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CYTOGENETIC STUDIES ON THE CHROMOSOMES OF TODA BUFFALOES

N. Murali*, P. Devendran and S. Panneerselvam

ABSTRACT

Karyological studies and sister chromatid exchange analysis were carried out in Toda buffaloes stationed at the Sheep Breeding Research Station, Sandynallah, Ooty, Tamil Nadu. Mitosis was induced by pokeweed mitogen in short term leucocyte cultures and bromodeoxyuridine was incorporated in the cultures to elucidate the sister chromatid exchanges. The modal chromosome number was found to be 50 (2n) as in other river type buffaloes, and the relative length of chromosomes ranged between 7.12 $\pm$ 0.01 and 2.51 $\pm$ 0.34. The mean sister chromatid exchange frequency was 7.8 $\pm$ 0.23, and the data on SCE frequency was found to follow the Poisson distribution.

Keywords: river buffalo, Toda buffalo, chromosomes, relative length, sister chromatid exchange

INTRODUCTION

The preference of buffaloes as milch animals in India is increasing over the years as they are considered to be a better converter of fibrous feeds into milk, more resistant to diseases and better adapted to local climatic conditions. Buffaloes contribute more than 54 percent to the total milk production in India. Buffalo cytogenetics could serve as an essential tool in implementation of breeding programmes, particularly in screening bulls used for artificial insemination programmes. Systematic cytogenetic investigation of breeding problems of buffaloes is still lacking, and hence most of the aberrations have escaped our attention.

Toda buffaloes, named after an aboriginal tribe the Toda of South India, are a genetically isolated group of animals found in the Nilgiris district of Tamil Nadu, India. This the only breed of buffalo being reared in this high rainfall and high altitude region has some phenotypic resemblance to the swamp buffaloes, but based on karyological studies, it is classified under the river buffalo (Nair *et al*., 1986).

This paper presents the karyotype, relative length of the chromosomes and sister chromatid exchange (SCE) frequency of Toda buffaloes.

MATERIALS AND METHODS

Blood samples were collected in vacutainers containing sodium heparin from seventeen male and three female Toda buffaloes maintained at the Sheep Breeding Research Station, Sandynallah, Ooty, Tamil Nadu, India. All the animals were apparently healthy and were above the age of 18 months. The cultures were set up using RPMI 1640 culture medium and buffy coat and autologous plasma from the blood samples. Mitosis was induced by the incorporation of pokeweed mitogen (10 μg/ml), and the cultures were incubated at 37.5°C for 72 h. The cultures were harvested with colchicine followed by hypotonic treatment (0.075 M KCl) and fixed in methanol: acetic acid (3:1). Air-dried slides were prepared and stained in 2 percent Giemsa (Kumar and Yadav, 1991). About 25 metaphase spreads were screened for chromosome complement. Those spreads with clear staining and non-overlapping chromosomes were photographed (x 1000) for the

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preparation of karyotypes and measuring relative length of the chromosomes.

Simultaneously, duplicate cultures were incubated with the incorporation of the bromodeoxyuridine (BrdU: 10 μg/ml) at 20 h of incubation and the samples were also harvested as per standard protocol (Iannuzzi *et al*., 1988). Air-dried slides were prepared, stained in Hoechst 33258 (10 μg/ml) for 15 minutes, incubated in 2x SSC buffer at 60°C for 1 h, exposed to sunlight in the same buffer and stained in 2 percent Giemsa (Perry and Wolff, 1974). About 25 metaphase spreads with complete chromosome complement and appreciable sister chromatid differentiation were counted for each animal to arrive at the mean SCE frequency.

RESULTS AND DISSCUSSION

The chromosomal complement revealed a diploid chromosome number (2n = 50) and the morphology resembles (first 5 pairs were submetacentric and the remaining 19 pairs were acrocentric) that of the river buffaloes (Nair *et al*., 1986; Iannuzzi, 1994). The relative length of chromosomes ranged between 6.74 ± 0.04 and 2.02 ± 0.00 (Table 1) and the ideogram is presented in Figure 1. The Y chromosome was one of the small acrocentrics and not always identifiable whereas the X chromosome was the largest acrocentric and was easily recognised in all metaphase spreads. The metaphase spread with complete chromosome complement and the karyotype are presented in Figures 2 and 3 respectively.

The comparative relative length of the chromosomes (from the longest to shortest) of Murrah, Surti and Mehsana were reported to range from 6.73 ± 0.28 to 2.24 ± 0.19, 6.92 ± 0.35 to 2.21 ± 0.24 and 6.46 ± 0.23 to 2.23 ± 0.16 respectively (Kumar and Yadav, 1991). Gupta and Chaudhuri (1978) reported the relative length of Indian Murrah buffaloes to range between 7.42 ± 0.08 and 1.69 ± 0.08 and Joshi and Govindaiah (1997) reported in South Kanara buffaloes of Karnataka as 6.8 ± 0.17 for the longest and 1.92 ± 0.7 for the shortest chromosome and these reports are comparable to the results obtained in the present study in Toda buffaloes.

The mean SCE frequency was estimated as 7.8 ± 0.23. The longer submetacentric chromosomes were observed to carry a greater number of exchanges when compared to the autosomal acrocentrics. The distribution of the SCEs was found to follow Poisson distribution. The metaphase spreads with SCEs in chromosomes of Toda buffalo are presented in Figure 4.

The SCE test has been used to detect the genome stability in most livestock species like cattle (Ciotola *et al*., 2005), sheep (Di Meo *et al*., 2000) and pigs (Peretti *et al.*, 2006). However, studies in buffaloes are comparatively few. The mean SCE frequency in indigenous buffaloes was reported to be 7.61 ± 0.18 (Joshi *et al.*, 1996) and 3.66 per cell (Vijh *et al.*, 1991) in Murrah buffaloes, 5.56 per cell (Vijh *et al.*, 1995) in Bhadawari buffaloes and 14.05 ± 0.12 (Murali *et al*., 1998) in Surti buffaloes. A detailed study of SCE in chromosome of river buffaloes reared in southern Italy revealed a mean SCE frequency of 8.8 ± 3.4 (Iannuzzi *et al*., 1988). The base line SCE frequency in Beheri and Saidi breed of Egyptian water buffaloes was reported as 8.3 ± 1.1 and 7.76 ± 0.8 respectively (Ahmed, 2001). The observations made in the present study and the data on SCE in the literature suggest that the SCEs in the chromosomes of buffaloes have a wide range and hence the technique has to be standardised in each laboratory so as to utilise it for assessing the effect of external agents.

Table 1. Relative length (mean ± s.e.) of chromosomes of Toda buffalo.

Chromosome	Mean ± S.E.
1	6.74 ± 0.04
2	6.34 ± 0.04
3	6.17 ± 0.08
4	5.53 ± 0.02
5	4.45 ± 0.18
6	4.56 ± 0.01
7	4.35 ± 0.02
8	4.03 ± 0.05
9	3.86 ± 0.03
10	3.79 ± 0.01
11	3.74 ± 0.00
12	3.60 ± 0.02
13	3.56 ± 0.02
14	3.48 ± 0.02
15	3.30 ± 0.00
16	3.07 ± 0.01
17	2.88 ± 0.07
18	2.82 ± 0.08
19	2.78 ± 0.06
20	2.70 ± 0.02
21	2.54 ± 0.01
22	2.32 ± 0.08
23	2.18 ± 0.05
24	2.02 ± 0.00
X	6.40 ± 0.00
Y	2.79 ± 0.03

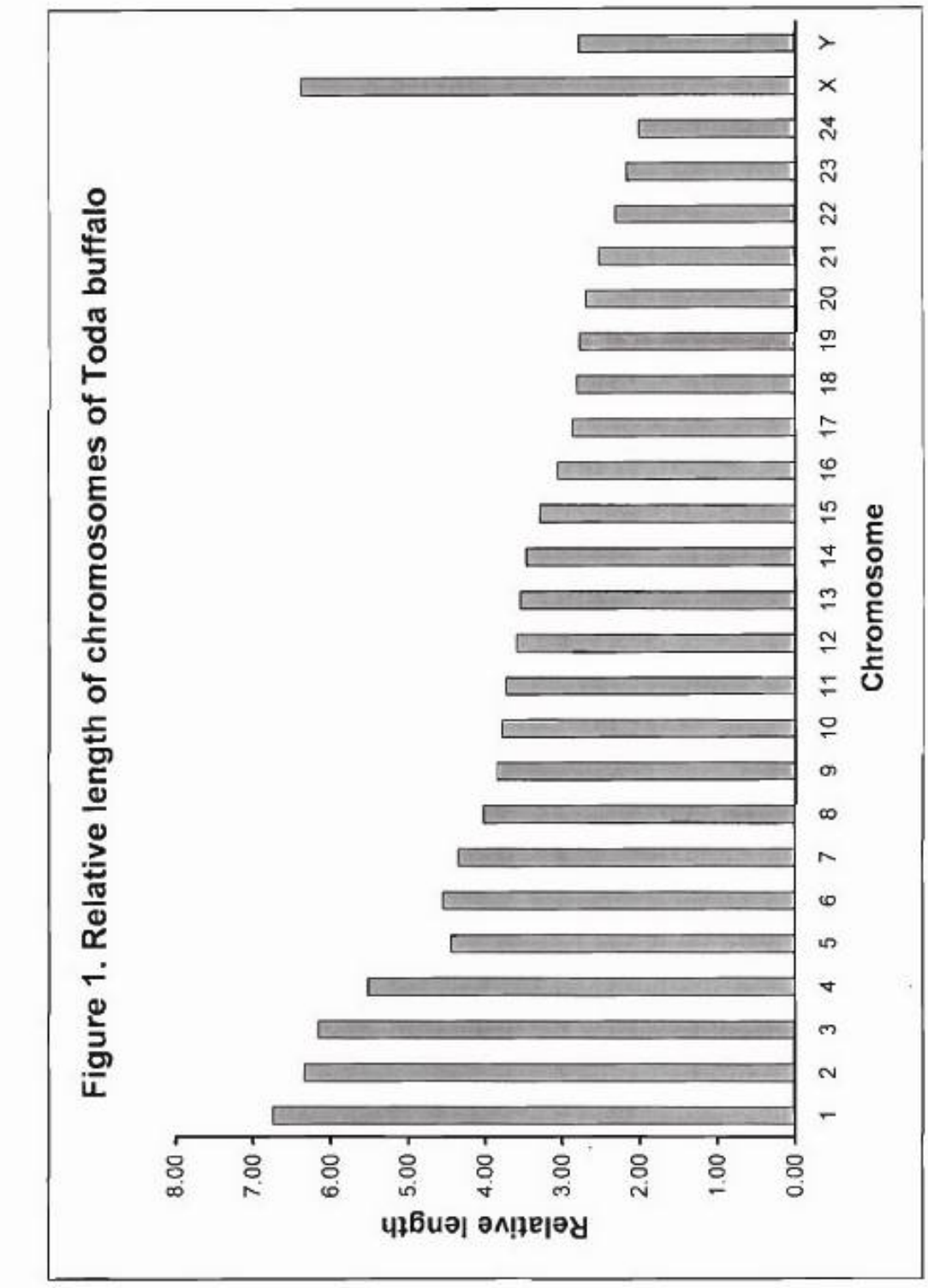
Figure 1. Relative length of chromosomes of Toda buffalo
Relative length
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 X Y
Chromosome

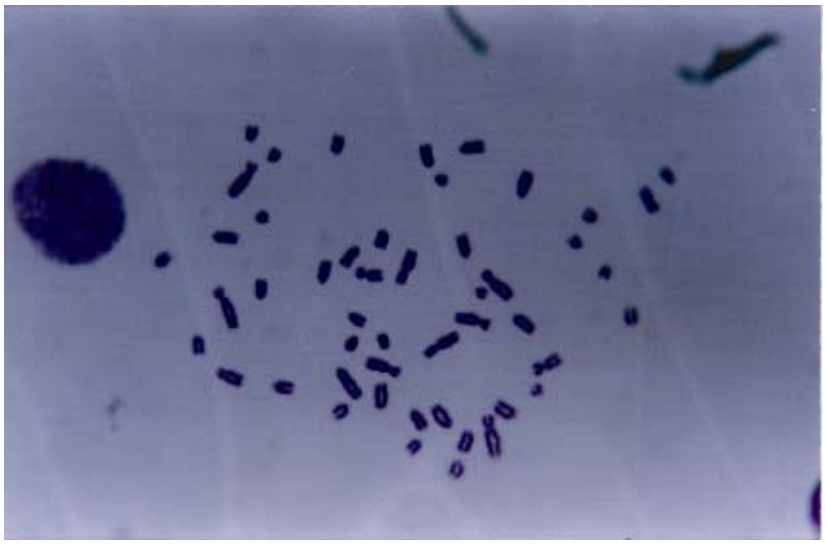

Figure 2. Metaphase spread (x 1000) of Toda buffalo (male).

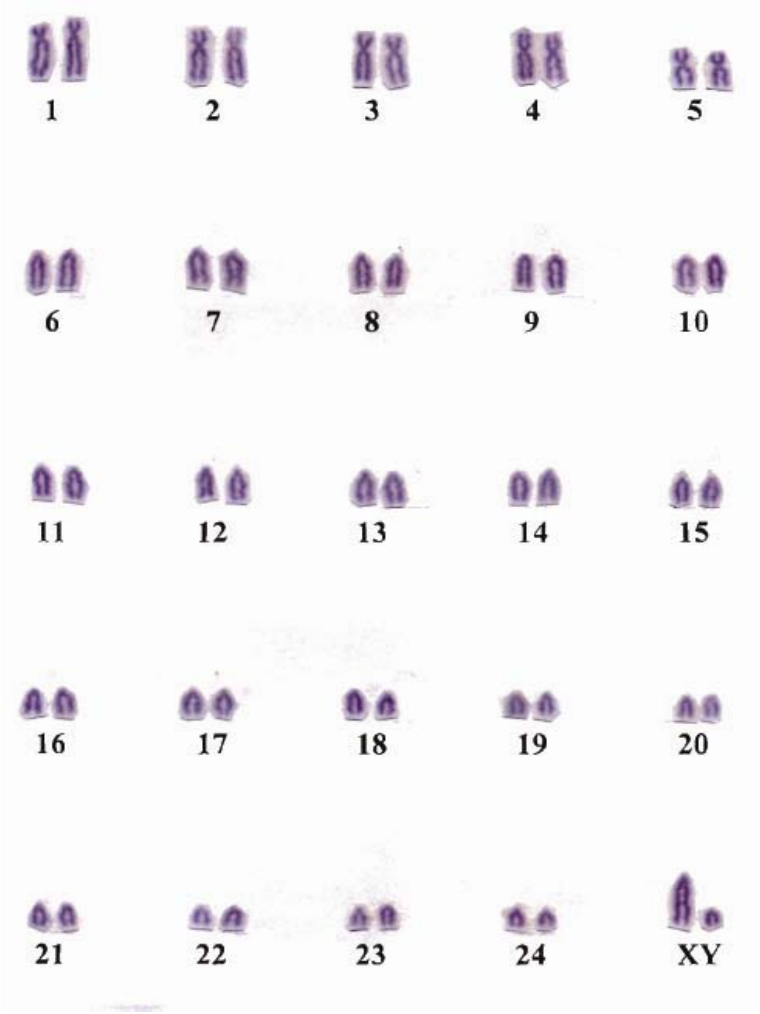

Figure 3. Karyotype of male Toda buffalo.

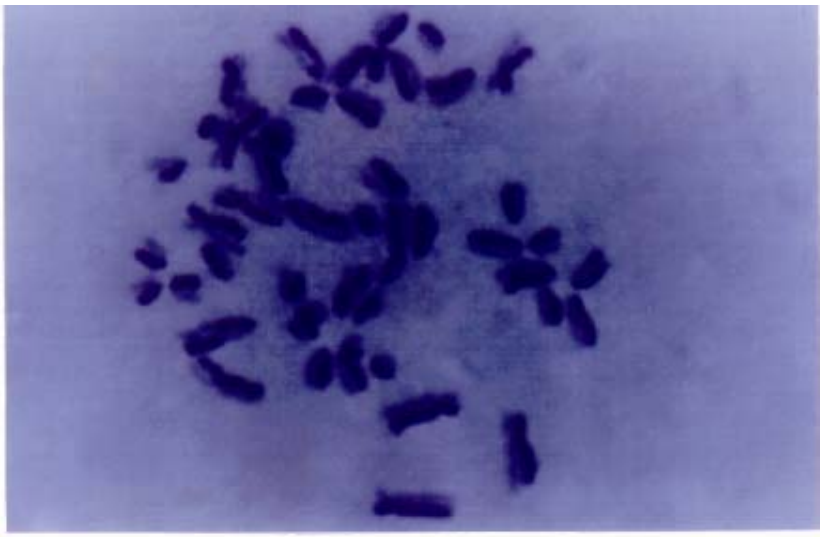

Figure 4. Sister chromatid exchange (SCE) in chromosomes of Toda buffalo (x 1000).

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