Variations in Karyotypic Characteristics of Different Breed Groups of Water Buffaloes (*Bubalus bubalis*)**

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ABSTRACT : Karyotype analysis was carried out on blood samples of 30 water buffaloes belonging to different breed groups (i.e. Philippine Carabao (PC), Indian Murrah (IM), Bulgarian Murrah (BM), "F_1 50% IM-50% PC", "F_1 50% BM-50% PC" and "75% IM-25% PC"), using the modified Leucocyte Culture Technique. The modal chromosome numbers of the PC, "F_1 50% IM-50% PC", "F_1 50% BM-50% PC", IM, BM and "75% IM-25% PC" were 2n=48, 49, 49, 50, 50 and 50, respectively. The water buffalo chromosomes are mostly acrocentric (79.67%) and the remainder submetacentric (20.33%). Results of the ordinary least square analysis showed significant breed effects ($p<0.01$) on other karyotypic characteristics (i.e. relative length, arm ratio and centromeric index). Significant correlation between karyotypic characteristics and some animal performance traits were also found. The significant correlation values imply that karyotypic characteristics can be used as important criteria to select potentially productive young water buffaloes. In the future, more production and reproduction traits from non-institutional herds should be included in the analysis to reveal meaningful correlations with various karyotypic characteristics. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 3 : 321-325)***

Key Words : Water Buffaloes (*Bubalus bubalis*), Karyotype, Chromosome Number, Relative Length, Centromeric Index, Arm Ratio

INTRODUCTION

Crossbreeding the swamp-type (e.g., Philippine Carabao) with river-type water buffaloes (*Bubalus bubalis*) such as the Murrah breed is widely known to produce fertile offspring with various karyotypes (2n=48, 2n=49, and 2n=50). Buffaloes with a 2n=48 chromosomal genotype could be swamp, F_2, or ¾ swamp; 2n=49 could be F_1, F_2, ¾ swamp or ¾ river; and 2n=50 could be river, F_2 or ¾ river (Bongso and Hilmi, 1982; Na-Chaingmai and Chavananikul, 1998; Parker, 1992). The unbalanced karyotypes suggested chromosomal variations within the crossbreds and may be linked to low fertility in crossbred buffaloes since they may affect gametogenesis (e.g., Majid et al., 1991; Songsri and Ramirez, 1979). The heterozygote 2n=49 in particular might be sub-fertile, although mutations (e.g., translocation) which became fixed in a population are unlikely to be selectively disadvantageous (Cooper, 1991).

While chromosome numbers and morphology are important to determine their relationships with incidence of reduced fertility in crossbred buffaloes, evaluation of their variability between breed groups can be especially useful in improving buffalo productivity. For example, indirect selection for overall productivity based on the karyotypic characteristics can be done at a much earlier age, thereby reducing generation interval considerably. Farmers could also have their animals tested at an early age in order to identify and cull those that are going to be unproductive and infertile. Karyotype analysis can be used to predict the purity or grade of animals when breeding history and records are not available to the farmer.

This study aimed to determine the variability of karyotypic characteristics (i.e. modal chromosome number, percent chromosome type based on centromeric location, centromeric index, arm ratio, and relative length) between 3 purebreds and 3 crossbreeds of water buffaloes. Relationships of the karyotypic characteristics with animal performance traits (i.e. body weight at 6 months, 1, 2 and 3 years old; heart girth, body length, and wither height, milk production, and semen characteristics) were also determined by correlation analysis.

MATERIALS AND METHODS

Thirty (30) water buffaloes at the Philippine Carabao Center (PCC) - U.P. Los Baños, belonging to six breed groups namely, Philippine Carabao (PC), Indian Murrah (IM), Bulgarian Murrah (BM), "F_1 50% IM-50% PC", "F_1 50% BM-50% PC" and "75% IM-25% PC", were used in the karyotype (chromosome) analysis.

Using the modified Leucocyte Culture Technique (Bongso and Hilmi, 1982), blood samples were cultured in the laboratory to induce the leukocytes to grow at metaphase stage. Photomicrographs of the metaphase spreads were taken at 400x total magnification from a microscope using black and white film. At least 5 pictures from 3 slide preparations from each buffalo were selected, scanned and stored in microcomputer files. The

** Supported by the National Research Council of the Philippines (NRCP), Department of Science and Technology (DOST), Republic of the Philippines.
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Received August 17, 2001; Accepted November 12, 2001

photomicrographs were further enhanced, magnified (4 to 5 times), and printed. Chromosome pairs were manually cut and pasted to form the photokaryotype.

The chromosomes were identified as pairs according to their length and position of the centromere. In the photokaryotype, the identified pairs of autosomes were arranged in decreasing order of length in each category, namely submetacentric, metacentric and acrocentric. The sex chromosome pair was placed at the last in the karyotype. The X chromosome was the largest acrocentric pair in the karyotype while the Y chromosome was one of the smallest acrocentric chromosomes. Examples of karyotype pictures in the PC, IM and 50% IM-50% PC are shown in figure 1.

Analysis of variance by ordinary least squares method was used to determine differences among breed groups in terms of karyotypic characteristics such as the modal chromosome number based on the highest chromosome frequency of 2n=47, 48, 49 or 50 percent chromosome type based on centromeric location, relative length, arm ratio, and centromeric index. Taking the total length of all chromosomes in the genome as 100, the relative length was computed as percentage of the total length. Centromeric index was computed as percentage of the short arm to the total length of the chromosome. The arm ratio was the length of the long arm divided by the length of the short arm.

Individual performance records of the experimental pure breed and crossbred buffaloes were also gathered from the PCC-UPLB. The production and reproduction records included body weight at 6 months, 1, 2 and 3 years old; heart girth, body length, and wither height, horn type, lactation yield, lactation length, and age at first calving. Pearson correlation analysis was then used to determine relationships between karyotypic characteristics and individual performance traits.

Philippine Carabao

Indian Murrah

50% IM×50% PC

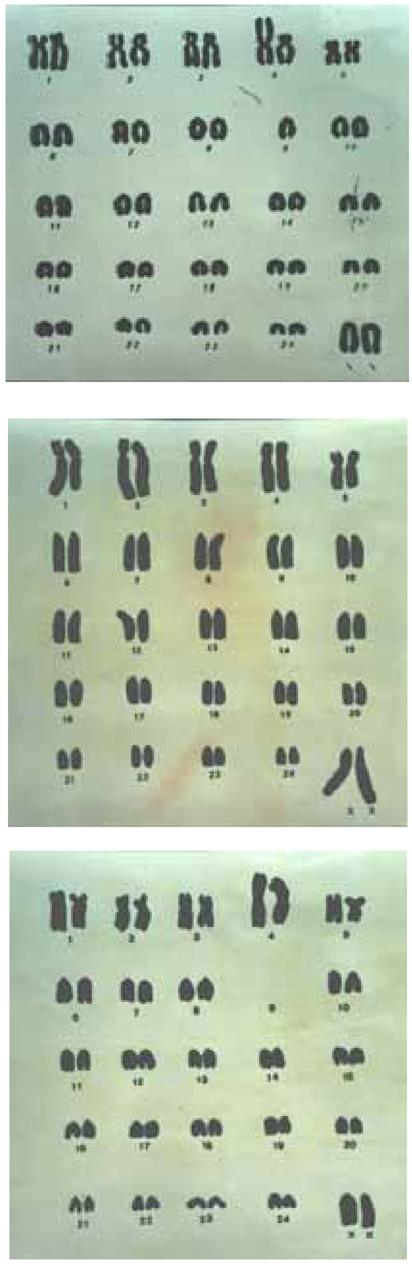

Figure 1. Sample karyotypes of PC (2n=48), M (2n=50) and 50%IM-50% PC (2n=49)

RESULTS AND DISCUSSION

Modal chromosome number

Table 1 shows that the modal chromosome number based on the highest chromosome frequency, for the PC is 2n=48. IM, BM and "75% IM–25% PC" had 2n=50 chromosomes, with chromosome frequency of 69.75%, 80.79% and 64.22% respectively. The diploid chromosome number of the F_1 crosses (i.e. "50% IM-50% PC" and "50% BM-50% PC" was 49, with chromosome frequency of 68.58% and 76.34% respectively. The chromosome complement of 2n=49 in the F_1 hybrid was consistent with the results reported by previous workers (e.g., Fischer and Ulbrich, 1968; Songsri and Ramirez, 1979; Bongso et al., 1984). The features of chromosomes without partners in "50% IM-50% PC" and "50% BM-50% PC" conform to the tandem fusion between chromosome numbers 4 and 9 of the river type described previously by Bongso and Hilmi (1982). Such information was commonly suggested to be used to predict the grade of animals when breeding history and records are not available to farmers.

Chromosome type based on centromeric location

Contrary to early published reports (e.g., Songsri and Ramirez, 1979), no metacentric chromosomes were found in all water buffalo breed groups. On the average, 20.33% of all chromosomes were submetacentric and 79.67% were acrocentric (table 2).

In the Philippine Carabao, 10 out of 48 chromosomes

Table 1. Modal chromosome number and average chromosome frequency

Breed group	Ave. No. of cells examined	Chromosome frequency (%)			
		47	48	49	50
Pure breeds					
Philippine Carabao (PC)	107.6	9.64	78.76	7.09	3.51
Indian Murrah (IM)	92.0	7.21	12.96	10.08	69.75
Bulgarian Murrah (BM)	93.0	8.28	5.50	5.42	80.79
Crosses					
50% IM-50% PC	99.6	0.00	19.44	68.58	11.98
50% BM-50% PC	82.0	0.00	13.63	76.34	10.03
75% IM-25% PC	101.8	0.00	20.54	15.24	64.22
Overall	96.0	4.19	25.14	30.46	40.05
Total cells examined	2,880				

Table 2. Chromosome type (in percent) based on centromeric location

Breed Group	Metacentric (%)	Submetacentric (%)	Acrocentric (%)
Pure breeds			
Philippine Carabao (PC)	0.00	21.01	78.99
Indian Murrah (IM)	0.00	20.92	79.08
Bulgarian Murrah (BM)	0.00	20.93	79.07
Crosses			
50% IM-50% PC	0.00	19.51	80.49
50% BM-50% PC	0.00	19.59	80.41
75% IM-25% PC	0.00	20.00	80.00
Overall	0.00	20.33	79.67

(20.8%) were submetacentric. The rest of the chromosomes were acrocentric. The type of chromosome based on the location of the centromere was similar for the BM, IM and 75% "IM-25% PC" whose modal chromosome number is 2n=50. The chromosome type was also similar for "F_1 50% IM-50% PC" and "F_1 50% BM-50% PC", both with chromosome number 2n=49.

Breed effects on karyotypic characteristics

Results of the analysis of variance revealed significant effects of breed on chromosome frequency (p<0.01) and chromosome location (p=0.08). Highly significant breed effects (p<0.01) were also found for relative length, arm ratio, and centromeric index of chromosome 4A and 4B (table 3). The relative length of chromosomes 1B, 2A, 2B, 3B, 5B, 8A, 8B, 16B, 22A, 22B and 23A were also different

Table 3. Significant breed effects on various karyotypic characteristics

Karyoptypic characteristic	Level of significance
Chromosome frequency (%)	
2n=47, 48, 49, 50	**
Centromeric location (%)	
Submetacentric, Acrocentric	p=0.8
Relative length	
1B, 2A, 2B, 3B, 4A, 4B, 5B,	**
8A, 8B, 16B, 22A, 22B, 23A	**
	**
Arm ratio	
4A, 4B	**
Centromeric index	
4A, 4B	**

** Statistically significant, p<0.01.
* Statistically significant, p<0.05.

between breed groups of water buffaloes. The significant variations in some karyotypic characteristics between breed groups provide extra information to previous reports (e.g., Azmi et al., 1990) showing trends mostly in chromosome numbers that were usually associated with differences in growth rates among crossbred water buffaloes.

Relationships between karyotypic characteristics and animal performance

Table 4 shows the list of correlation coefficients (r) between karyotypic characteristics and some animal performance traits. Significant correlations were found to be more common between animal performance and relative length than with animal performance and chromosome frequency, arm ratio or centromeric index.

Other important findings were as follows:

1. Some measures of body weight appeared to be related with arm ratio of chromosomes 3A and 4B, and with centromeric index of chromosome 2B.
2. Heart girth was mostly negatively correlated to relative length of chromosomes 3B, 13B, 14A, 14B, and 23A.
3. Withers height was significantly correlated with relative length of chromosomes 3B, 5B, 22A, and 23A.
4. Lactation yield and lactation length seemed to be associated with chromosome frequency (2n=50) and with relative length, arm ratio, and centromeric index of chromosome 4A and 4B.

The significant correlation values may imply that some karyotypic characteristics can be used as important criteria to select potentially productive young water buffaloes. Hence, indirect selection for overall productivity based on the karyotypic characteristics of breeding animals can be done at a much earlier age, thereby reducing generation interval considerably. The physiological mechanisms that determine the correlation between karyotypic characteristics and some performance traits however, need to be elucidated further.

Table 4. Significant correlations (p<0.05) between karyotypic characteristics and some animal performance traits

Karyoptypic characteristic	Animal performance trait	No. of paired observations	Corr. coeff. (r)	p value
Chromosome frequency (%)				
(2n=47)	>3 year weight, kg	17	-0.64	0.0059
(2n=47)	Age at 1st calving, months	17	+0.87	0.0108
(2n=50)	Lactation yield, kg	8	+0.86	0.0066
(2n=50)	Lactation length, days	8	+0.74	0.0368
Relative length				
3B	Withers height, cm	27	+0.53	0.0047
3B	Heart girth, cm	27	+0.42	0.0283
3B	Body length, cm	27	+0.53	0.0047
3B	Lactation yield, kg	8	+0.73	0.0388
3B	Lactation length, days	8	+0.87	0.0049
4A	Lactation yield, kg	8	-0.81	0.0149
5B	Withers height, cm	27	+0.38	0.0481
12B	>3 year weight, kg	8	+0.82	0.0135
13B	Heart girth, cm	14	-0.66	0.0107
14A	Heart girth, cm	14	-0.63	0.0157
14B	Heart girth, cm	14	-0.57	0.0322
22A	Withers height, cm	14	-0.54	0.0437
22A	Heart girth, cm	14	-0.66	0.0108
22B	2 year weight, kg	6	+0.84	0.0368
23A	Withers height, cm	14	-0.57	0.0327
23A	Heart girth, cm	14	-0.66	0.0103
24A	6 month weight, kg	4	-0.98	0.0224
24B	6 month weight, kg	3	-0.99	0.0360
24B	Age at 1st calving, months	4	+0.95	0.0465
Arm ratio				
3A	Birth weight, kg	18	-0.64	0.0039
3A	1 year weight, kg	17	-0.55	0.0211
3A	2 year weight, kg	15	-0.60	0.0181
3B	Withers height, cm	27	+0.39	0.0420
4A	Lactation yield, kg, kg	8	+0.88	0.0037
4A	Lactation length, days	8	+0.71	0.0448
4B	3 year weight, kg	22	+0.58	0.0042
Centromeric index				
2B	6 month weight, kg	12	-0.60	0.0390
4A	Lactation yield, kg	8	-0.88	0.0041

ACKNOWLEDGEMENT

The financial support through a research grant provided by the National Research Council of the Philippines (NRCP) of the Department of Science and Technology (DOST) is gratefully acknowledged. The authors also thank Dr. Arnel N. del Barrio and PCC-UPLB staff for their kind assistance during blood collection.

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