ABSTRACT

Standardized karyotype and idiom of the Mehsani buffalo (*Bubalus bubalis*) at Burirum Livestock Research Station, Thailand, was studied. Blood samples were taken from two male and two female buffaloes. After standard whole blood lymphocytes were cultured at 37°C for 72 h in the presence of colchicine, the metaphase spreads were prepared on microscopic slides and air-dried. Conventional staining, GTG-banding, CBG-banding and Ag-NOR banding techniques were applied to stain the chromosomes. The results showed that the diploid chromosomes number of Mehsani buffaloes was $2n=50$; the fundamental numbers (NF) were 60 in both male and female. The types of autosomes were four large metacentric, six large submetacentric, eight large telocentric, eight medium telocentric and 22 small telocentric chromosomes. The X chromosome was a large telocentric chromosome and the Y chromosome was a small telocentric chromosome. In GTG-banding, each chromosome pair appeared clearly differentiated. CBG-banding showed dark bands on the centromere of all telocentric chromosomes (autosome pairs 6-24 and the X chromosome) but light band on the others (autosome pairs 1-5 and the Y chromosome). Ag-NOR banding exhibited six position of autosomes (four telocentric and two submetacentric chromosomes) in Mehsani buffaloes. The karyotype formula of Mehsani buffaloes was as follows: $2n$ (diploid) $50 = L^m_4 + L^un_6 + L^t_8 + M^c_6 + S^t_22 + sex$ chromosomes.

Keywords: karyotype, idiom, Mehsani buffalo (*Bubalus bubalis*), chromosome

INTRODUCTION

The river buffalo, *Bubalus bubalis* (Figure 1) is an economically important livestock species in many Asian and Mediterranean countries, and its genetic improvement, especially in reproductive performance and quantity of meat and milk production, ranks high among the agricultural research needs of these countries (El Nahas et al., 2001).

The first international conference which established standard karyotypes in domestic animals was the Reading Conference (1976). GTG-banded karyotypes at medium (cattle and buffalo) and low band (sheep and goat) resolutions were presented without diagrammatic representations of the banding patterns. Later, the use of R-banding techniques and of prometaphase preparations made it neces-

---

1Applied Taxonomic Research Center, Department of Biology, Faculty of Science, Khon Kaen University, Muang, Khon Kaen, 40002, Thailand, *E-mail: tanomtong@hotmail.com
2Burirum Livestock Research Station, Pakhum, Burirum, 31220, Thailand
3Biology Program, Faculty of Science and Technology, Surindra Rajabhat University, Muang, Surin, 32000, Thailand
sary to organize a second international conference (ISCNDA 1989). Karyotypes with more elongated chromosomes (450 band levels) were proposed using various banding techniques: GTG, QFQ, RBA and RBG for cattle (BTA), RBA and RBG for both sheep and goat (Di Berardino et al., 2001).

As is known, the buffalo (B. bubalis) includes two cytotypes commonly referred to as the river buffalo \( (2n=50) \) and the swamp buffalo \( (2n=48) \). Several cytogenetic studies have been carried out to define the conventional karyotype (Fischer and Ulbrich, 1968; Chandra, 1968; De Hondt and Ghanam, 1971) as well as the distribution of constitutive heterochromatin and G-banding pattern of this species (Gupta and Ray-Chaudhuri, 1978; Cribiu and Obeidah, 1978).

Previous cytogenetic studies of river buffalo include Dutt and Bhattacharya (1952); Chandra (1968); Fischer and Ulbrich (1968); De Hondt and Ghanam (1971); Bongso et al. (1977, 1982); Cribiu and Obeidah (1978); Gupta and Ray-Chaudhuri (1978); Di Berardino et al. (1979); Di Berardino and Iannuzzi (1981, 1984); Chavananikul (1989); Yadav et al. (1991); Iannuzzi (1994); Iannuzzi and Di Meo (1995); Iannuzzi et al. (1987, 1990, 1998, 2003); Di Meo et al. (2002); Tanaka et al. (1999, 2000); El Nahas et al. (2001); Patel et al. (2006); Chauhan et al. (2009) and Murali et al. (2009).

MATERIALS AND METHODS

Blood samples were collected from two male and two female Mehsani buffaloes, kept at the Burirum Livestock Research Station (BLRS), Thailand by aseptic technique. The samples were kept in 10 ml vacuum tubes containing heparin to prevent blood clotting and cooled on ice until arriving at the laboratory.

Cell preparation

The lymphocytes were cultured using the whole blood microculture technique adapted from Rooney (2001) and Campiranont (2003).

Cell culture

The 5 ml of RPMI 1640 medium was prepared with 2% PHA (phytohemagglutinin) as a mitogen and kept in blood culture flasks. A blood sample of 0.5 ml was dropped into a medium bottle and mixed well. The culture bottles were loosely capped, incubated at 37°C under a 5% carbon dioxide environment and shaken regularly in the morning and evening. When harvest time was nearly reached at 72 h of incubation, colchicine was added and mixed well, followed by further incubation for 30 minutes.

Cell harvest

The blood sample mixture was centrifuging at 3,000 rpm for 5 minutes and the supernatant was discarded. Ten milliliters of hypotonic solution (0.075 M KCl) was applied to the pellet and the mixture incubated for 30 minutes. KCl was discarded from the supernatant after centrifugation again at 3,000 rpm for 5 minutes. Cells were fixed in fresh cool fixative (3 methanol: 1 glacial acetic acid) gradually added up to 8 ml before centrifuging again at 3,000 rpm for 5 minutes, 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 by micropipette followed by the air-dry technique. The slide was conventionally stained with 20% Giemsa’s solution for 30 minutes.

GTG-banding method

G-banding technique was adapted from
Campiranont (2003). The slide was well dried and then soaked in working trypsin (0.025% trypsin EDTA) at 37°C until the termination of trypsin activity by washing the slide with sorenson buffer. The slide was stained with 20% Giemsa’s solution for 30 minutes.

**CBG-banding method**

Slides were heated at 60°C for 2-3 days, soaked in 0.2 N HCl for 10-15 minutes, rinsed with distilled water then soaked in 0.05 N Ba(OH)₂ for 15 minutes at 37°C, rinsed with distilled water at 60°C, and then soaked in 2X SSC at 60°C for 1-2 h. The slides were stained with 20% Giemsa’s solution for 30 minutes.

**Ag-NOR staining method**

Two drops of 50% silver nitrate and 50% gelatin were placed on slides, and then they were sealed with cover glasses and incubated at 60°C for 3 h. Then, they were soaked in distilled water until the cover glasses were separated. The slides were then stained with 20% Giemsa’s solution for 1 minute.

**Chromosomal checks, karyotyping and idiograming**

Chromosome counting was performed on mitotic metaphase cells under a light microscope. Twenty clearly observable and well spread chromosomes from the males and 20 from the females were selected and photographed. The length of short arm chromosomes (Ls) and the lengths of long arm chromosomes (Ll) were measured and total arm length of the chromosomes were calculated (LT, LT = Ls+Ll). The relative length (RL) and the centromeric index (CI) were estimated. CI was also computed to classify the types of chromosomes according to Chaiyasut (1989). All parameters were used in karyotyping and idiograming.

**RESULTS AND DISCUSSION**

Cytogenetic study of the Mehsani buffalo using lymphocyte culture revealed that the chromosome number is 2n (diploid) = 50 (Figures 2 and 6). This is the same chromosome number reported for river buffaloes in previous studies (Dutt and Bhattacharya, 1952; Chandra, 1968; Fischer and Ulbrich, 1968; De Hondt and Ghanam, 1971; Bongso et al., 1977, 1982; Cribiu and Obeidah, 1978; Gupta and Ray-Chaudhuri, 1978; Chavanankul, 1989; Yadav et al., 1991; Iannuzzi, 1994; Iannuzzi and Di Meo, 1995; Iannuzzi et al., 1987, 1990, 1998, 2003; Di Meo et al., 2002; Tanaka et al., 1999, 2000; El Nahas et al., 2001; Patel et al., 2006; Chauhan et al., 2009 and Murali et al., 2009).

The domestic buffalo (*B. bubalis*) has been classified into two general types according to geographical distribution: one is the river-type buffalo, raised in most areas from India to Egypt and in some southern and eastern European countries; the other is the swamp-type buffalo of South-east Asia (Mason, 1974). The karyotypes differ in the two types of buffalo, and their diploid chromosome numbers are 48 and 50 in the swamp type buffalo and the river type buffalo, respectively (Fischer and Ulbrich, 1968). The karyotypes of the two types of buffaloes differ due to tandem fusion translocation; the swamp-type chromosome 1 resulted from a telomere-centromere tandem fusion between the river type chromosome 4, and 9, with a loss of the centromere of river-type chromosome 9 (Bongso and Hilmi 1982; Di Berardino and Iannuzzi 1981; Tanaka et al., 1999).
This examination also revealed that the fundamental number (NF, number of chromosome arms) of the Mehsani buffalo was 60 in both the male and the female. This is the same NF for the river buffalo as reported by Chandra (1968); De Hondt and Ghanam (1971); Bongso et al. (1977) and Iannuzzi (1994). The family Bovidae includes several species demonstrating variable diploid chromosome numbers but having similar fundamental numbers (NF=60), which, with the exception of a few cases, vary between 58 and 62. The karyotype contains variable numbers of centric fusions, or Robertsonian translocations, which have changed the diploid number but not the NF (Wurster and Benirschke, 1968). These rearrangements of a basic karyotype consisting of one-armed chromosomes have later been confirmed by studies using banding techniques in various species of Bovidae (Evans et al., 1973; Buckland and Evans, 1978; Bunch and Nadler, 1980; Di Berardino and Iannuzzi, 1981, 1984).

The types of autosomes were four large metacentric, six large submetacentric, eight large telocentric, eight medium telocentric and 22 small telocentric chromosomes. These features are similar to the reports of Chandra (1968); De Hondt and Ghanam (1971); Bongso et al. (1977); Iannuzzi (1994) and Murali et al. (2009). The X chromosome of the Mehsani buffalo is a large telocentric chromosome, and the Y chromosome is the small a telocentric chromosome. These features are similar to the reports of Di Berardino and Iannuzzi (1981) and Di Meo et al. (2005) that revealed river buffalo have telocentric X and Y chromosomes. In comparison, in the other ruminant species in the family Bovidae in Thailand, the X chromosomes of swamp buffalo (B. bubalis), gaur (B. gaurus), banteng (B. javanicus), cattle (Bos taurus) and cattle (Bos indicus) are telocentric, submetacentric, submetacentric, submetacentric and submetacentric chromosome, respectively. The Y chromosomes of all these species are telocentric, metacentric, submetacentric, submetacentric and acrocentric chromosome, respectively (Wurster and Benirschke, 1968).

From GTG-banding technique, each chromosome pair appears with distinctively differentiated. The G-banded revealed that the number of bands on 1 set of haploid chromosomes, which includes autosomes, X and Y chromosomes, are 346 bands for the Mehsani buffalo (Figures 3 and 7). The G-banded provide a clearly chromosome band which represent in black and white regions on chromosome. The level of band numbers is defined by a visible and in a haploid set which compose of autosomes, X and Y chromosome. Thus, the haploid set of the Mehsani buffalo consist of 24 autosomes include X and Y chromosome.

CBG-banding technique demonstrated dark bands (C-positive) on centromere of all telocentric chromosomes (pairs 6-24 autosome and X chromosome) but other appears as light bands or C-negative (pairs 1-5 autosome and Y chromosome) (Figures 4 and 8). The C-banding can provide a dark region on chromosome which represents the constitutive heterochromatin of chromosome that can be found at all centromeres and some telomeres of normal chromosomes. C-banding is being accepted technique for the sex chromosome studying, especially for the identification of Y chromosome because of its individual characteristics that normally cannot provide a dark region on the centromere (Campiranont, 2003).

In this investigation, the six nucleolar organizer regions, NORs (satellite chromosomes), which represent the chromosome marker, are located on the long arm near telomere of two pairs
Table 1. Mean of the short arm chromosome length (Ls), the long arm chromosome length (Ll), total arm chromosome length (LT), relative length (RL), centromeric index (CI), chromosome size and chromosome type from metaphase chromosomes of 20 cells in male and female the Mehsani buffalo (*Bubalus bubalis*), 2n (diploid) = 50.

<table>
<thead>
<tr>
<th>Chromosome Pair</th>
<th>Ls (cm)</th>
<th>Ll (cm)</th>
<th>LT (cm)</th>
<th>RL</th>
<th>CI</th>
<th>Size</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.359</td>
<td>0.820</td>
<td>1.179</td>
<td>0.071</td>
<td>0.695</td>
<td>L</td>
<td>sm</td>
</tr>
<tr>
<td>2</td>
<td>0.342</td>
<td>0.736</td>
<td>1.078</td>
<td>0.063</td>
<td>0.682</td>
<td>L</td>
<td>sm</td>
</tr>
<tr>
<td>3</td>
<td>0.453</td>
<td>0.554</td>
<td>1.007</td>
<td>0.060</td>
<td>0.550</td>
<td>L</td>
<td>m</td>
</tr>
<tr>
<td>4</td>
<td>0.347</td>
<td>0.638</td>
<td>0.985</td>
<td>0.053</td>
<td>0.647</td>
<td>L</td>
<td>sm</td>
</tr>
<tr>
<td>5</td>
<td>0.373</td>
<td>0.486</td>
<td>0.859</td>
<td>0.045</td>
<td>0.565</td>
<td>L</td>
<td>m</td>
</tr>
<tr>
<td>6</td>
<td>0.000</td>
<td>0.647</td>
<td>0.647</td>
<td>0.047</td>
<td>1.000</td>
<td>L</td>
<td>t</td>
</tr>
<tr>
<td>7</td>
<td>0.000</td>
<td>0.616</td>
<td>0.616</td>
<td>0.045</td>
<td>1.000</td>
<td>L</td>
<td>t</td>
</tr>
<tr>
<td>8</td>
<td>0.000</td>
<td>0.591</td>
<td>0.591</td>
<td>0.043</td>
<td>1.000</td>
<td>M</td>
<td>t</td>
</tr>
<tr>
<td>9</td>
<td>0.000</td>
<td>0.564</td>
<td>0.564</td>
<td>0.041</td>
<td>1.000</td>
<td>M</td>
<td>t</td>
</tr>
<tr>
<td>10</td>
<td>0.000</td>
<td>0.545</td>
<td>0.545</td>
<td>0.040</td>
<td>1.000</td>
<td>M</td>
<td>t</td>
</tr>
<tr>
<td>11</td>
<td>0.000</td>
<td>0.528</td>
<td>0.528</td>
<td>0.039</td>
<td>1.000</td>
<td>M</td>
<td>t</td>
</tr>
<tr>
<td>12</td>
<td>0.000</td>
<td>0.499</td>
<td>0.499</td>
<td>0.036</td>
<td>1.000</td>
<td>M</td>
<td>t</td>
</tr>
<tr>
<td>13</td>
<td>0.000</td>
<td>0.469</td>
<td>0.469</td>
<td>0.034</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>14</td>
<td>0.000</td>
<td>0.451</td>
<td>0.451</td>
<td>0.033</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>15</td>
<td>0.000</td>
<td>0.427</td>
<td>0.427</td>
<td>0.031</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>16</td>
<td>0.000</td>
<td>0.404</td>
<td>0.404</td>
<td>0.029</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>17</td>
<td>0.000</td>
<td>0.384</td>
<td>0.384</td>
<td>0.028</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>18</td>
<td>0.000</td>
<td>0.366</td>
<td>0.366</td>
<td>0.027</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>19</td>
<td>0.000</td>
<td>0.348</td>
<td>0.348</td>
<td>0.025</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>20</td>
<td>0.000</td>
<td>0.327</td>
<td>0.327</td>
<td>0.024</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>21</td>
<td>0.000</td>
<td>0.313</td>
<td>0.313</td>
<td>0.023</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>22</td>
<td>0.000</td>
<td>0.293</td>
<td>0.293</td>
<td>0.021</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>23</td>
<td>0.000</td>
<td>0.269</td>
<td>0.269</td>
<td>0.019</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>24</td>
<td>0.000</td>
<td>0.240</td>
<td>0.240</td>
<td>0.017</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
<tr>
<td>X</td>
<td>0.000</td>
<td>0.892</td>
<td>0.892</td>
<td>0.065</td>
<td>1.000</td>
<td>L</td>
<td>t</td>
</tr>
<tr>
<td>Y</td>
<td>0.000</td>
<td>0.306</td>
<td>0.306</td>
<td>0.027</td>
<td>1.000</td>
<td>S</td>
<td>t</td>
</tr>
</tbody>
</table>

Remarks: L = large chromosome, M = medium chromosome, S = small chromosome, m = metacentric chromosome, sm = submetacentric chromosome, and t = telocentric chromosome.
Figure 1. Mehsani buffalo, *Bubalus bubalis* (Artiodactyla, Bovidae).
Figure 2. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo (*Bubalus bubalis*) $2n$ (diploid) = 50 by conventional staining technique, showing sex chromosomes (arrows), scale bars 10 $\mu$m.
Figure 3. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo (*Bubalus bubalis*) 2n (diploid) = 50 by GTG-banding technique, showing sex chromosomes (arrows), scale bars 10 μm.
Figure 4. Metaphase chromosome plates (left) and karyotypes (right) of Mehsani buffalo (*Bubalus bubalis*) 
$2n$ (diploid) = 50 by CBG-banding technique, showing sex chromosomes (arrows), scale bars 
10 μm.
Figure 5. Metaphase chromosome plates of male (top) and female (bottom) Mehsani buffalo (*Bubalus bubalis*) $2n$ (diploid) = 50 by Ag-NOR banding technique, showing nucleolar organizer regions, (arrows), scale bars 10 μm.
Figure 6. Idiogram of Mehsani buffalo (Bubalus bubalis) 2n=50 by conventional staining technique.
Figure 7. Idiogram of Mehsani buffalo (*Bubalus bubalis*) 2*n*=50 by GTG-banding technique.
Figure 8. Idiogram of Mehsani buffalo (*Bubalus bubalis*) $2n=50$ by CBG-banding technique.
telocentric autosome (four positions) and on the short arm near telomere of one pair submetacentric autosome (two positions) (Figure 5). In contrast, Di Berardino and Iannuzzi (1981) indicated that NORs of the swamp buffalo and river buffalo appear on the long arm near centromere of the pair autosomes 4p, 8, 20, 22, 23 (10 positions) and 3p, 4p, 8, 21, 23, 24 (12 positions), respectively. By comparing the two types of buffalo, it was concluded that all of the chromosomes are similar in banding patterns that chromosome 1 of swamp results from a telomere-centromere tandem fusion between two chromosomes identified as 4p and 9, respectively, in the river buffalo karyotype, thus accounting for the reduced diploid number of swamp buffalo; that the fusion causes the loss of NOR’s on the telomeres of chromosome 4, thus accounting for the reduced number of NOR chromosome pairs of swamp; that the presence of a pale C-banded are in the region of junction between chromosome 4 and 9 involved in the fusion suggests that the centromeric region of the later has been.

The chromosome of mitotic metaphase cells and the karyotypes of Mehsani buffalo in male and female by conventional staining, GTG-banding, CBG-banding and Ag-NOR banding technique are shown in Figures 2, 3, 4 and 5. The lengths of chromosomes in centimeters of (20 male and 20 female) cells, in mitotic metaphase were measured. The mean length of short arm chromosome (Ls), length of long arm chromosome (Ll), total length of arm chromosome (LT), relative length (RL), centromeric index (CI), size and type of chromosome are presented in Table 1. The idiogram of Mehsani buffalo shows gradually decreasing length of the autosomes (Figures 6, 7 and 8).

The Mehsani buffalo revealed that the chromosome marker is the chromosome pair 1, which is the largest telocentric chromosome. The important karyotype feature of Mehsani buffalo is the asymmetrical karyotype, which is all three types of chromosomes were found (metacentric, submetacentric and telocentric chromosome). The largest and smallest chromosomes show difference size (approximately 5 fold). The karyotype formula of Mehsani buffalo was as follows:

\[
2_n \text{ (diploid)} = L_m^4 + L_m^6 + L_s^8 + M_s^8 + S'_{22} + \text{sex chromosomes.}
\]

ACKNOWLEDGEMENTS

This work was supported by the Applied Taxonomic Research Center, Khon Kaen University grant ATRC_R5304. The authors gratefully acknowledge the help given by the staff of the Burirum Livestock Research Station.

REFERENCES


Campiranon, A. 2003. Cytogenetics, 2nd ed. Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand.

Chaiyasut, K. 1989. Cytogenetics and cytotaxonomy of the family Zephyranthes. Department of Botany, Faculty of Science, Chulalongkorn
University, Bangkok, Thailand.


Iannuzzi, L. and G.P. Di Beo. 1995. Chromosomal evolution in bovids: a comparison of cattle, sheep and goat G- and R-banded chromo-


