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Mapping fragile-sites in the standard karyotype of River Buffalo (*Bubalus bubalis,* 2n=50)

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ABSTRACT: A fragile-site map has been preliminarily established in the standard karyotype of river buffalo (*Bubalus bubalis*, 2n=50) with the aim of unmasking 'weak' chromosomal regions in the karyotype of the species. The majority of the breakages took place in the RBA/RBG-negative bands or at the band-interband regions. The most fragile chromosomes were identified as the inactive X, chromosomes 9 and 8, and the active X, with 42, 32, 31 and 30 breakages, respectively. The 400 breakages were distributed in 106 breaksites (BS), with an average intensity of 4 breaks per chromosomal site; (b) the most fragile bands of the river buffalo karyotype were identified as 9q213 with 24 breaks, band 19q21 with 16; inacXq24 with 15; bands 15q23 and 17q21 with 13; band 13q23 with 12, and so on. Preliminary gene mapping analysis revealed that the closest loci to these fragile sites contain genes such as RASA1 and CAST (9q214), NPR3 and C9 (19q19), OarCP09 (15q24), PLP and BTK (Xq24-q25) and EDNRB (13q22), whose mutations are responsible for severe phenotypic malformations and immunodeficiency in humans and mice, and meat quality in pigs. Further cytogenetic and molecular studies are needed to fully exploit the biological significance of the fragile sites in the karyotypes of domestic animals and their relationships with productive and reproductive efficiency.

Key words: Genetic diversity, *Bubalus bubalis*, Fragile sites, Chromosomes.

INTRODUCTION - Cytogenetic characterization of aphidicolin-induced fragile sites in metaphase chromosomes of river buffalo (*Bubalus bubalis*, 2n=50) has been scarcely docu-

mented in the literature. Balakrishnan et al. (1988) reported gaps on the X chromosome of anestrous females suggestive of a fragile site, whereas Pires et al.(1998) found fragile sites on the X chromosome in three breeds of river buffalo, but -so far- no detailed information is available on the fragile site expression in the karyotype of this species. Recently, a preliminary report has been published by our laboratory (Nicodemo et al.,2007) on the chromosomal characterization of the fragile sites on this species. The present study extends previous data by reporting on the localization of the fragile sites on the standard karyotype of this species. The definition of a species-specific 'fragile site map' for the various domestic animals species represents an important step toward a more precise characterization of the degree of chromosome stability/instability of the species, which could be used as "bio-indicator" for monitoring environmental pollution on behalf of environmental and nutritional security.

MATERIAL AND METHODS - A sample of eight clinically healthy river buffaloes belonging to the Italian Mediterranean breed was used for the investigation: four (two males and two females) reared in a farm located in the province of Naples, and four (two males and two females) reared in a farm located in the province of Salerno. Conventional lymphocyte cultures were performed; after 48 hours of growth, Aphidicolin (Sigma) was added to the cultures at 0.15 mM (final concentration), as recommended by Rodriguez et al.(1990), for other 24 hours; 6 hours before the end, BrdU + H33258 (20 μg/ml, f.c. each) was added to the cultures for labelling late replication regions of the genome. The slides were treated for RBA and RBG-banding and examined under fluorescence and bright field optics. Only metaphases with clear RBA or RBG-banding and -at least- one clear fragile site were considered. A total of 400 fragile sites was scored, 50 for each animal, and localized on the standardized ideogram of the river buffalo (Iannuzzi, 1994).

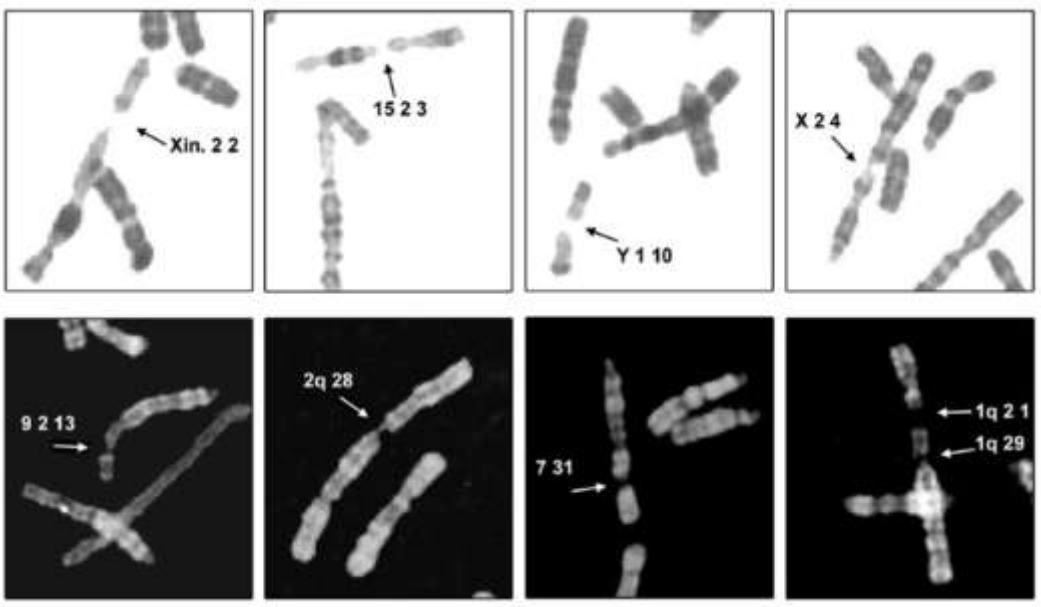
RESULTS AND CONCLUSIONS - Figure 1 shows breaks on various river buffalo chromosomes from partial and enlarged RBG-banded and RBA-banded prometaphase plates. Preliminary results are as follows: (a) the 400 breaks were distributed in 106 breaksites, with an average intensity of nearly 4 breaks per chromosomal site; (b) the most fragile bands of the river buffalo karyotype were identified as 9q213 with 24 breaks, band 19q21 with 16; inacXq24 with 15; bands 15q23 and 17q21 with 13; band 13q23 with 12, and so on (Table 1). χ^2 analysis was performed to identified fragile sites in the river buffalo karyotype. Based on 438-band standard RBG karyotype, and assuming each band had an equal probability of breakage, the expected number of breaks from the 400 aberrations induced by APD was 0.91 breaks/band. χ^2 analysis indicated that any band with 3 or more breakage events was significantly damaged. Band locations of fragile sites with 3 or more breakage are listed in Table 1. On 106 different breakpoints, 51 locations were significantly damaged, distributed as follows: 5 fragile sites in the chromosome 8; 4 FS in the chromosomes Xact., Xinac, Y and 7; 3 FS in the chromosomes 1, 2,10 and 13; 2 FS in the chromosomes 3, 9, 12, and 22; 1 FS in the chromosomes 5, 14, 15, 16, 17, 18, 19, 20, 21 and 23. No FS were found on chromosomes 4, 6, 11 and 24. Preliminary gene mapping analysis reveal that the closest loci to some of these fragile sites contain genes such as RASA1 and CAST (9q214), NPR3 and C9 (19q19), OarCP09 (15q24), PLP and BTK (Xq24-q25) and EDNRB (13q22), whose mutations are responsible for severe phenotypic malformations and immunodeficiency in humans and mice; CAST gene has been considered a good candidate influencing the meat

Table 1. Identification of aphidicolin-induced fragile sites on RBG-banded prometaphase chromosomes of river buffalo.

N of breaks	Band locations
24[c]	9q213
16[c]	19q21
15[c]	17q21, inacXq24
13[c]	15q23
12[c]	13q23
11[c]	inacXq31
9[c]	8q16
8[c]	1q21, 10q12, inacXq22
7[c]	7q24, 8q31, Xq24, Xq31
6[c]	7q14, 7q31, 10q14, 13q21, 23q12, Xq22, inacXq13
5[c]	1q29, 2q13, 2q28, 12q21, 12q31, 13q12, Yq14, Yq110
4[b]	8q21, 8q33, 9q21, 10q21, 14q12, 16q14, 18q21, Xq13, Yq12, Yq16
3[a]	1q44, 2q26, 3q14, 3q16, 5q15, 7q22, 8q14, 20q12, 21q21, 22q13, 22q21

[a]P<0.05, [b]P<0.005, [c]P<0.001.

Figure 1. Chromosome breaks on river buffalo chromosomes from RBG-banded (upper line) and RBA-banded (lower line) prometaphase plates.



quality in pigs. Further cytogenetic and molecular studies are needed to fully exploit the biological significance of the fragile sites in karyotype evolution (Ruiz Herrera et al., 2006) and their relationships with animal biodiversity, environmental and nutritional security, and productive and reproductive efficiency of livestocks.

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