GENETIC VARIABILITY OF KARYOTYPIC CHARACTERISTICS IN RELATION TO THE GENETIC IMPROVEMENT OF SOME ECONOMICALLY-IMPORTANT TRAITS OF WATER BUFFALOES

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Abstract

One hundred and thirty (130) water buffaloes grouped into: purebreeds [i.e. Philippine carabao (PC), Indian Murrah (IM), and Bulgarian Murrah (BM)], two-way crosses (i.e. 50%IM-50%PC, 50%BM-50%PC, 50%Nili-Ravi-50%C), backcross (i.e. 75%IM–25%PC), and three-way crosses among PC, IM, and Nili-Ravi, were considered for karyotype analysis using the modified Leukocyte Culture Technique and evaluation of their production and reproductive performance using the ordinary least squares method.

Karyotypic characteristics (i.e. modal chromosome number based on chromosome frequency, chromosome type, and percent relative length, centromeric index, and arm ratio for some chromosomes) were significantly different between breed groups. Significant differences between breed groups were also found for production traits (i.e. 1-year and 3-year old body weights, withers height, heart girth, and body length) and reproductive parameters (i.e. total milk yield, lactation length, age at first calving, and semen volume). Significant linear correlation between karyotypic characteristics and some economically important traits in water buffaloes are also highlighted in the study. The significant correlation values imply that some karyotypic characteristics can be used as important markers or criteria to select potentially productive young water buffaloes.

The high coefficient of variation (C.V.) values in karyotypic characteristics, production and reproductive traits imply potential and practical basis to accurately distinguish genetic differences between breed groups, especially in the absence of breeding history and factual pedigree and identification records on-farm.

Results of the karyotype analyses and their relationships with production and reproductive traits in water buffaloes can be applied in the design and development of local selection, crossbreeding, and/or conservation programs. Differences between purebreeds and their crosses in terms of percent heterosis and advantage over the Philippine carabao will be useful in the design and development of systematic crossbreeding programs useful in the dominant local production and marketing systems. Indirect selection for overall productivity based on the karyotypic characteristics of breeding animals can be practiced at a much earlier age, thereby reducing generation interval considerably.

Because of the requirements for laboratory facilities and expertise, the use of karyotype analysis is however recommended for the gene pool and institutional herds such as those maintained by the Philippine Carabao Center (PCC) in aid of the establishment of a national buffalo registry and implementation of a national breeding program for water buffaloes.

Keywords: karyotype analysis, chromosome number, chromosome type, relative length, centromeric index, arm ratio, production and reproductive traits, water buffaloes

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Introduction

The Philippine carabao, a swamp type of buffalo (*Bubalus bubalis*), has long been an important draft animal in local agriculture. About 99% of the more than 3 million buffaloes in the country are raised and bred by small hold farmers. Despite the serious threats of extinction due to the rinderpest disease problem brought about by the early livestock importation from India, the Philippine carabao has survived. Perpetuation of the carabao species was successfully aided by the yearly introduction of tens of thousands of similar swamp type buffaloes from French Indo-China (now Cambodia) in the early 20th century (Evaristo, 1915).

In 1981, the Philippine Carabao Research and Development Center (PCRDC), the predecessor of the now Philippine Carabao Center (PCC) under the Department of Agriculture, initiated a large scale hybridization project which aimed to produce crossbred buffaloes that possess improved genetic potentials for buffalo milk and meat production. Through the help of the Food and Agriculture Organization/ United Nations Development Program (FAO/UNDP), the project successfully produced crossbred buffaloes by mating the male river-type buffaloes (such as the Murrah and Nili Ravi breeds from India and Pakistan) to the Philippine carabao through artificial insemination (A.I.).

Unlike other reports of inter specific hybridization in farm animals, the crossbred buffaloes are not sterile. Filipino farmers had overwhelmingly approved the crossbreeding project as this has offered a convenient breeding approach to "improve" the carabao. The usual procedure in buffalo crossbreeding to take advantage of heterosis is to mate the female swamp buffalo to male river buffalo and then the F_1 offspring are either back-crossed to river buffalo or *inter se* mated. The weight gain and milk production of F_1 crossbred buffalo is usually higher than the swamp purebred (Shrestha, 1992).

However, with poor and inadequate recording on-farm, the mass production of swamp x river type crosses makes it difficult to evaluate the genetic profiles and potentials of various breed groups and their fractional crosses. With continuous upgrading using Murrah bulls, the Philippine carabao in its pure genetic form may eventually be lost irretrievably.

In this regard, karyotype analysis is proposed to generate baseline information that is useful to the design and development of a local breeding (i.e. genetic improvement and conservation) program anchored on institutional herds such as in the PCC. Chromosome numbers and morphology may be used to predict the grade of animals when breeding history and records are not available to the farmers. 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. The purpose of this study is to determine the variability and inherent relationships between chromosome characteristics and productivity of common purebred and crossbred water buffaloes and make suggestions concerning the applications of this correlation on the design and development of a local breeding or genetic improvement program.

The 150 million buffaloes in the world are divided into two species: the numerous Asiatic water buffalo (*Bubalus bubalis*) and the rarer African buffalo (*Syncerus caffer*). According to Iannuzzi (1994), the river buffaloes represent 80% of the world's buffalo population. These include the Murrah, Jaffarabadi, Surti, Menshana, Egyptian and Mediterranean breeds, which are found in many countries of Asia, Africa, Europe and South America, while the swamp buffaloes are limited to a few far eastern countries, like the Philippines, Thailand, Vietnam and Southern China.

Karyotyping using the conventional staining (without banding) was previously demonstrated to differentiate pure breeds and crossbreeds (F_1, F_2 crosses and backcrosses) of water buffalo. Of the Asiatic water buffalo two subspecies are known, the river (2n=50) and the swamp (2n=48) buffalo. The reduction in the number of chromosomes in the Philippine carabao (swamp-type) is the result of a tandem fusion between the telomeric end of the short arm of the submetacentric chromosome number 4 with the centromeric end of the acrocentric chromosome number 9 in the Murrah (river-type) buffalo (Hilmi, 1991).

Na-Chaingmai and Chavananikul (1998) recently summarized that the diploid number of the river buffalo is 50 of which five pairs are submetacentric and 20 are acrocentric including the sex chromosomes. The swamp buffalo is found to have 48 chromosomes of which five are submetacentric pairs and 19 are acrocentric pairs including sex chromosomes. In both types, the female has two of the larger X-chromosomes while the male has one larger X-chromosome and one second smaller Y-chromosome.

Crossbreeding the Philippine carabao with the Murrah breed is also widely known to produce fertile offspring with various karyotypes (2n=48, 2n=49, and 2n=50). Crossbred buffaloes with 2n=48 chromosomal genotype could be F_2 or ¾ swamp; 2n=49 could be F_1, F_2, ¾ swamp or ¾ river; and 2n=50 could be F_2 or ¾ river (Bongso and Hilmi, 1982; Parker, 1992; Na-Chaingmai and Chavananikul, 1998). Data summarized by Harisah et al. (1989) indicated that the segregation in the F_2 and the backcross animals are Mendelian. 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., Songsri and Ramirez, 1979; Majid et al., 1991). The heterozygote in particular, might be sub-fertile although mutations (e.g., translocation) that become fixed in a population are unlikely to be selectively disadvantageous (Cooper, 1991). Similar centric fusions involving various pairs on non-homologous chromosomes have been reported in domestic cattle, sheep, goats, pigs, and dogs, and in wild ungulates of various species (Nicholas, 1989). However, except for the 1/29 translocation in cattle, centric fusions have not been found to have any adverse effects on reproductive ability.

Chavananikul (1989) suggested that the loss or gain of genetic material during chromosome division may lead into a decline in reproductive fitness of various kinds of crossbreeds and backcrosses. On the other hand, Fischer (1989) reported that buffalo crossbreds, in spite of diverse blood compositions, show no reduction in fertility.

In many previous reports, variations in chromosome number and morphology between breed groups are deemed to be useful in the design and development of local crossbreeding programs especially when breeding history and records are not available to the farmer. On the other hand, the variability of karyotypic characteristics and their correlation with individual production and reproductive performance of water buffaloes has not been established before. Bondoc et al. (2001) suggested that karyotypic characteristics in young animals may be used as indirect traits to select potentially productive water buffaloes for economically traits that are measured late in the animal's productive life such as those related to adult body weight, milk production, and reproductive traits.

Materials and Methods

Experimental Animals

One hundred and thirty (130) water buffaloes (40 males and 90 females) mainly from the institutional herd of Philippine Carabao Center at U.P. Los Baños (PCC-UPLB), Batangas Dairy Cooperative (BADACO) in Lipa City Batangas, and small hold farms in Sta. Cruz and Pagsanjan, Laguna were considered in the study. In Table 1, the water buffaloes were grouped into: purebreeds [i.e. Philippine carabao (PC), Indian Murrah (IM), and Bulgarian Murrah (BM)], two-way crosses [i.e. 50%IM-50%PC (F_1, F_2, and F_3), 50%BM-50%PC, 50% Nili-Ravi-50%PC (F_1 and F_2)], backcross (i.e. 75%IM–25%PC), and three-way crosses [i.e. 12.5%IM-62.5% PC-25%N, 25%IM-50%PC-25%N (F_1 and F_2), 50%IM-12.5%PC-37.5%N, 50%BM-25%PC-25%IM, 50%IM-25%PC-25%N)].

The origin of the Indian Murrah buffalo can be traced back to the early importations in 1917 (Bondoc, 1998). The Nili-Ravi breed was imported from Pakistan in the form of frozen semen in 1983. Murrah buffaloes were imported from Bulgaria in 1995.

Animal identification, sire and dam breed, and date of birth were recorded for each experimental animal. Individual production records consisted of body weight at different ages [i.e. at birth (N=74), 6 months (N=45), 1 year (N=62), 2 years (N=53), 3 years (N=52), and greater than 3 years old (N=76)] and external body measurements (i.e. withers height, heart girth, and body length) of buffaloes that are at least 3 years old (N=84). Withers height was measured at the highest point above or around the scapulas. The heart girth crossed both the fifth and sixth coastae in a right angle to the ground. Body length was taken as the horizontal distance from the frontal edge of the scapula to the caudal end of the plane.

The reproductive traits included total milk yield (N=30), lactation length (N=30), and age at first calving (N=35) for females while semen characteristics (N=4 for semen volume, mass activity, sperm motility and sperm concentration) were taken for males.

The type of horns (i.e. crescent-like shape or semi-circle; ram-like refers to horns that are curved backward and up; and intermediate between crescent and ram-like) was also noted for each animal (i.e. N=130).

Karyotype Analysis

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

Photokaryograms were successfully developed and evaluated for 96 out of the 130 experimental animals. No chromosome spreads was obtained and evaluated from blood samples taken at least one (1) hour away from PCC-UPLB. The use of karyotype analysis is, therefore, to be conducted only in a nucleus/ elite breeding farm that is adequately equipped with laboratory facilities.

The chromosomes were identified as pairs according to their length and position of the centromere. In the photokaryotype, the identified pairs of autosomes are arranged in decreasing order of length in each category, namely metacentric (i.e. centrally located centromere, arms of equal length), submetacentric (i.e. centromere positioned above the median, one arm longer than the other), acrocentric (i.e. acrocentric – centromere near at one end, arms are very unequal in length, and telocentric (i.e. terminally located centromere and only one arm). The sex chromosome pair is placed at the last in the karyotype. The X chromosome was the largest acrocentric chromosome in the karyotype while the Y chromosome was one of the smallest acrocentric chromosomes.

Individual karyotypes were assessed in terms of the modal chromosome number based on the highest chromosome frequency of 2n=47, 48, 49, or 50, 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 for each chromosome 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 is the length of the long arm divided by the length of the short arm. The average relative length, centromeric index, and arm ratio for each animal was computed from the 5 best photokaryotypes developed per animal.

Statistical Analysis

Analysis of variance by ordinary least squares method was used to determine differences among breed groups in terms of karyotypic characteristics (i.e. modal chromosome number, chromosome type, average relative length, centromeric index, and arm ratio) and production and reproductive traits. Variability of the karyotypic characteristics and animal performance records was measured in terms of coefficient of variation (C.V.). Means were compared between breed groups using the Duncan's Multiple Range Test (DMRT).

The fixed linear model (with missing data) included the breed group as the independent variable. The average relative length, centromeric index, and arm ratio were analyzed separately for each chromosome. The least square means (LSM) for production and reproductive traits were used to compute percent heterosis and percent advantage of crossbred over the Philippine carabao using the following formulas:

$$\% \text{ Heterosis} = \frac{[\text{LSM of cross} - (\text{Average LSM of parental breeds})] \times 100}{(\text{Average LSM of parental breeds})}$$

$$\% \text{ Advantage over the Phil. carabao} = \frac{[\text{LSM of cross} - \text{LSM of Philippine carabao}] \times 100}{(\text{LSM of Philippine carabao})}$$

Pearson (linear) correlation analysis was used to determine significant relationships (P<.05 to test Ho: r=0) between karyotypic characteristics (including chromosome frequency for 2n=47, 48, 49, 50, percent relative length, centromeric index, and arm ratio for all chromosomes) and production and reproductive records

Results and Discussion

Variations in Karyotypic Characteristics

- Modal Chromosome Number

Based on a total of 9,283 cells examined from 96 animals, the modal chromosome number was 2n=48 for the Philippine carabao (PC), 2n=49 for the two-way and three-way crosses, and 2n=50 for the Indian Murrah (IM), Bulgarian Murrah (M), and backcrosses (i.e. 75%IM - 25%PC), (Table 2, next page). The absence of chromosomes 9A and 9B in the Philippine carabao confirms that chromosomes 4 and 9 have "tandemly fused" (Bongso and Hilmi, 1982), thus leading to a diploid number of 48 compared to 2n=50 in the Indian and Bulgarian Murrah. The results in the crosses were consistent with those reported by Songsri and Ramirez (1979) and Bongso et al. (1984).

Chromosome frequency was significantly different (P<.01) between breed groups for each of the chromosome numbers, i.e. 2n=47, 48, 49, and 50. The modal chromosome numbers were determined based on the highest chromosome frequency ranging from 47.4% to 83.7%. The highest maximum chromosome frequency was observed in the purebreeds (71.0% to 83.7%) and two-way crosses (74.5% to 78.5% for 2n=49). A lower maximum chromosome frequency was however, found in the backcrosses (i.e. 57.1% for 2n=50) and three-way crosses (i.e. 47.4% for 2n=49). Chromosome number 2n=47 was also encountered in all breed groups except for the 50%BM – 50%PC. The maximum chromosome frequency for 2n=47 was however low, ranging from 6.0% to 9.1% in the purebreeds and even lower (0% to 3.1%) in the crosses.

Table 2. Chromosome frequency and modal chromosome numbers, by breed group

Breed Group	Ave. no. of cells Examined per animal	Chromosome Frequency (%) 47	48	49	50	Modal Chromosome No.
Pure breeds:						
Philippine Carabao (PC)	106.3	9.1[a]	82.8[a]	5.2[c]	2.5[c]	48
	(5.3)	(1.0)	(2.7)	(4.1)	(3.1)	
Indian Murrah (IM)	95.2	6.0[ab]	12.8[bc]	10.2[c]	71.0[b]	50
	(7.1)	(1.3)	(3.7)	(5.5)	(4.2)	
Bulgarian Murrah (BM)	94.7	7.2[a]	4.2[c]	4.9[c]	83.7[a]	50
	(6.6)	(1.2)	(3.4)	(5.1)	(3.9)	
Two-way crosses:						
50%IM – 50%PC	96.4	0.6[c]	14.7[b]	74.5[a]	10.2[e]	49
	(3.7)	(0.7)	(1.9)	(2.9)	(2.2)	
50%BM – 50%PC	85.5	0.0[c]	11.8[bc]	78.5[a]	9.7[e]	49
	(6.2)	(0.0)	(3.2)	(4.8)	(3.6)	
50%Nili-Ravi –50%PC	91.0	0.8[c]	12.5[bc]	75.1[a]	11.6[e]	49
	(7.1)	(1.3)	(3.7)	(5.5)	(4.2)	
Backcross (75%IM – 25%C)	94.2	1.0[c]	10.8[bc]	31.1[c]	57.1[c]	50
	(3.3)	(0.6)	(1.7)	(2.6)	(1.9)	
Three-way crosses	114.4	3.1b[c]	8.8[bc]	47.4[b]	40.7[d]	49
	(6.2)	(1.1)	(3.2)	(4.8)	(3.6)	

Note: A total of 9,283 cells from 96 animals were examined to determine chromosome numbers in the research project.
Numbers in parenthesis are standard errors of the least square means.
Least square means in the same column with different letter superscripts are significantly different from one another, ($P<.05$).

- Chromosome Type

Chromosomes of the purebred and crossbreed groups averaged 20.2% submetacentric (C.V.=3.0%) and 79.7% acrocentric (C.V.=.9%), (Table 3). This is equivalent to about 10 submetacentric chromosomes for all breed groups and 38 acrocentric chromosomes for Philippine carabao, 39 acrocentric chromosomes for two-way crosses, and 40 acrocentric chromosomes for Indian and Bulgarian Murrah, backcrosses, and three-way crosses. No metacentric and telocentric chromosomes were found for all breed groups examined

- Relative Length

Results of the ordinary least square analysis showed significant effects of the breed group ($P<.01$) on relative length for chromosomes pairs 1, 2, 3, 4, 5 (C.V.= 8.8% to 19.6%), chromosome 9B (C.V.=84.4%), XB (C.V.=56.2%) and YB (C.V.=21.2%), see Table 4.

For chromosomes 1A, 1B and 4B, the percent relative length was significantly larger in the Philippine carabao than any other breed group considered in the study. The female Indian Murrah was distinctly different from the Bulgarian Murrah in terms of the sex chromosome b in terms of percent relative length (i.e. 0.0% vs. 2.0%). For chromosomes 3A, 3B and 5B, percent relative length was generally higher in the purebreds than the two-way crosses, backcross, and three-way cross.

Table 3. Least square means and standard error of the frequency of submetacentric and acrocentric chromosomes, by breed group

Breed Group	%Submetacentric	%Acrocentric
Pure breeds:		
Philippine Carabao (PC)	20.9 ± .2 (10)	79.1 ± .2 (38)
Indian Murrah (IM)	20.7 ± .3 (10)	79.3 ± .3 (40)
Bulgarian Murrah (BM)	20.0 ± .3 (10)	80.0 ± .3 (40)
Two-way crosses:		
50%IM – 50%PC	20.2 ± .2 (10)	79.8 ± .2 (39)
50%BM – 50%PC	19.9 ± .3 (10)	80.1 ± .2 (39)
50%Nili-Ravi – 50%PC	20.4 ± .3 (10)	79.6 ± .3 (39)
Backcross (75%IM-25%C)	20.1 ± .1 (10)	79.8 ± .1 (40)
Three-way crosses	20.3 ± .3 (10)	79.7 ± .3 (40)
N animals	96	96
Overall mean ± std. dev.	20.2 ± 0.3	79.7 ± 0.3
C.V., %	3.70	0.9

Note: No metacentric chromosome was observed.
Number in parenthesis is the number of chromosomes of a particular type and was estimated relative to the modal chromosome number.

Table 4. Least square means for percent relative length of each chromosome, by breed group

Chrom. No.	Pure Breeds			Crosses					C.V.
	C	IM	BM	IMxC	BMxC	NxC	BKX	3WC	%
$1A^{**}$	4.5^{a}	4.0^{b}	4.0^{b}	3.9^{b}	4.1^{b}	4.0^{b}	3.9^{b}	4.0^{b}	7.1
$1B^{*}$	4.4^{a}	4.0^{b}	3.9^{b}	3.9^{b}	3.9^{b}	3.8^{b}	3.9^{b}	3.9^{b}	9.4
$2A^{ns}$	4.0	3.8	3.9	3.8	3.8	3.3	3.6	3.8	11.6
$2B^{**}$	4.0^{a}	3.8^{ab}	3.6^{abc}	3.4^{bc}	3.8^{ab}	3.3^{bc}	3.2^{c}	3.4^{bc}	13.5
$3A^{*}$	3.6^{a}	3.5^{ab}	3.6^{ab}	3.1^{b}	3.1^{b}	3.3^{ab}	3.1^{b}	3.2^{ab}	12.8
$3B^{**}$	3.4^{a}	3.7^{a}	3.4^{a}	3.0^{b}	3.0^{b}	3.0^{b}	3.0^{b}	3.0^{b}	8.8
$4A^{**}$	4.2^{bc}	3.0^{d}	3.1^{d}	4.3^{ab}	4.8^{ab}	5.0^{a}	3.4^{cd}	$4.1b^{c}$	19.6
$4B^{**}$	4.2^{a}	2.8^{b}	3.1^{b}	3.0^{b}	3.0^{b}	3.0^{b}	3.0^{b}	3.0^{b}	16.1
$5A^{ns}$	3.0	2.8	3.0	2.9	2.9	3.0	2.7	3.0	12.0
$5B^{**}$	2.5^{abc}	2.8^{ab}	3.0^{a}	2.4^{bc}	2.4^{bc}	2.3^{c}	2.2^{c}	2.6^{ab}	19.0
$6A^{ns}$	2.2	2.5	2.3	2.3	2.6	2.3	2.5	2.5	20.6
$6B^{ns}$	2.0	2.3	2.3	2.2	2.2	2.0	2.2	2.4	18.8
$7A^{ns}$	2.0	2.2	2.1	2.1	2.2	2.0	2.1	2.0	14.1
$7B^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	7.3
$8A^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
$8B^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
$9A^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
$9B^{**}$		2.0^{a}	2.0^{a}	0.1^{c}	0.0^{c}	0.0^{c}	1.3^{b}	0.5^{c}	84.4
$10A^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	10.1
$10B^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
$11A^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
$11B^{ns}$	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0

Table 4. *continuation*

Chrom. No.	PureBreeds			Crosses					C.V.
	PC	IM	BM	IMxPC	BMxPC	NRxPC	BKX	3WC	%
12Ans	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
12Bns	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
13Ans	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	5.3
13Bns	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
14Ans	2.0	1.8	2.0	2.0	2.0	2.0	2.0	2.0	7.2
14Bns	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	0.0
15Ans	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	7.5
15Bns	2.0	2.0	1.9	2.0	1.9	2.0	2.0	2.0	12.3
16Ans	1.9	1.8	1.8	2.0	1.9	1.7	1.9	1.9	16.3
16Bns	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	22.5
17Ans	1.5	1.7	1.4	1.6	1.6	1.3	1.6	1.5	31.9
17Bns	1.2	1.5	1.0	1.3	1.6	1.3	1.4	1.4	35.1
18Ans	1.3	1.3	1.1	1.2	1.5	1.2	1.2	1.1	35.0
18Bns	1.1	1.2	1.0	1.1	1.2	1.2	1.2	1.1	31.8
19Ans	1.0	1.0	1.1	1.0	1.1	1.0	1.1	1.0	21.6
19Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	14.5
20Ans	1.0	1.0	1.0	1.0	1.1	1.0	1.0	1.0	9.9
20Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
21Ans	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
21Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	10.3
22Ans	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
22Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
23Ans	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	10.5
23Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
24Ans	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
24Bns	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0
Xans	3.0	3.0	3.1	3.0	3.1	3.0	3.0	3.0	5.8
XB**	1.5^{abc}	0.0^{c}	2.0^{ab}	2.5^{ab}	1.0^{bc}	3.0^{a}	2.3^{ab}	3.0^{a}	56.2
Ybns	1.0	1.0	1.0	0.8	0.5	-	1.0	1.0	21.2

Note: PC - Philippine Carabao; IM – Indian Murrah; BM – Bulgarian Murrah; IMxPC – IM x C cross; BMxPC – BM xP C cross; NxPC – Nili-Ravi x P C cross; BKX – Backcross (75%IM-25%C); 3WC – 3 way cross among the Indian Murrah, Nili-Ravi, and Carabao.

N=96 animals for all chromosomes except for XB (N=56) and YB (N=40).

Least square means in the same row with different superscripts are significantly different from one another.

"ns" means no significant difference, (P>.05).
* means significant difference, (P<.05).
** means highly significant difference, (P<.01).

- Centromeric Index

Table 5 shows that the centromeric index was significantly different between breed groups (P<.01) for chromosomes 1A (C.V.=16.8%), 1B (C.V.=16.5%), 4A (C.V.=32.7%), 4B (C.V.=34.2%), and 5B (C.V.=5.6%).

For chromosomes 1A and 1B, the centromeric index was significantly higher in the Philippine carabao than any other breed group considered in the study. The centromeric index for chromosomes 4A and 4B are significantly greater in the Philippine carabao than the Indian and Bulgarian Murrah. For chromosome 5B, centromeric index was generally higher in the purebreds than any of the crosses.

Table 5. Least square means for percent centromeric index of each chromosome, by breed group

Chrom. No.	**Pure Breeds**			**Crosses**					**C.V.**
	PC	**IM**	**BM**	**IMxPC**	**BMxPC**	**NRxPC**	**BKX**	**3WC**	**%**
1A**	1.662^a	1.220^b	1.189^b	1.151^b	1.229^b	1.070^b	1.100^b	1.091^b	16.8
1B**	1.606^a	1.158^b	1.184^b	1.141^b	1.148^b	1.085^b	1.089^b	1.055^b	16.5
2A^ns	1.178	1.148	1.153	1.135	1.094	1.050	1.106	1.068	11.2
2B^ns	1.143	1.150	1.144	1.124	1.306	1.002	1.078	1.124	16.6
3A^ns	1.182	1.173	1.177	1.088	1.086	1.100	1.084	1.049	11.8
3B^ns	1.169	1.092	1.137	1.046	1.110	1.023	1.044	1.081	10.9
4A**	2.504^ab	1.070^d	1.110^cd	1.854^ab	2.284^a	2.002^ab	1.267^cd	1.618^bc	32.7
4B**	2.054^a	1.037^b	1.161^b	.999^b	1.031^b	.970^b	1.015^b	1.068^b	34.2
5A^ns	1.005	1.017	1.027	.945	.889	1.020	.913	1.001	17.3
5B*	.988^ab	.985^ab	1.034^a	.879^bc	.799^c	.880^bc	.884^bc	.871^bc	5.6

Note: P C - Philippine Carabao; IM – Indian Murrah; BM – Bulgarian Murrah; IMxPC – IM x PC cross; BMxPC – BM x PC cross; NRxPC – Nili-Ravi x PC cross; BKX – Backcross (75%IM-25%C); 3WC – 3 way cross among the Indian Murrah, Nili-Ravi, and Carabao.

Least square means in the same row with different superscripts are significantly different from one another.

"ns" means no significant difference, (P>.05).
* means significant difference, (P<.05).
** means highly significant difference, (P<.01).

> Arm Ratio

The percent arm ratio was found to be significantly different between breed groups (P<.01) for chromosomes 1A (C.V.=15.1%), 1B (C.V.=14.8%), 4A (C.V.=32.7%), 4A (C.V.=24.4%), and 4B (C.V.=17.1%).

For chromosomes 1A, 1B, and 4B, the arm ratio was significantly lower in the Philippine carabao than all other breed groups. The arm ratio for chromosomes 4A and 4B are significantly lower in the Philippine carabao than the Indian and Bulgarian Murrah.

Variations in Some Productive and Reproductive Traits

> Body Weight at Different Ages

Table 7 shows the least square mean and standard errors of body weight at birth (C.V.=15.8%), 6 months (C.V.=23.1%), 1 year (27.2%), 2 years (24.2%), 3 years (18.0%), and greater than 3 years old (C.V.=20.4%). (No data on the birth weight, 6-month weight, and 1-year weight of the Bulgarian buffaloes and 6-month weight of the Philippine carabao was included in the data set.) Significant differences between breed groups (P<.05) were found for 1-year and 3-year old weights.

Table 7. Least square means for body weight measurements at different ages, by breed group

Breed Group	Body weight (kg) at different ages					
	Birth	6 mos	1 yr	2 yrs	3 yrs	>3 yrs
Pure breeds:						
Philippine Carabao (PC)	29.0 (5.2)	*na*	174.5^{b} (38.9)	263.0 (52.1)	283.0^{b} (69.3)	499.7 (35.2)
Indian Murrah (IM)	32.2 (2.1)	147.0 (18.7)	297.0^{a} (24.6)	390.5 (30.1)	471.0^{a} (28.3)	526.7 (43.1)
Bulgarian Murrah (BM)	*na*	*na*	*na*	306.0 (73.7)	435.8^{a} (31.0)	541.9 (20.3)
Two-way crosses:						
50%IM – 50%PC	32.2 (1.2)	146.8 (8.1)	193.4^{b} (12.6)	301.9 (18.4)	372.2^{ab} (16.3)	506.6 (23.0)
50%BM – 50%PC	34.2 (2.1)	124.3 (13.2)	201.1^{b} (20.8)	282.0 (73.7)	351.0^{ab} (34.7)	533.0 (74.6)
50%Nili-Ravi – 50%PC	34.2 (2.1)	136.2 (14.5)	195.2^{b} (24.6)	258.8 (33.0)	358.5^{ab} (34.7)	556.0 (47.2)
Backcross (75%IM – 25%PC)	33.4 (1.0)	166.0 (13.2)	190.2^{b} (12.0)	292.8 (17.4)	384.4^{a} (20.9)	473.7 (61.0)
Three-way crosses	33.2 (1.9)	166.0 (13.2)	228.7^{ab} (31.8)	313.8 (36.9)	349.3^{ab} (40.0)	404.7 (61.0)
N observations	74	45	62	53	52	76
Overall mean	33.2	140.3	202.8	303.9	386.0	518.5
Standard deviation	5.1	34.3	60.1	76.7	76.7	105.3
C.V.,%	15.8	23.1	27.2	24.2	18.0	20.4
F-test result	ns	ns	*	ns	*	ns

Note: Least square means in the same column with different letter superscripts are significantly different from one another, (P<.05).

"ns" means no significant difference, (P>.05).
* means significant differences, (P<.05).
"*na*" means data is not available.

The 1 year old weight of the Indian Murrah (297 kg) was significantly heavier than the Philippine carabao (174.5 kg), two-way crosses (50%IM – 50%PC = 193.4 kg; 50%BM - %PC = 201.1 kg; 50% Nili-Ravi – 50%PC = 195.2 kg), and backcrosses (75%IM – 25%PC = 190.2 kg). The 1-year weight of the three-way crosses (228.7 kg) was not significantly different (P>.05) compared to the 1-year weight of all breed groups considered in the study.

Similarly, the 3-year old weight of the Indian Murrah (471.0 kg) and Bulgarian Murrah (435.8 kg) and backcrosses (75%IM – 25%PC = 384.4 kg) were significantly higher than the Philippine carabao (283.0 kg). The 3-year weight of the two-way crosses and three-way crosses were not significantly different (P>.05) compared to the Philippine carabao, Indian and Bulgarian Murrah, and backcrosses.

Table 8 shows that heterosis expressed as superiority of the cross over the average of the purebred parents, was negative for 1-year weight (-18.0% to –22.4%) and 3-year weight (-1.3% to –8.8%). However, compared to the Philippine carabao, crossbreds had higher 1-year weight (9.0% to 31.1%) and 3-year weight (23.4% to 35.8% heavier). Using IM, BM, or Nili-Ravi in the two-way cross or their combinations in the three-way crosses recorded the greatest advantage over the Philippine carabao in terms of 1-year weight. Greater percent advantage over the Philippine carabao in terms of 3-year weight was noted in crosses that involve the Indian Murrah.

Table 8. Percent heterosis and advantage of crosses over the Philippine Carabao in terms of body weight at different ages

	Birth	6 mos	1 yr	2 yrs	3 yrs	>3 yrs
Percent Heterosis:						
50%IM–50%PC	52	*na*	-18.0	-7.6	-1.3	-1.3
50%BM– 50%PC	*na*	*na*	*na*	-0.9	-2.3	2.3
50%Nili-Ravi – 50%PC	*na*	*na*	*na*	*na*	*na*	*na*
Backcross (75%IM– 25%PC)	3.7	-16.1	-22.4	-8.8	-8.8	-8.3
Three-way cross	*na*	*na*	*na*	*na*	*na*	*na*
Advantage over the Philippine Carabao (%):						
50%IM–50%PC	11.0	-	10.8	14.8	31.5	1.4
50%BM– 50%PC	17.9	-	15.2	7.2	24.0	6.7
50%Nili-Ravi – 50%PC	17.9	-	11.9	-1.6	26.7	11.3
Backcross (75%IM– 25%PC)	15.2	-	9.0	11.3	35.8	-5.2
Three-way cross	14.5	-	31.1	19.3	23.4	-19.0

Note: "na" means that heterosis cannot be computed due to unknown value of one parental purebred involved in the cross.

- Withers Height, Heart Girth, Body Length

Withers height (C.V.=5.4%), heart girth (C.V.=6.4%), and body length (C.V.=15.4%) of buffaloes that are at least 3 years old were significantly different between breed groups (P<.01). Withers height was largest in the Philippine carabao, Indian Murrah, and 50%IM – 50%PC than the Bulgarian Murrah, 50%BM – 50%PC, 50%Nili-Ravi – 50%PC, and three-way crosses (see Table 9, next page). Heart girth was however, highest in the 50%Nili-Ravi – 50%PC (i.e. 206.0 cm) and smallest in the Bulgarian Murrah (176.7 cm), backcross (178.5 cm), and the three-way cross (176.2 cm). Body length was notably shortest in the three-way cross than the other breed groups.

Among the pure breeds, the Philippine carabao and Indian Murrah were larger in size than the Bulgarian Murrah in terms of withers height and heart girth. The average body length of the Philippine carabao (138.8 cm) was however shorter than the Indian (161.8 cm) and Bulgarian Murrah (141.7 cm).

Table 9. Least square means for external body measurements (cm), by breed Group

Breed Group	Withers Height (cm)	Heart Girth (cm)	Body Length (cm)
Pure breeds:			
Philippine Carabao (PC)	136.7^{a} (2.4)	194.7^{ab} (4.0)	138.8^{ab} (7.6)
Indian Murrah (IM)	143.3^{a} (2.9)	197.8^{ab} (4.9)	161.8^{a} (9.3)
Bulgarian Murrah (BM)	127.0^{b} (1.8)	176.7^{c} (3.0)	141.7^{ab} (5.7)
Two-way crosses:			
50%IM – 50%PC	137.8^{a} (1.5)	199.5^{ab} (2.6)	159.4^{a} (4.9)
50%BM – 50%PC	128.2^{b} (3.2)	186.0^{bc} (5.4)	152.4^{a} (10.2)
50%Nili-Ravi – 50%PC	141.6^{a} (3.2)	206.0^{a} (5.4)	165.0^{a} (10.2)
Backcross (75%IM – 25%PC)	126.9^{b} (1.7)	178.5^{c} (2.9)	147.8^{a} (5.6)
Three-way crosses	126.2^{b} (3.6)	176.2^{c} (6.1)	117.5^{b} (11.5)
N observations	84	84	84
Overall mean	132.9	188.8	145.3
Standard deviation	9.2	15.8	24.8
C.V.,%	5.4	6.4	15.4
F-test result	**	**	**

Note: Numbers in parenthesis are standard errors of the least square means
Least square means in the same column with different letter superscripts are significantly different from one another, (P<.05).
"ns" means no significant difference, P>.05.
** means significant differences, P<.01.

Percent heterosis was highest in the 50%IM – 50%PC for withers height (-1.6%) and heart girth (1.6%). On the other hand, percent heterosis for body length was highest in the 50%BM – 50%PC (8.7%). The superiority of the crossbreds over the Philippine carabao was highest for the 50% Nili-Ravi – 50%PC and lowest for the backcross and three-way cross (see Table 10).

➢ Total Milk Yield, Lactation Length, Age at First Calving

The reproductive performance of the female water buffaloes measured in terms of total milk yield (C.V.=34.2%), lactation length (C.V.=24.8%), and age at first calving (C.V.=16.2%) were significantly different between breed groups (see Table 11). No data however was obtained for the 50%Nili-Ravi – 50%PC, backcross, and three-way cross.

Among the pure breeds, total milk yield was highest while lactation length and age at first at calving were shortest in the Indian and Bulgarian Murrah than the Philippine carabao. The total milk yield and lactation length were not significantly different between the 50%IM – 50%PC and the Philippine carabao. Age at first at calving in the 50%IM – 50%PC was however, similar to that of the Indian and Bulgarian Murrah.

Table 10. Percent heterosis and advantage of crosses over the Philippine Carabao in terms of external body measurements

	Withers Height	Heart Girth	Body Length
Percent Heterosis:			
50%IM–50%PC	-1.6	1.6	6.0
50%BM – 50%PC	-2.8	0.2	8.7
50%Nili-Ravi – 50%PC	*na*	*na*	*na*
Backcross (75%IM – 25%PC)	-9.7	-10.1	-8.0
Three-way cross	*na*	*na*	*na*
Advantage over the Philippine Carabao (%):			
50%IM–50%PC	0.8	2.5	14.8
50%BM – 50%PC	-6.2	-4.5	9.8
50%Nili-Ravi – 50%PC	3.6	5.8	18.9
Backcross (75%IM – 25%PC)	-7.2	-8.3	6.5
Three-way cross	-7.7	-9.5	-15.3

Note: "na" means that heterosis cannot be computed due to unknown value for the Nili-Ravi.

Table 11. Least square means for total milk yield, lactation length and age at first calving, by breed group

Breed Group	Total Milk Yield, kg	Lactation Length, d	Age at 1st Calving, mos
Pure breeds:			
Philippine Carabao (PC)	397.5^{b} (291.8)	217.0^{c} (38.7)	72.0^{b} (6.0)
Indian Murrah (IM)	1,348.0^{a} (291.7)	305.0^{a} (38.7)	53.7^{a} (4.9)
Bulgarian Murrah (BM)	1,539.1^{a} (100.0)	244.2^{ab} (13.3)	49.3^{a} (1.9)
Two-way crosses:			
50%IM – 50%PC	724.8^{b} (137.5)	189.8^{bc} (18.2)	53.9^{a} (2.8)
50%BM – 50%PC	-	-	48.0a (8.5)
N observations	30	30	35
Overall mean	1,206.0	230.1	52.1
Standard deviation	579.6	60.8	9.6
C.V.,%	34.2	24.8	16.2
F-test result	**	**	*

Note: Numbers in parenthesis are standard errors of the least square means.
Least square means in the same column with different letter superscripts are significantly different from one another, (P<.05).
* means significant differences, (P<.05).
** means significant differences, (P<.01).

Percent heterosis was negative for total milk yield (-14.4%), lactation length (-11.4%), and age at first calving (-14.2%), see Table 12. However, when compared to the Philippine carabao, the percent advantage of the 50%IM – 50%PC was 220.9% and 43.6% for total milk yield and lactation length, respectively. The age at first calving was also earlier by 25.1% compared to the Philippine carabao.

Table 12. Percent heterosis and advantage of crosses over the Philippine Carabao in terms of total milk yield, lactation length and age at first calving

Breed Group	Total Milk Yield, kg	Lactation Length, d	Age at 1st Calving, mos
Percent Heterosis:			
50%IM – 50%PC	-14.4	-11.4	-14.2
50%BM – 50%PC	*na*	*na*	.23.6
Advantage over the Philippine Carabao (%)			
50%IM – 50%PC	220.9	43.6	-25.1
50%BM – 50%PC	*na*	*na*	2.5

Note: "na" means that heterosis cannot be computed due to unknown value of Bulgarian Murrah - Carabao cross.

- Semen Volume and Semen Characteristics

Data on the semen characteristics of Philippine carabao and Indian Murrah were the only available data used in the analysis. Purebred (and not crossbred) bulls are being used for semen collection at the PCC-UPLB for conservation purposes and production of crosses through artificial insemination. Table 13 shows that semen volume per ejaculate was significantly higher in the Indian Murrah (5.08 ml) than the Philippine carabao (2.52 ml). No significant differences in mass activity (C.V. =22.1%), sperm motility (C.V.=2.9%) and sperm concentration (C.V.=18.1%) was found between the two pure breeds.

Table 13. Comparisons of least square means for semen volume and semen characteristics in the Philippine Carabao and the Indian Murrah

Breed Group	Volume (ml)	Mass Activity (%)	Sperm Motility (%)	Sperm Concen. (%)
Philippine Carabao (PC)	2.52[b] (0.1)	1.49 (0.3)	57.34 (1.2)	954.30 (114.0)
Indian Murrah (IM)	5.08[a] (0.1)	1.87 (0.3)	57.95 (1.2)	824.10 (114.0)
(Advantage of IM over PC, %)	101.6	25.5	1.1	-13.6
N observations	4	4	4	4
Overall mean	3.80	1.68	57.64	889.20
Standard deviation	1.48	0.38	1.42	151.57
C.V., %	3.20	22.12	2.92	18.13
F-test result	**	ns	ns	ns

Note: Numbers in parenthesis are standard errors of the least square means.

Least square means in the same column with different letter superscripts are significantly different from one another, (P<.05).

ns means no significant differences, (P>.05).
** means significant differences, (P<.01).

On the contrary, Ramakrishnan et al. (1989) reported that the semen quality (except for spermatozoa concentration), was significantly better (P<.05) in the swamp compared to the Murrah buffalo. The mean values of the swamp and Murrah buffalo for (i) ejaculation time was 467 and 112 seconds, (ii) semen volume, 1.79 and 2.19 ml, (iii) progressive motility, 73.43 and 71.52%, (iv) spermatozoa concentration, 1177.80 and 1140.60 x 10^6 cells/ml, live spermatozoa, 89.24 and 86.87%, (vi) intact acrosome 89.41 and 86.33%, and vii) spermatozoa abnormalities, 9.07 and 12.28%, respectively.

- Type of Horns

The Philippine carabao had the crescent-like shape or semi-circle horns. The Indian and Bulgarian Murrah had the ram-like horns that are curved backward and up (sometimes spiral and strong tendency to curl). All crosses (i.e. two-way crosses, backcrosses, and three-way crosses) had horns that are intermediate between the crescent and ram-like shapes, i.e. crescent-shaped but with slight to marked upward turn. Similar horn features in crossbred buffaloes, were described by Azmi et al. (1989).

Correlation Between Karyotypic Characteristics and Reproductive Traits

Significant linear correlation between karyotypic characteristics and productive traits (see Table 14) and reproductive traits (see Table 15) were found. The significant correlation values imply that some karyotypic characteristics can be used as important markers or criteria to select potentially productive young water buffaloes.

Table 14. Significant linear correlation between some production traits and karyotypic characteristics

Production Trait	Karyotypic Characteristic		Corr. Coefficient	P value	N paired obs.
Birth weight	3A	Arm ratio	-.23	*	50
Birth weight	5B	Rel. length	+.24	*	73
6 month weight	6B	Rel. length	+.31	*	45
1 year weight	3A	Arm ratio	-.29	*	62
1 year weight	5B	Rel. length	+.26	*	62
1 year weight	XB	Rel. length	+.31	*	39
2 year weight	3A	Arm ratio	-.29	*	53
3 year weight	%cells, 2n=49		-.33	**	52
3 year weight	%cells, 2n=50		+.46	*	52
3 year weight	4A	Rel. length	-.30	*	52
3 year weight	4A	Arm ratio	+.27	*	52
3 year weight	4B	Rel. length	+.28	*	52
>3 year weight	1B	Arm ratio	-.29	*	50
>3 year weight	3A	Arm ratio	-.35	**	50

Table 14. *continuation...*

Production Trait	Karyotypic Characteristic		Corr. Coefficient	P value	N paired obs.
Heart girth	%cells, 2n=50		-.38	**	70
Heart girth	1B	Rel. length	+.24	*	70
Heart girth	3A	Arm ratio	-.26	*	70
Heart girth	4A	Rel. length	+.27	*	70
Heart girth	4A	Centr. Index	+.23	*	70
Heart girth	6A	Rel. length	-.29	*	70
Heart girth	7B	Rel. length	+.41	*	70
Withers height	%cells, 2n=50		-.32	**	70
Withers height	3A	Centr. Index	+.30	**	70
Withers height	3A	Arm ratio	-.31	**	70
Withers height	3B	Rel. length	+.23	*	70
Withers height	5B	Rel. length	+.23	*	70
Body length	%cells, 2n=47		-.42	**	70
Body length	4B	Rel. length	+.24	*	70

Note: P value = * means significant difference from 0, (P<.05).
P value = ** means highly significant difference from 0, (P<.01).

Table 15. Significant linear correlation between some reproductive traits and karyotypic characteristics

Reproduction Trait	Karyotypic Characteristic		Correlation Coefficient	P value	N paired obs.
Total milk yield	cells, 2n=50		+.68	*	13
Lactation length	2A	Rel. length	+.63	*	13
Age at first calving	%cells, 2n=47		+.56	*	15
Age at first calving	4B	Rel. length	-.56	*	15
Semen volume	%cells, 2n=47		+.99	**	4
Semen volume	3A	Rel. length	-.99	**	4
Semen volume	17B	Rel. length	+.99	*	4
Sperm mass activity	1A	Rel. length	+.23	*	4
Sperm mass activity	1A	Centr. Index	-.99	**	4
Sperm mass activity	1B	Arm ratio	+.96	*	4
Sperm motility	2A	Centr. Index	-.99	**	4
Sperm motility	2B	Arm ratio	+.98	**	4
Sperm motility	3B	Arm ratio	+.99	**	4
Sperm motility	5A	Rel. length	+.98	*	4
Sperm concentration	%cells, 2n=48		+.95	*	4
Sperm concentration	4A	Centr. Index	+.96	*	4
Sperm concentration	4A	Rel. length	-.98	*	4

Note: P value = * means significant difference from 0, (P<.05).
P value = ** means highly significant difference from 0, (P<.01).

> Relationship With Some Production Traits

The body weights at different ages (i.e. birth weight, 1 year, 2 years, and > 3 years old) were positively correlated with percent relative length of some chromosomes but are negatively correlated with arm ratio. This may suggest that a larger chromosome (especially chromosomes 3A and 4A) may contain extra genes that act additively and are associated with higher values in various weight traits. Three-year weight in particular, is negatively correlated with chromosome frequency for 2n=49 (i.e. r = +.46) but positively correlated with chromosome frequency for 2n=50 (i.e. r = -.33).

Withers height and heart girth of water buffaloes that are at least 3 years old, are positively correlated with percent relative length and centromeric index, but is negatively correlated to arm ratio (especially chromosomes 3A and 4A). Chromosome frequency for 2n=50 is negatively correlated with withers height (i.e. r = -.32) and heart girth (i.e. r = -.38). Genotypes with greater frequency of cells 2n=48 (purebred swamp) and 2n=49 (crosses) are likely have to be large in terms of withers height and heart girth.

The significant correlation between karyotypic characteristics of chromosomes 3A and 4A with the weight traits and withers height and heart girth, may imply pleiotropy (i.e. one gene affects the expression of two or more traits).

> Relationship With Some Reproductive Traits

For the female water buffaloes, total milk yield was positively correlated to chromosome frequency for 2n=50 (i.e. r = +.68). The relative length of chromosome 2A was positively associated with lactation length (i.e. r = .63). The relative length of chromosome 4B was related to early age at first calving (i.e. r = -.56).

In males, semen volume was positively correlated to chromosome frequency 2n=50. Mixed results however, were noted on the association between percent relative length, centromeric index and arm ratio with the other semen characteristics, perhaps due to the small data size considered in the analysis. More data will therefore be required in order to arrive at more meaningful relationships between karyotypic and semen characteristics.

Applications and Recommendations

Results of the karyotype analyses and their relationships with production and reproductive traits in water buffaloes can be applied in the design and development of local selection, crossbreeding, and/or conservation programs.

The high C.V. values in each trait (karyotypic, production, or reproductive) imply potential and practical basis to accurately distinguish genetic differences between breed groups, especially in the absence of breeding history and factual pedigree and identification records on-farm. Information on the purity of the breed such as the Philippine carabao (or distinct differences between pure breeds) is especially useful in prioritizing the limited funds available to conserve them in their pure form *in situ* (live animal gene pool) or *ex situ* (frozen semen and/or embryos). For example the relative length, centromeric index and arm ratio of chromosomes 1A and 1B are distinctly different in the Philippine carabao than any other breed group.

Differences between pure breeds and their crosses in terms of percent heterosis and advantage over the Philippine carabao will be useful in the design and development of systematic crossbreeding programs. Crossbred animals in smallholder farmer and institutional herds, where recording is usually lacking, could be tested at an early age in order to identify and cull those that are going to be unproductive.

The promotion and production of specific breed groups (purebred or crossbred) can then be matched to specific but common breeding objectives and dominant local production and marketing systems. However, future studies should consider comparisons of different generations within the two-way cross (i.e. F_1, F_2, F_3, etc.) and different blood compositions within the three-way crosses.

The significant correlation between karyotypic characteristics and some production and reproduction traits can be used in the design of a "marker"-assisted selection. 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. For example, karyotypic characteristics for chromosomes 3A and 4A may be used as the selection criterion to improve various weight traits, withers height, and heart girth. Chromosome frequency for 2n=50, relative length of chromosomes 2A and 4B may be considered as indirect traits to select for total milk yield, lactation length, and age at first calving, respectively.

An advantage of karyotype analysis in water buffaloes is that blood samples can be obtained in both sexes, young or old. However, to successfully obtain photokaryotypes of each animal evaluated, laboratory facilities and expertise are required to be developed and established on-farm. In this regard, karyotype analysis is especially recommended for institutional (nucleus or elite) herds such as those maintained in the national gene pool and various PCC centers. This activity must be implemented in aid of the national breeding program for water buffaloes. It may even initiate the important establishment of a national buffalo registry.

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Literature Cited

Azmi, T.J., M.K. Vidyadaran and N. Hardy. 1989. Phenotypic identification of the grade (karyotype) of crossbred buffaloes. In Vidyadaran, M.K., Azmi, T.I. and Hilmi, ed. Symposium on Buffalo Genotypes for Small Farms in Asia. Centre for Tropical Animal Production and Disease Studies, Universiti Pertanian, Malaysia and International Development Research Centre, Canada. pp. 191-193.

Bondoc, O.L. 1998. *Biodiversity of Livestock and Poultry Genetic Resources in the Philippines.* Los Baños: IAS-CA/UPLB and PCARRD/DOST. 141 pp.

Bondoc, O.L., M.C.G.T. Flor, A.G. Albarece and S.G.N. Rebollos. 2002. Variations in karyotypic characteristics of different breed groups of water buffaloes (*Bubalus bubalis*). Asian Australian Journal of Animal Sience 15 (3): 321 - 325.

Bongso, T.A. and M. Hilmi. 1982. Chromosome banding homologies of a tandem fusion in river, swamp and crossbred buffaloes (*Bubalus bubalis*). Canadian Journal of Genetics and Cytology. 24: 667-673.

Chavananikul, V. 1989. Cytogenetic aspects of crossbreeding in buffaloes. In: Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia. (eds. M.K. Vidyadaran, T.I. Azmi, and P.K. Basrur). 1990. Universiti Pertanian Malaysia. pp. 281-288.

Cooper, D.W. 1991. Cytogenetic aspects of crossbreeding river and swamp buffalo. In: Buffalo and Goats in Asia. Genetic Diversity and Its Application. Proceedings of a Workshop in Kuala Lumpur, Malaysia. 10-14 February 1991. Tulloh, N.M. (Ed.) ACIAR Procedings No. 34. pp. 48-52.

Evaristo, G. 1915. Study of Philippine carabao. The Philippine Agriculturist and Forester. 4(7): 123-141.

Fischer, H. 1989. Karyotype research: a review. In: Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia. (eds. M.K. Vidyadaran, T.I. Azmi, and P.K. Basrur). 1990. Universiti Pertanian Malaysia. pp. 277-279.

Harisah, M., T.K. Azmi, T.A. Bongso, M. Hilmi, M.K. Vidyadaran, Z.M. Nava, V.G. Momongan and P.K. Basrur. 1989. Chromosome make-up of water buffalo breedtypes in South East Asia. In Vidyadaran, M.K., Azmi, T.I. and Hilmi, ed. Symposium on Buffalo Genotypes for Small Farms in Asia. Centre for Tropical Animal Production and Disease Studies, Universiti Pertanian, Malaysia and International Development Research Centre, Canada. pp. 289-300.

Hilmi, M. 1991. Karyotypes of water buffalo crosses (swamp x river). In: Buffalo and Goats in Asia. Genetic Diversity and Its Application. Proceedings of a Workshop in Kuala Lumpur, Malaysia. 10-14 February 1991. Tulloh, N.M. (Ed.) ACIAR Procedings No. 34. pp. 53-56.

Iannuzzi, L. 1994. Standard karyotype of the river buffalo (*Bubalus bubalis L.*, 2n=50). Cytogenet. Cell. Genet. 67: 102-113.

Majid, M.A., Z.M. Nava, B.A. Parker, V. G. Momoñgan and M.N. Islam Faridi. 1991. Chromosomal analysis of buffalo (*Bubalus bubalis*): swamp type (Philippine carabao) and river types (Murrah and Nili Ravi) and their crosses (F_1 and F_2). Philippine Agriculturist. 74(4): 543-556.

Na-Chaingmai, A. and V. Chavananikul. 1998. Performance and cytogenetic aspects of swamp x river crossbred buffaloes. Proc. 5th World Congress on Genetics Applied to Livestock Production. University of New England, Armidale, NSW, Australia. 451-458.

Nicholas, F.W. 1989. Veterinary Genetics. Oxford Science Publications, Clarendon Press, Oxford.

Parker, B.A. 1992. Genetic improvement. In Carabao Production in the Philippines. (Eds. S.K. Ranjhan and P.S. Faylon). PCARRD and FAO/UNDP. Los Baños, Laguna, Philippines. pp. 25-62.

Ramakrishnan, P., S. Adnan, Y. Nordin and S. Shanmugavelu. 1989. A comparison of semen characteristics of the swamp and Murrah buffaloes. In: Proceedings of Seminar on Buffalo Genotypes for Small Farms in Asia. (eds. M.K. Vidyadaran, T.I. Azmi, and P.K. Basrur). 1990. Universiti Pertanian Malaysia. pp. 235-238.

Shrestha, N.P. 1992. Genetic evaluation of Philippine carabao (PC), Murrah Buffalo (MB) and the F_1 hybrids MB x PC and Nili-Ravi x PC. Unpublished Ph.D. Thesis Institute of Animal Science, University of the Philippines Los Baños, Laguna.

Songsri, S. and D.A. Ramirez. 1979. The cytology of swamp and river type of water buffaloes and their hybrids. Philippine Agriculturist. 62: 262-275.