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**A CHROMOSOMAL ANALYSIS BASED ON THE
G AND C BAND STAINING TECHNIQUES OF
THE BUFFALO (*BUBALUS BUBALIS*)**

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A chromosomal analysis of 3 swamp buffaloes (2 females & 1 male) was carried out by G and C band pattern staining techniques. The diploid number of the swamp buffalo was 48, and the karyotype consisted of 10 meta- or submetacentric chromosomes and 38 acrocentric chromosomes. The X chromosome was the largest acrocentric and the Y chromosome was the smaller acrocentric. Chromosomal pairing and identification were made possible by the distinct G band patterns, which showed clearly that the Y chromosome was the second smallest acrocentric. While the C band technique stained the centromeric region of the acrocentric chromosomes, it failed to stain that of the meta- or submetacentric chromosomes. The X chromosome had the broadest C band in the centromeric region, and a greater part of the Y chromosomal arm was stained darkly by the C band technique.

INTRODUCTION

Buffalo (family Bovidae & tribe Bovini) are distinguished into Bubalia and Syncerina (tab. 1), which consist of the arni buffalo, the tamarao buffalo and the anoa buffalo in Bubalia, and the red buffalo and the black buffalo in Syncerina. The arni buffalo is classified further into two groups, the river buffalo and the swamp buffalo according to its habitat¹²⁾. The swamp buffalo (2 females & 1 male) was introduced into Japan from Taiwan to Ishigaki Island, Okinawa-ken, in 1933, and thereafter the progeny of these buffaloes have been widely bred in the various islands of Okinawa-ken¹⁵⁾. The swamp buffalo of Okinawa-ken have been used as draft animals in agriculture; however, recently, their milk and meat have attracted attention as sources of food. The biological aspects of the swamp buffalo in Okinawa-ken have been reported in detail by SHINJO ('77), and a cytogenetical study has been performed by MURAMATSU et al. ('79); however, a detailed analysis of the buffalo chromosomes has not yet been adequately accomplished.

Herein we described a chromosomal analysis of three swamp buffaloes (2 females & 1 male) in Hokkaido which was based on the G and C band pattern staining techniques. The animals were brought to Hokkaido from Okinawa-ken in 1972.

MATERIALS AND METHODS

Three swamp buffaloes (2 females & 1 male) were used for this chromosomal analysis. Blood samples were aseptically taken from the jugular vein into tubes containing heparin. Blood leucocytes were cultured using Eagle's minimum essential medium (MEM), calf serum and Bacto-phytohemagglutinin-M (PHA-M); all other preparations were made in the usual manner (MOORHEAD *et al.*, '60).

The chromosomal karyotype was analyzed with 50 metaphase figures per animal; the well-spread metaphase plates were examined and the karyotyping was arranged with a slight modification of the method used by ULBRICH & FISCHER ('67).

The procedure for G band pattern staining followed by the technique of MIYAKE & ISHIKAWA ('78) with some modifications of the method by SEABRIGHT ('71), which were, namely that the air dried preparations were treated with 0.1 % trypsin solution for 10-20 minutes at 4°C, rinsed in running water, and then stained with 2 % giemsa solution diluted with phosphate buffer (pH 6.8) for 5-10 minutes.

The C band pattern method for staining the constitutive heterochromatic components was carried out according to the BSG method of SUMNER ('72). The preparations were treated with 0.2 N HCl for 60 minutes at room temperature, immersed in 5 % Ba(OH)₂·8H₂O for 10-15 minutes at 50°C, and rinsed in running water. These samples were then immersed in 0.6 M sodium chloride containing 0.06 M tri-sodium citrate (2 × SSC) for 60 minutes at 60°C and stained with 2 % giemsa solution.

RESULTS

Figures 1-A and 2-A show a photograph of the female and the male swamp buffalo, and the female and male metaphase karyotypes and spreads, which had a diploid number of 48 chromosomes, are given (figs. 1-B, 1-C, 2-B & 2-C). The autosomes consisted of 10 meta- or submetacentric chromosomes (pair Nos. 1-5) and 36 acrocentric chromosomes (pair Nos. 6-23). The pair of sex chromosomes was XX in the female and XY in the male. From this karyotype it appeared that the X was the largest acrocentric chromosome and that the Y was one of the smaller acrocentric chromosomes.

The trypsin-giemsa banding pattern technique resulted in the characteristic G band pattern for each chromosome, and thus all chromosomes of the swamp buffaloes could be identified correctly. In addition, the pairing of chromosomes which cannot ordinarily be obtained by the conventional staining technique was possible with the G band pattern staining technique. The G band patterns of the female and the male buffalo and their schematic diagrams are shown in figures 3, 4 and 5. Table 2 gives the number of bands in each chromosome pair. It was possible to confirm by the G band staining that the X chromosome was the largest acrocentric chromosome, and that the Y chromosome was the second smallest acrocentric chromosome.

TABLE 1 A comparison of karyotypes among different buffaloes

CLASSIFICATION*1	2n	AUTOSOME		SEX CHROMOSOME		THE NUMBER OF FUNDAMENTAL ARMS	REFERENCES			
		m/sm*2	a*3	m/sm	a					
—Bubalina										
Bovidae	1	arni buffalo (<i>Bubalus bubalis</i>)	swamp b.	48	10	36	0	2	58	2, 4, 8, 10, 11, 17-19
			river b.	50	10	38	0	2	60	1, 4, 10
	2	tamarao buffalo*4 (<i>Bubalus mindrorensis</i>)	46	12	32	0	2	58	3	
	3	anoa buffalo (<i>Bubalus anoa depressicornis</i>)	48	12	34	0	2	60	9	
	—Syncerina									
1	red buffalo (<i>Syncerus caffer nanus</i>)	54	6	46	0	2	60	5, 20		
2	black buffalo (<i>Syncerus caffer caffer</i>)	52	8	42	0	2	60	19		

*1: According to ROUSE ('72)

*2: meta- or submetacentric

*3: acrocentric

*4: It has not been confirmed whether the sex chromosomes are meta- or acrocentric.

TABLE 2 *The number of G bands in each chromosome pair*

PAIR NO.	SHORT ARM	LONG ARM	PAIR NO.	SHORT ARM	LONG ARM
1	5	9	14		6
2	2	9	15		5
3	3	8	16		5
4	4	8	17		5
5	2	3	18		3
6		6	19		4
7		7	20		4
8		6	21		2
9		6	22		2
10		5	23		2
11		4	X		8
12		8	Y		1
13		7			

Figures 6 and 7 show the C band pattern of a female and a male swamp buffalo. The C band staining technique can stain the constitutive heterochromatin, which lead us to conjecture that the constitutive heterochromatin of mammals may be present in the centromeric region of the chromosome. A clear C band appeared only in the acrocentric chromosomes of the buffalo and did not appear in the meta- or submetacentric chromosomes. The X chromosome had the broadest C band in the centromeric region and was clearly distinguishable from the other acrocentric chromosomes. In the Y chromosome, the greater part of the chromosome was stained darkly, and it showed a characteristic picture as compared with the other smaller acrocentric chromosomes.

DISCUSSION

The present karyotypes of the swamp buffaloes showed a similar karyotypic picture to that obtained by ULBRICH & FISCHER ('67) and TOLL & HALNAN¹⁷⁾ ('76) and it is clear that this karyotypic pattern is common to the swamp buffalo in Thailand, Malaysia and Australia. It has also been clearly recognized that the sex chromosomes of the swamp buffalo are acrocentric; however, their size, particularly that of the Y chromosome, has not been clarified. ULBRICH & FISCHER ('67) and TOLL & HALNAN¹⁷⁾ ('76) have reported that the Y chromosome was the smallest acrocentric chromosome; however, this finding could not be proved from the karyotype demonstrated in the present experiment. The G band pattern staining showed clearly that the X chromosome was the largest acrocentric and the Y chromosome the second smallest acrocentric in the karyotype.

Compared with the G band idiogram of TOLL & HALNAN¹⁸⁾ ('76), the schematic diagram shown in figure 5 is somewhat different with regards to the numbering of chromosomes and the number or position of the bands. Probably, it appears that chromosome Nos. 2, 4, 13 and 14 coincide with Nos. 4, 2, 14 and 13 in the idiogram by TOLL & HALNAN¹⁸⁾ ('76) respectively. Moreover, the number of bands in our diagram did not coincide exactly with that of the idiogram by TOLL & HALNAN¹⁸⁾ ('76). In almost all of the chromosomes, some bands showed a difference; nevertheless, our diagram was more or less similar to that of TOLL & HALNAN¹⁸⁾ ('76).

It has been presumed that the Y chromosome is the smallest acrocentric; however, from the C band pattern staining results of this experiment, it was clear that the Y chromosome was the second smallest acrocentric chromosome. Our results also coincided with the opinion of ROMMELT-VASTERS et al. ('78), who reported that the C banding pattern does not give any indication that the centromeric region of pair No. 9 is incorporated into the chromosome pair No. 2; in this experiment it corresponded to No. 1 in our karyotype of the swamp buffaloes.

Many types and breeds of the domestic buffalo exist in South East Asia, Australia, North Africa, South America, the Middle East, and the Mediterranean Coasts. Because cytogenetical studies of the domestic buffaloes have not been adequate, the origin of buffaloes in these various countries is not clearly understood. According to available data, we know that the whole chromosome sets are 46 in the tamarao buffalo³⁾, 48 in the swamp buffalo^{2,4,8,10,11,17~19)}, 48 in the anoa buffalo⁹⁾, 50 in the river buffalo^{1,4,10)}, 52 in the black buffalo¹⁹⁾ and 54 in the red buffalo^{5,20)} (tab. 1). Furthermore, in one study the chromosomal number of a male mountain anoa (*Bubalus anoa depressicornis quarlesi*) was found to be 45 (SCHEURMEN et al., '77). The river buffalo and the swamp buffalo belong to the same genus, although the whole chromosome sets differ in each buffalo. In the case of the swamp buffalo ($2n=48$), the set has two less chromosomes than that of the river buffalo ($2n=50$). The chromosome sets of the swamp buffalo ($2n=48$) and the anoa buffalo ($2n=48$) are the same, but the number of meta- or submetacentric in the swamp buffalo is two less than that of the anoa buffalo, and the number of acrocentrics is vice versa. In the other buffaloes showing a difference in the whole chromosome sets, the karyotype also varies. Moreover, there are two types in the number of the fundamental arms. The swamp buffalo and the tamarao buffalo have 58 and the remaining buffaloes have 60, respectively. Accordingly, all buffaloes have a different chromosome karyotype. The biological significance of the differences in the chromosomal number of karyotype is a subject for further study.

In the future we expect an increase in the number of cytogenetical studies of many kinds of buffaloes using the various chromosomal band pattern staining techniques, and we hope that through these the origin of unknown buffalo breeds or the correlation of the various breeds will also be confirmed.

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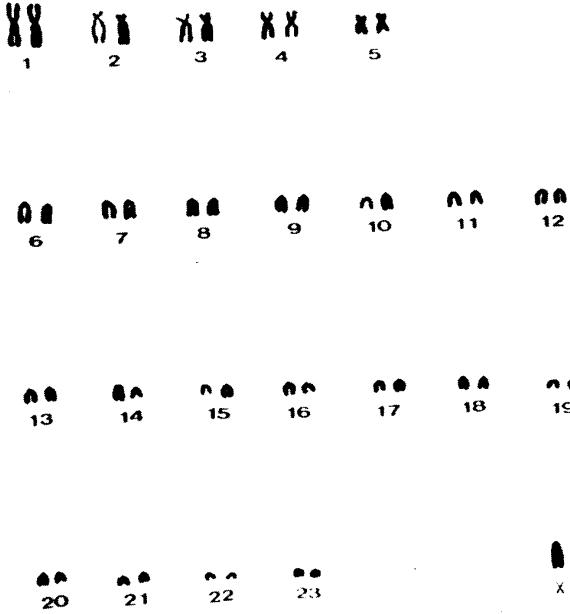
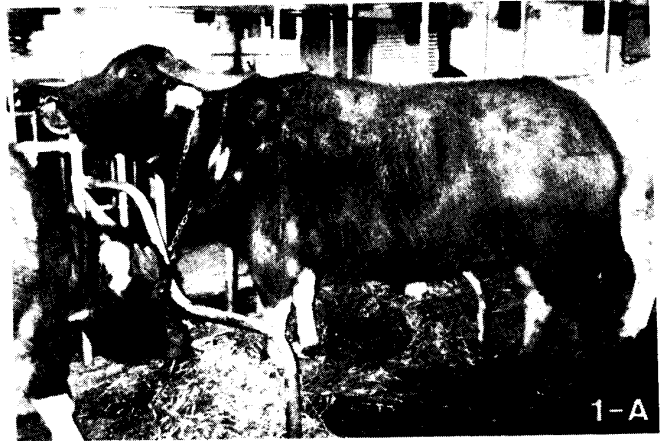
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EXPLANATION OF PLATES

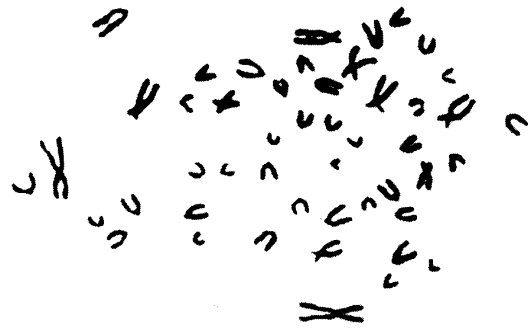
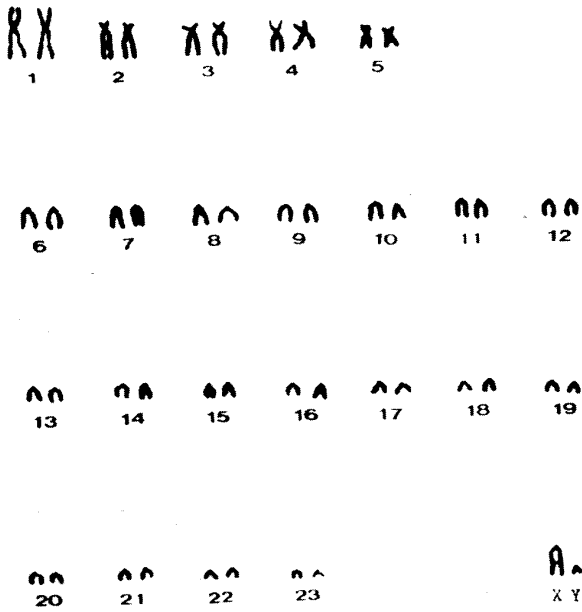
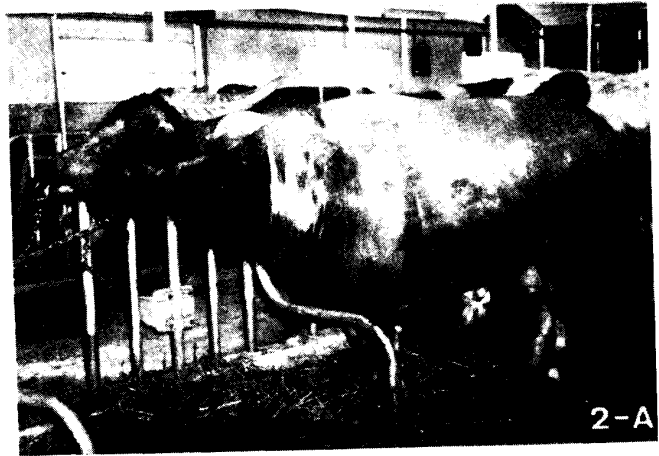
PLATE I

- | | | |
|--------|-----|---|
| Fig. 1 | 1-A | Photograph of a female swamp buffalo |
| | 1-B | A chromosomal karyotype
approx. $\times 2,000$ |
| | 1-C | A chromosomal spread
approx. $\times 2,000$ |
| Fig. 2 | 2-A | Photograph of a male swamp buffalo |
| | 2-B | A chromosomal karyotype
approx. $\times 2,000$ |
| | 2-C | A chromosomal spread
approx. $\times 2,000$ |



1-B

1-C



2-B

2-C

PLATE II

Fig. 3 G band pattern of female swamp buffalo
approx. $\times 3,200$

Fig. 4 G band pattern of male swamp buffalo
approx. $\times 4,300$

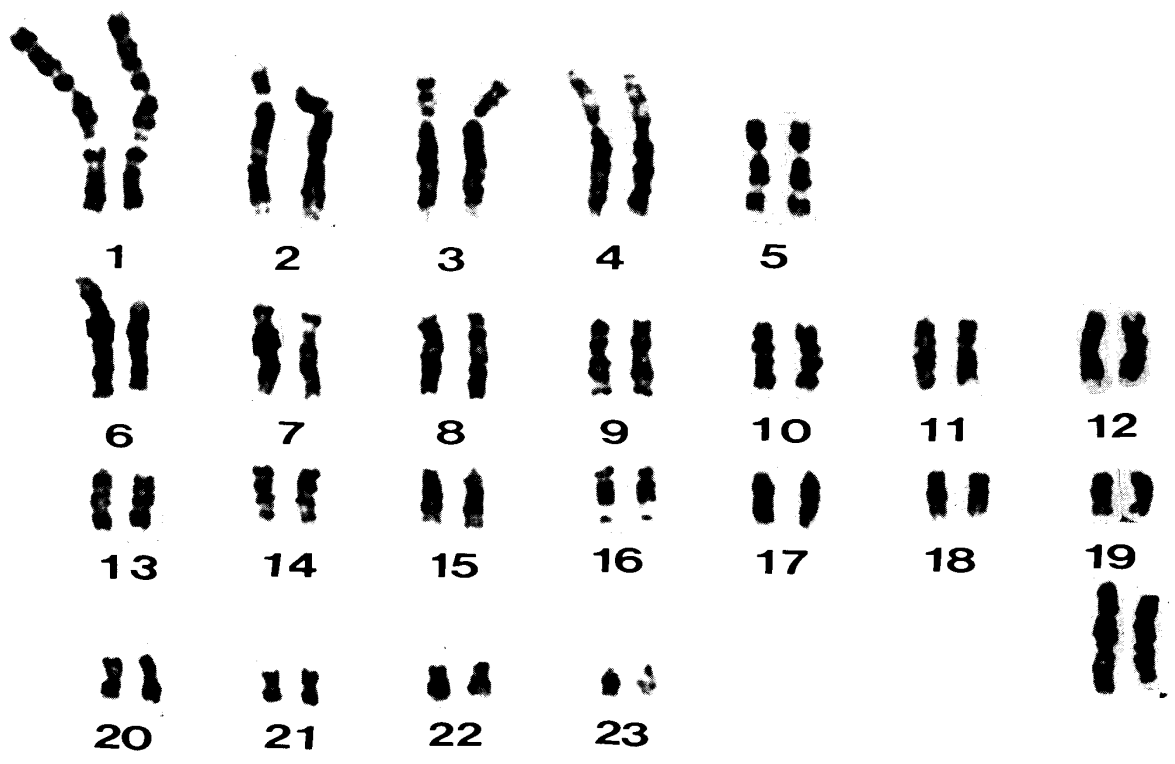


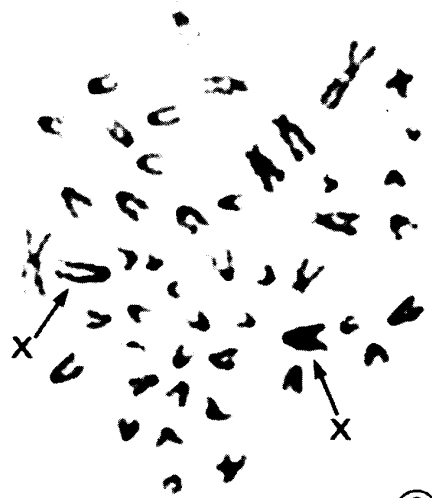
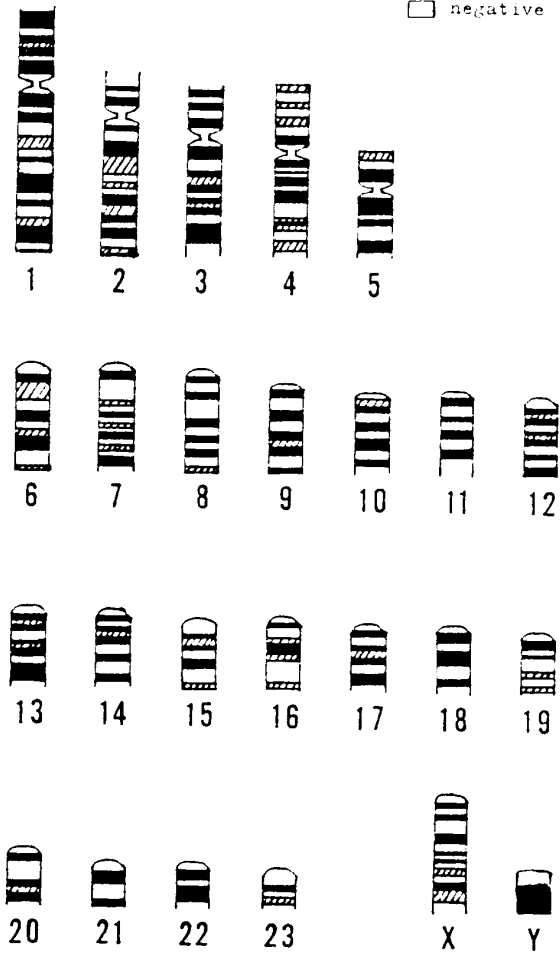
PLATE III

Fig. 5 A schematic diagram of the G band pattern

Fig. 6 C band pattern of a female swamp buffalo
X; X chromosome
approx. $\times 2,800$

Fig. 7 C band pattern of a male swamp buffalo
Y; Y chromosome
approx. $\times 1,700$

■ dark band
▨ pale band
□ negative band



⑥



⑦

⑤