3 hours before harvesting. After trypsination, the cells were treated with hypotonic NaCl solution, 0.30%, for 5 min. The tissue cultures, however, ceased to grow before metaphases were harvested in sufficient quantities. For blood cultures a few cc of fetal blood were taken from the umbilical vessels and the great vessels of the fetal membranes. The blood cells were cultured in the same medium as the tissue cultures, but for 72 hours at 38°C. Hypotonic treatment by KCl solution, 0.30%, for 5 min.

Most of the fibroblast cells in metaphases were burst by the hypotonic treatment, and the chromosomes of the blood metaphase plates did not spread very well. The metaphase plates from the blood were stained by conventional Giemsa stain, those of the tissue cultures were first stained by quinacrine mustard (QM) and then after fluorescence microscopy restained by Giemsa stain. These methods have been described previously (HANSEN 1972).

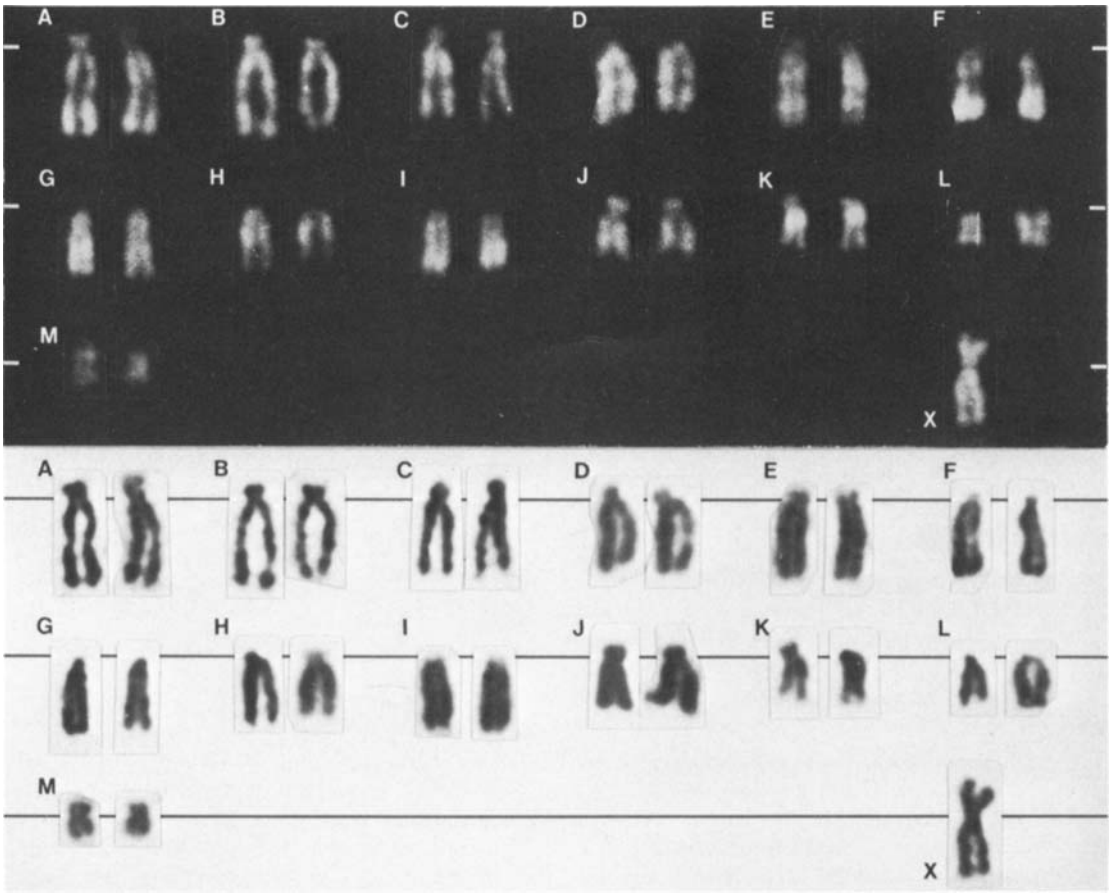

Fig. 1. Q-bands of thirteen pairs of autosomes and the X chromosome of the White Rhinoceros (*Diceros simus*). The pairs were marked by letters to avoid misinterpretations by a possible later band identification of the whole karyotype.

Results

In 10 blood metaphase plates the chromosome number was 84. The group of subtelo- and submetacentric autosomes seemed to include 9—16 pairs of chromosomes with one or two small and nearly metacentric pairs (Fig. 1, pair M). The other pairs of autosomes were all acro- or telocentric. The X chromosome was the longest submetacentric chromosome of the karyotype ($\frac{q}{p}=1.7$), and for that reason it was easy to distinguish, even by conventional Giemsa staining. The Y chromosome was not identified.

It was not possible to identify the Q-band pattern of all the chromosome pairs, because the material was very restricted, but 13 pairs of the autosomes and the X chromosome were found to show characteristic Q-band patterns (Fig. 1). To avoid misinterpretation in relation to a possible future identification of the total band karyotype, the pairs were marked by letters. Only pairs identified in four to six cells or fragments of cells are shown. Most of the telo- or acrocentric chromosomes showed bands on the proximal or distal part of the long arm. As these chromosomes possibly differ only in length, and the whole karyotype was not identified, they were not depicted in Fig. 1. The fluorescence terms recommended by the Paris Conference (1971) were used. The short arm is indicated by (p), the long arm by (q).

Pair A. The longest chromosome pair of the karyotype. (p): Medium to pale; (q): By a broad negative band divided into two medium blocks, which possibly are subdivided. The distal one is flask like. *Pair B.* The second longest pair. (p): Medium; (q): Almost uniform fluorescence, but possibly subdivided into five medium bands. *Pair C.* The third longest

Table 1. Chromosome numbers of different Rhinoceros

Subfamily	Species	2n	Authors
Rhinocerotinae			
	Great Indian Rhinoceros		
	Rhinoceros unicornis	82	WURSTER and BENIRSCHKE (1968)
		82	HSU and BENIRSCHKE (1973)
	Javan Rhinoceros		
	Rhinoceros sondaicus	not examined	
Dicerorhininae			
	Sumatran Rhinoceros		
	Didermocerus sumatrensis	not examined	
	White Rhinoceros		
	Diceros simus	82	HEINICHEN (1967, 1971)
		84	HSU and BENIRSCHKE (1973)
		84	HANSEN, present study
	Black Rhinoceros		
	Diceros bicornis	84	HUNGERFORD et al. (1967)

pair. (p): Medium to pale; (q): By a broad negative band divided into two fluorescent blocks. The proximal block is medium to intense, the distal one medium to pale. *Pair D.* (q): Three medium bands. *Pair E.* (p): Pale; (q): A pale, a negative, a medium, a broad negative, a medium and a pale band. *Pair F.* (q): A proximal medium band, a broad negative band, and a broad intense band subdivided into two bands. *Pair G.* (q): A pale, a medium, a pale, an intense, and a pale band. *Pair H.* (q): Proximal half medium, distal part pale to negative fluorescence. *Pair I.* (q): Proximal half pale to medium, divided into two bands, distal part with two medium bands. *Pair J.* (p): Medium; (q): Two medium bands and one pale band. *Pair K.* (p): Pale; (q): Proximal half intense, distal half pale to medium. *Pair L.* (q): Two medium bands. *Pair M.* (p) and (q): Very pale fluorescence. *The X chromosome.* (p): A pale, a medium, and a pale to medium band; (q): Three medium bands.

Discussion

As shown in Table 1, the chromosome number of the Black Rhinoceros is 84, and that of the Great Indian Rhinoceros 82. The chromosome numbers of the Javan Rhinoceros and of the Sumatran Rhinoceros are unknown.

The conventional Giemsa-stained karyotypes of the Great Indian Rhinoceros and of the White Rhinoceros are very similar, because many pairs of chromosomes are acrocentric or telocentric (HSU and BENIRSCHKE 1973). The morphology of some of the subtelocentric and submetacentric chromosomes seems to differ, but the morphology of the X chromosomes of the two species is very similar.

The conventional Giemsa-stained karyotype of the Black Rhinoceros seems to differ from that of the two species mentioned above, since the karyotype includes many subtelo- and submetacentric chromosomes. The X and Y chromosomes of the Black Rhinoceros were not identified (HUNGERFORD et al. 1967).

It is presumed that the original X of a common ancestor has been preserved in its entirety by diverse species of placental mammals (OHNO 1967). In the family Rhinocerotoidae the Great Indian Rhinoceros and the White Rhinoceros have very similar X chromosomes of about the same length. If the X chromosomes of the Great Indian Rhinoceros are measured on the karyotypes published by WURSTER and BENIRSCHKE (1968) and HSU and BENIRSCHKE (1973), the centromere index $(\frac{q}{p})$ is about 1.7, the same as for the White Rhinoceros. In the family Equidae (order Perissodactyla) the X chromosome of Böhm's or Grant's zebra (Common zebra) also has a centromere index of 1.7 (HANSEN 1975). Thus, the gross morphology of the X chromosomes of these three species is very similar.

The Q-band patterns of the short arms of the X chromosomes of the White Rhinoceros and of the Common zebra are very similar, but those of the long arms differ considerably. In White Rhinoceros the long arm is divided into three broad Q-bands; in Common zebra the long arm has four bands, one broad and three small Q-bands. These differences reflect the 50 million years or more which have passed since the two species had a common ancestor (COLBERT 1969).

The Q-band pattern of the autosomes of the White Rhinoceros compared to that of other species of the order Perissodactyla will be discussed elsewhere.

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