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New Robertsonian Translocation Chromosomes in Captive Thai Gaur (*Bos gaurus readei*)

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Abstract: Robertsonian translocation have been well documented in domestic cattle, with the most commonly occurring fusion involving chromosomes 1 and 29. The widespread nature of this translocation is indicative of its ancient origin. Fifty Giemsa's stained metaphase spreads derived from lymphocyte cultures of the Thai gaur were analyzed for each animal. The Thai gaur had diploid chromosome number of 2n = 57 in male and 2n = 56 in female instead of the normal 2n = 58. The 2n = 57 in male chromosomes presence of an extra submetacentric chromosome and loss of two acrocentric chromosomes was observed [XY, 57, rob (1;29)]. The 2n = 56 in female chromosomes presence of two extra submetacentric chromosomes and loss of four acrocentric chromosomes was observed [XX, 56, rob (1;29)]. Results from the Giemsa's stained analyses confirm that the two autosomes (2n = 57) and four autosomes (2n = 56) involved in the translocation are the bovine homologues 1 and 29.

Key words: Robertsonian translocation, centric fusion, Thai Gaur (*Bos gaurus readei*)

INTRODUCTION

About at the same time, two very original studies showed the importance of centric fusion translocations in bovids from both an evolutionary (Wurster and Benirschke, 1968) and clinical (Gustavsson, 1969) point of view. By analyzing the karyotypes of many bovid species, Wurster and Benirschke (1968) concluded that the differences in the diploid number were essentially due to the common use of Robertsonian translocation (rob) which reduced the diploid number and conserved, with few exceptions, the fundamental number (NF). Gustavsson (1969) demonstrated that the presence of rob (1;29) in Swedish cattle reduced fertility in the carriers when compared with cattle showing normal karyotypes.

After these two important studies, many others demonstrated a high degree of banding homologies among bovids by using banding techniques, confirming the importance of centric fusion translocations in the autosomal karyotype evolution of bovids which originated from one common bovid ancestor (Buckland and Evans, 1978; Hayes *et al.*, 1991; Gallagher and Womack, 1992; Iannuzzi and Di Meo, 1995; Mastromonaco *et al.*, 2004). Evidence indicates that the domestic cattle karyotype (2n = 60) consisting of 58 acrocentric autosomes and 2 sex chromosomes is the primitive karyotype from which all modern bovid species are descended (Wurster and Benirschke, 1968; Buckland and Evans, 1978).

Robertsonian translocations have been well documented in domestic cattle, with cases reported in numerous breeds (Popescu, 1984). Although various chromosomes have been shown to be involved in translocations (14/20, Logue and Harvey, 1978; 14/24, Di Berardino *et al.*, 1979; 16/20, Rubers *et al.*, 1996; 16/18, Iannuzzi *et al.*, 1993; 13/19, Molteni *et al.*, 1998; 1/21, Tateno *et al.*, 1994; 15/25, Iannuzzi *et al.*, 1992; 21/27, Berland *et al.*, 1988), the most commonly occurring fusion involves chromosomes 1 and 29, rob(1;29) has been found in more than 60 different cattle breeds (Popescu and Pech, 1991) throughout the world and its correlation with reduced fertility in the heterozygous carries has been confirmed (Dyrendhal and Gustavsson, 1979; Rangel-Figueiredo and Iannuzzi, 1993; Molteni *et al.*, 1996). With the systematic cytogenetic screening of cattle populations, especially for the bull, other centric fusion translocations involving different chromosomes were discovered (Long, 1985).

The Thai gaur (*Bos gaurus readei*), native cattle of the rainforests of southeast Asia, is one of the many wild cattle species currently listed as vulnerable or endangered (IUCN Red, 2002). Phenotypic features of Thai gaur were

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similar to those of Indian and Malayan gaur. The dark brown coat was short, dense and shiny with large shoulder hump and white stockings. The head was high with a bulging forehead ridge between the horns (Lekagul and McNeely, 1977, 1988). As with other Bovidae, chromosome studies indicate that gaur evolved from the wild ancestor from which the domestic European and Zebu cattle originated (Wurster and Benirschke, 1968; Buckland and Evans, 1978).

A centric fusion between chromosome 2 and 28 of the ancestral cattle karyotype of 58 acrocentric chromosomes gave rise to the gaur karyotype (2n = 58), consisting of 27 pairs of acrocentric chromosomes, 1 pair of submetacentric chromosomes and the submetacentric sex chromosomes (Winter *et al*., 1984; Gallagher and Womack, 1992; Riggs *et al*., 1997) Q-banding indicate that extensive band homologies exist between gaur and domestic cattle (Gallagher and Womack, 1992).

Recent cytogenetic analysis of a female gaur at Toronto Zoo (central of Canada) found that the individual contained 2n = 57 chromosomes instead of the normal 2n = 58, with an extra submetacentric and the loss of two acrocentric chromosomes being observed (Mastromonaco *et al*., 2004). Vadhanakul *et al*. (2004a) cytogenetic study of a Thai gaur sire of Khao Kheow Open Zoo, (central of Thailand) the results revealed that the 2n = 56. Two pairs of submetacentric autosomes of different size and 25 pairs of acrocentric autosomes were found whereas sex chromosomes were submetacentric (X) and metacentric (Y). A comparison between GTG-banded karyotype of Thai gaur with domestic cattle standard showed the 2 pairs of submetacentric autosomes of Thai gaur occurred from centric fusion of 1;29 and 2;28 acrocentric autosomes, respectively.

In this study we report the cytogenetics characterization of a new centric fusion translocation involving Thai gaur chromosomes 1 and 29 [XX, 56 and XY, 57, rob (1;29)] in a female and male from Songkhla Zoo (south of Thailand). This study demonstrates the importance of cytogenetic analysis for the establishment of screening protocols for the assessment of reproductive potential in this and other exotic Bovidae.

MATERIALS AND METHODS

Blood samples from the jugular vein were collected from one male and one female Thai gaur, which were kept in Songkhla Zoo (Thailand), using aseptic technique. The samples were kept in 10 mL vacuum tubes containing heparin to prevent blood clotting and they were cooled on ice until arriving at the laboratory. The lymphocytes were cultured using the whole blood microculture technique adapted from Kampiranont (2003).

Cell culture: The RPMI 1640 medium was prepared with 2% PHA (Phytohemagglutinin) as a mitogen and kept in blood culture bottles of 5 mL each. A blood sample of 0.5 mL was dropped into a medium bottle and well mixed. The culture bottle was loosely capped, incubated at 37°C under 5% of carbondioxide environment and regularly shaken in the morning and evening. When reaching harvest time at the 72 h of incubation, colchicine was introduced and well mixed, followed by further incubation for 30 min.

Cell harvest: The blood sample mixture was centrifuged at 1,200 rpm for 10 min and the supernatant was discarded. Ten milliliter of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture was incubated for 30 min. KCl was discarded with the supernatant after centrifugation again at 1,200 rpm for 10 min. Cells were fixed by fresh cold fixative (methanol: glacial acetic acid = 3:1) gradually added up to 8 mL before centrifuging again at 1,200 rpm for 10 min and the supernatant was discarded. The fixation was repeated until the supernatant was clear and the pellet was mixed with 1 mL fixative. The mixture was dropped onto a clean and cold slide using a micropipette followed by the air-dry technique. The slide was conventionally stained with 20% stock Giemsa's solution for 30 min.

RESULTS

Fifty Giemsa's stained metaphase spreads derived from lymphocyte cultures were analyzed for each animal. The Thai gaur had diploid chromosome number of 2n = 57 in male (Fig. 1 and 2) and 2n = 56 in female (Fig. 3 and 4) instead of the normal 2n = 58. The 2n = 57 in male Thai gaur chromosomes presence of an extra submetacentric chromosome and loss of two acrocentric chromosomes was observed [XY, 57, rob (1;29)]. The 2n = 56 in female Thai gaur chromosomes presence of two extra submetacentric chromosomes and loss of four acrocentric chromosomes was observed [XX, 56, rob (1;29)]. The translocation was detected in all metaphase spreads. Identification of the centric fusion translocation in a captive Thai Gaur by Giemsa's stained determined that fusion had occurred between the bovine homologues of chromosome 1 and 29. The karyotype shown in Fig. 2 and 4 was arranged, as far as possible, according to the ISCNDB (2000) recommendations.

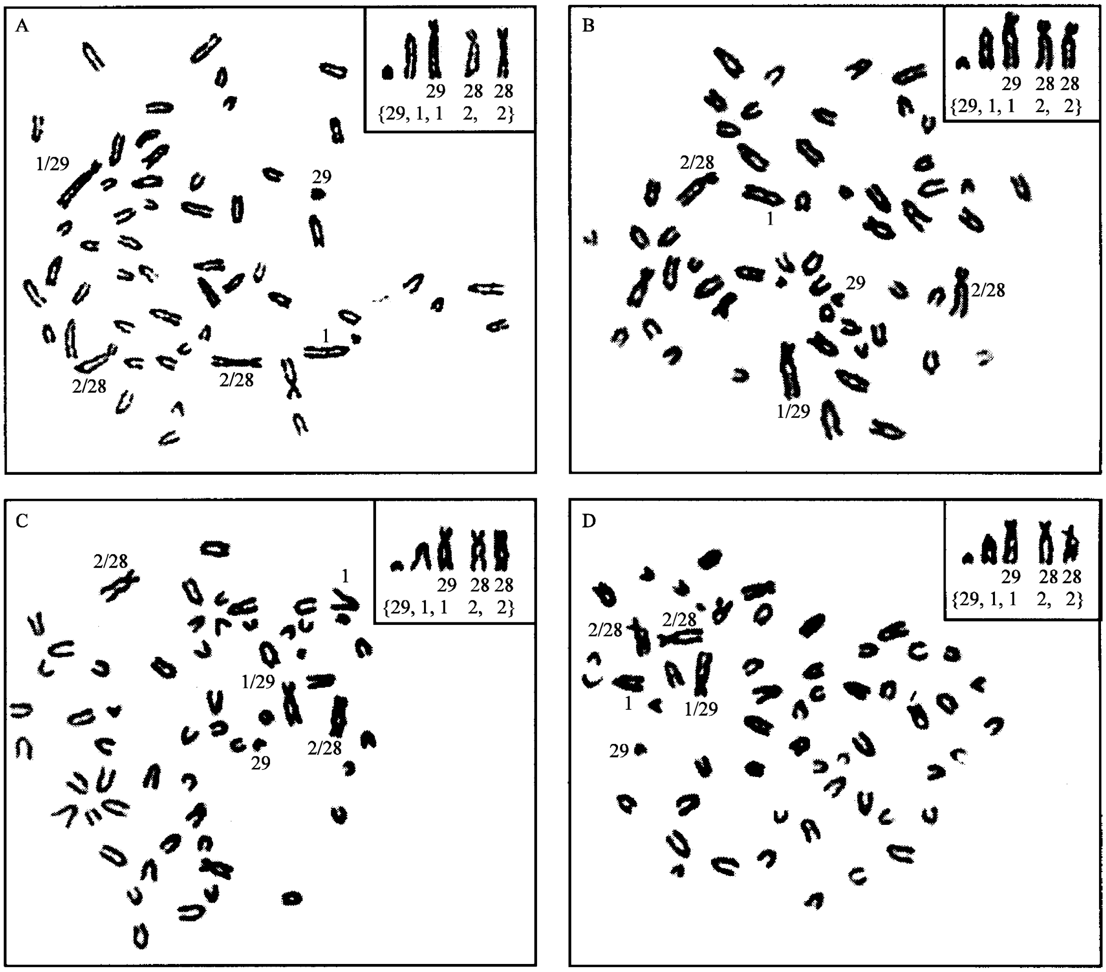

Fig. 1(A-D): Chromosome analysis of the male Thai gaur (*Bos gaurus readei*) with 2n = 57 chromosomes by conventional stained metaphase plates

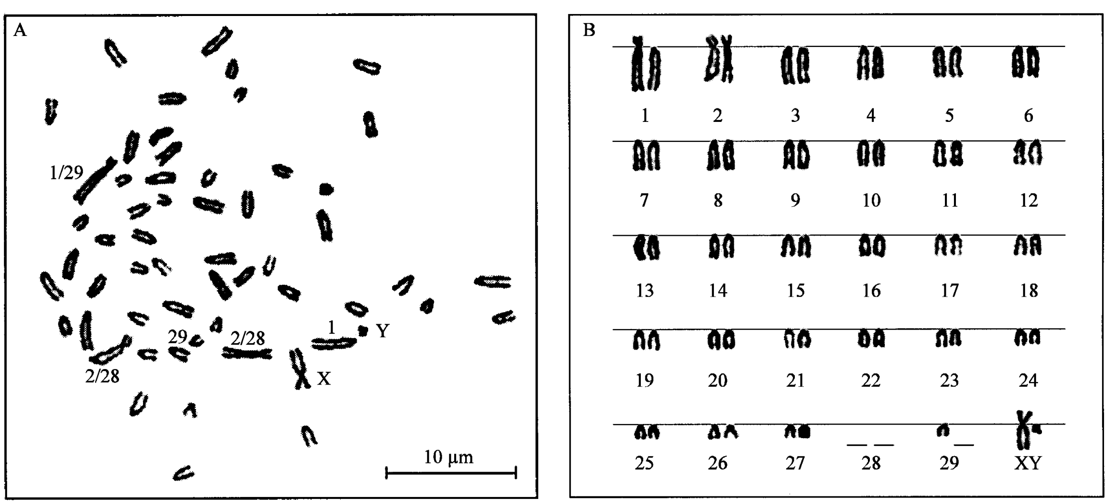

Fig. 2(A and B): Metaphase chromosome plates and karyotype of male Thai gaur (*Bos gaurus readei*) 2n = 57, by conventional staining

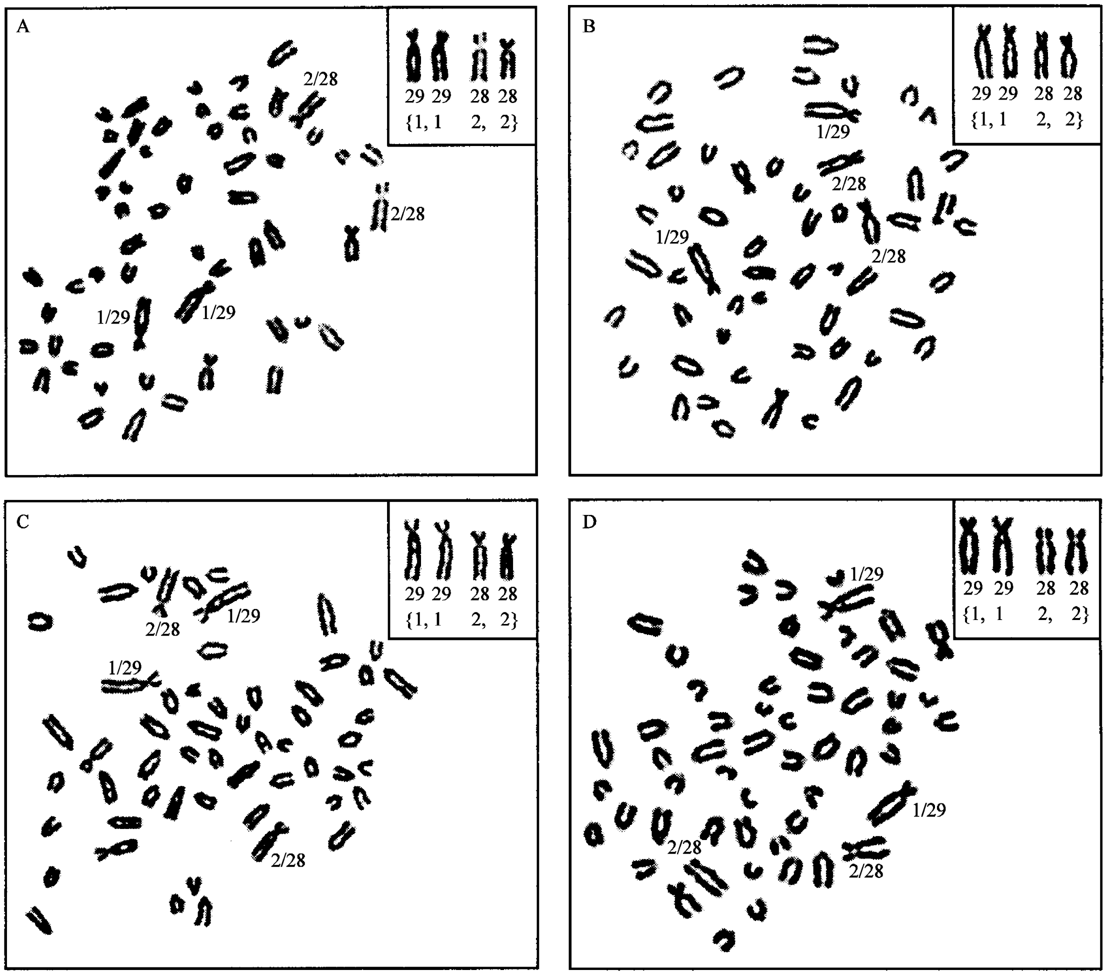

Fig. 3(A-D): Chromosome analysis of the female Thai gaur (*Bos gaurus readei*) with 2n = 56 chromosomes by conventional stained metaphase plates

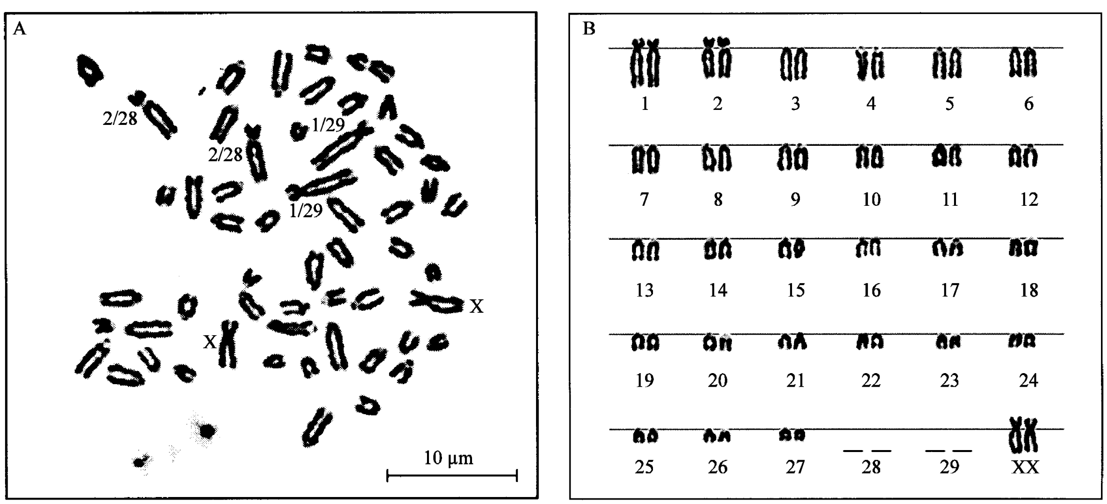

Fig. 4(A and B): Metaphase chromosome plates and karyotype of female Thai gaur (*Bos gaurus readei*) 2n = 56, by conventional staining

DISCUSSION

The identification of a translocation in a non-domestic cattle species (Thai gaur, *Bos gaurus readei*). Robertsonian translocation are one of the more common chromosome abnormalities in numerous species; cattle (Long, 1985) and human (Hamerton *et al.*, 1975). In domestic cattle, more than 25 centric fusion have been documented, with cases occurring on all continents (Long, 1985). The Robertsonian translocation reported in Bovidae by Gustavsson in 1964, rob (1;29), has now found in over 60 cattle breeds (Popescu, 1990). The translocation detected in the captive male (2n = 57) and female (2n = 56) Thai gaur in this study consisted of a fusion between the bovine homologoues of chromosome 1 and 29, as shown by Giemsa's stained analyses. Gustavasson (1979) postulated that there are two hypotheses for the worldwide distribution of rob (1;29); recurrent mutation or common ancient origin. The lack of identification of de novo cases and the monocentric nature of rob (1;29) support the latter theory.

Riggs *et al.* (1997) reported that the gaur (2n = 58) was characterized karyotypically by 2;28 Robertsonian translocation with respect to the cattle karyotype (2n = 60). This was consistent with an earlier report of Gallagher and Womack (1992) which examined chromosome comparisons between gaur and domestic cattle basing upon Q-banded karyotype. Giemsa's stained of Thai gaur in this study which had different chromosome diploid number (2n = 57 in male and 2n = 56 in female) from those two report that the first pair of submetacentric autosome were from centric fusion of 1 and 29 whereas the second pair were from centric fusion of 2 and 28 compared to G-banded standard of domestic cattle. Bongso *et al.* (1988) report the same diploid number (2n = 56) of Malayan gaur as in Thai gaur that also possessed 2 pair of submetacentric autosomes but the G-banded and C-banded karyotype was not studies. In the evolution of the Bovidae, this may explain the occurrence of fusion between chromosomes 1 and 29 in the karyotype of several species (*Bubalus bubalis*, DiBerarrdino and Iannuzzi, 1981; *Oryx* spp., *Antilope* spp., Gallagher and Womack, 1992) and its emergence in domestic cattle and the gaur.

Robertsonian translocation are thought to play a primary role in the evolution and speciation of the Bovidae (Wurster and Benirschhe, 1968; Buckland and Evans, 1978). The widespread nature of this translocation is indicative of its ancient origin. This is supported by the fact that rob (1;29) is monocentric, with a reduction in the heterochromatin block of the centromere region (Iannuzzi *et al.*, 1992) and that no spontaneous cases have been reported (Popescu, 1990). Dicentric fusion, with two distinct blocks of constitutive heterochromatin, are considered of more recent origin and can be less stable (Iannuzzi *et al.*, 1992). These have been shown to arise spontaneously in individuals from chromosomally normal parents (Iannuzzi *et al.*, 1993; Rubers *et al.*, 1996; Molteni *et al.*, 1998). Although the mechanism for the formation of Robertsonian translocation has not been elucidated, evidence indicates that they may arise as a result of the following possibilities; loss of telomeric sequences due to telomere shortening or chromosome breakage, loss of telomeric function or recombination between homologous sequences on non-homologous chromosomes (Page *et al.*, 1996; Slijepcevic, 1998).

Buckland and Evans (1978) suggested that bovid chromosomal evolution had proceeded from a primitive karyotype of 58 acrocentric autosome (2n = 60) as seen domestic cattle, domestic goat and many other bovids. They also agreed that centric fusion played an important role in reducing the ancestral diploid number to the range of values currently seen. Robertsonian translocation is better tolerated among the Bovidae than structural rearrangements such as inversion or alterations in sequence (Toll and Halnan, 1976). Most type of centric fusion reported for dairy cattle involve 1;29 Robertsonian translocation (Berland *et al.*, 1988) which has the main effect on impairing fertility in all studied breeds (Kovacs, 1989; Popescu, 1980).

Numerous studies provide evidence for the role of Robertsonian translocation in reduced fertility (Kawarsky *et al.*, 1996; Rubers *et al.*, 1999; Gustavsson, 1969; Dyrendahl and Gustavsson, 1979; Rangel-Figueiredo and Iannuzzi, 1991; Vadhanakul *et al.*, 2004b). Despite phenotypically normal, translocation carriers experience a decrease in reproductive potential, pseudominantly due to the production of chromosomally unbalanced gametes which subsequent to fertilization result in early embryonic death (Poppescu, 1990). This is supported by studies which detected monosomic (2n-1) and trisomic (2n+1) embryos among embryos sired by bulls who were heterozygous carriers of rob (1;29) (King *et al.*, 1980, 1981; Popescu, 1980), whereas no live offspring with these deleterious aneuploidies have been reported.

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